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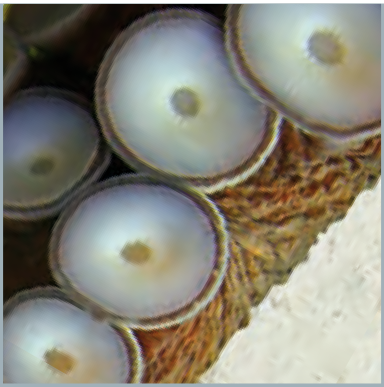
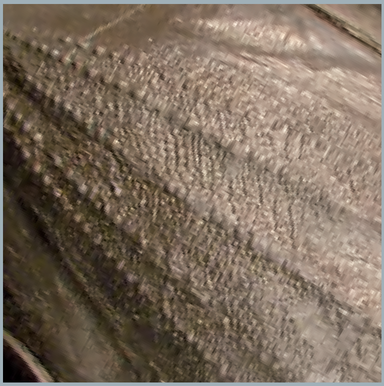


Proceedings of the 3rd international symposium Coleoid Cephalopods Through Time



Luxembourg
8th - 11th October 2008

Dirk Fuchs (editor)

59 2010

Travaux scientifiques
du Musée national
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Coleoid Cephalopods Through Time

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In Memory of Winfried Haas (*1934 - †2007)

Organizing institution:
Musée national d'histoire naturelle, Luxembourg (MnhnL)

Venue:
Centre Neumünster

Administrative responsibility:
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Scientific responsibility:
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Organising committee:
Alain Faber
Robert Weis
Dirk Fuchs

Preface

"It is our aim here to show how fruitful the combination of zoological and paleontological research can be for the creation of a scientific paleozoology."

Adolf Naef (1922)

Coleoidea, typified by squids, cuttlefishes and octopods, is a subclass of the invertebrate class Cephalopoda. Whereas publications on present-day coleoids often start with positive attributes such as "highly interesting group" or "fascinating animals", publications on fossil coleoids repeatedly start with introductive phrases such as "…the fossil record of coleoids is very poor…" etc. We should disengage this negative attitude, because it is out-of-date and most notably counteracts the primary goal of the International Symposium "Coleoids Through Time": to provide a platform for combined projects of both paleontologists and neontologists.

Many new localities from all over the world containing extraordinarily well preserved coleoids have been discovered since the pioneering works of Adolf Naef. Many time gaps without any morphological information are closed today. Prof. Naef would have been astonished!

Participants of the International Symposium "Coleoids Through Time" in Berlin (2002), Prague (2005) and Luxembourg (2008) probably have felt the motivating atmosphere? The triennial meeting has been established along with the main Cephalopod meetings "CIAC" (Cephalopod International Advisory Council) and "Cephalopods – Present & Past". While the latter meetings are focused on biological or paleontological topics respectively, the Coleoid Symposium is the only opportunity to meet colleagues from both fields of work. The particular charm of the Coleoid meetings has been the intimate size of the group (Berlin: 41; Prague: 35; Luxembourg: 42). Furthermore, the proceedings volumes of the Berlin and Prague meetings represent an important literary resource. This clearly reflects the success of the initiators of the first coleoid meeting: Kerstin Warnke, Sigurd von Boletzky and Helmut Keupp.

Volume 59 of the FERRANTIA, contains products of presentations held during the 3rd meeting at the Musée national d'histoire naturelle in Luxembourg (MnhnL). The proceedings volume is well-balanced and includes 8 neontological and 11 paleontological peer-reviewed contributions. The alphabetically ordered papers are covering various biological aspects such as behaviour, embryology, cytology, ontogeny, systematics, taphonomy, paleobiogeography, or phylogenetics.

Nevertheless, it is worthwhile to note that contributions are still separated. Paleontologists publish with Paleontologists and Neontologists with Neontologists. Interdisciplinary approaches are still limited and can be further improved; and this should be one of our goals for the next meeting in Stuttgart (SW Germany). We dedicate therefore the present volume to our late colleague Prof. Dr. Winfried Haas (1934 – 2007), who well understood how to span both paleontological and neontological knowledge.

As the editors of the symposium, we cordially thank Georges Bechet, Director of the MnhnL for his support and particularly the Font National de la Recherche Luxembourg (FNR) for their funding of the symposium. Many thanks go to Larisa Doguzhaeva, Jan Strugnell, Nino Mariotti, Martin Kostak, and Alexander Arkhipkin, the chairmen of the scientific sessions. We are also indebted to Isabelle Keller from the graphical service of the museum for her excellent conceptual design of the logo and the poster. Finally, we are grateful to the authors for their interesting contributions, to the reviewers for their thorough revisions of the submitted manuscripts and to Thierry Helminger and Romain Bei for the layout of this volume.

Dirk Fuchs, a child of the 1st Coleoid-Symposium.

Robert Weis

Alain Faber

In memoriam Winfried Haas (1934-2007)

Klaus Bandel

Dr. Winfried Haas, former Professor of Paleontology at the University of Bonn, was one of the initiators of the meetings "Coleoid cephalopods through time".

Winfried Haas was born and grew up near Frankfurt in Neuisenburg. He was attracted by fossils when still in school collecting the famous Teritiary fauna of the Mainzer Basin with its well preserved molluscs. After school he joined the University of Frankfurt and started to study geology from 1955 to 1958. During this time he became interested in the Devonian of the Rheinische Schiefergebirge, especially the Eifel. He changed the university to Bonn and became a student of Prof. Dr. H. K. Erben. Throughout his succeeding career as a paleontologist he was closely associated and influenced by Erben. Winfried remained a Frankfurt patriot as his local accent portrayed. Throughout his carreer he maintained close contacts with collegues in the Senckenberg Museum, including Wolfgang Stuve who worked on the stratigraphy and faunas of the Devonian of the Eifel. Many of Winfried's students in Bonn received their training in this region where he himself was a colorful leader for groups both from Germany and abond.

His doctorate thesis was based on field mapping, stratigraphy and palaeontology of the Silurian and Devonian rocks of the Bosporus region in northwest Turkey. It was a difficult area which he negotiated on a battered motorcycle and made impressive collections of trilobites of which group he first made his name. This was the starting point to become an internationally renowned specialist of trilobite systematics and an expert on asteropygines in particular. Most of his published work dealt with this group and he was expected to contribute a major revision for the forthcoming new issue of the Treatise. His passion for trilobites did not cease during all his lifetime when he became engaged in various topics including Permian trilobites from Afghanistan and more recently morphofunctional studies on the eye, muscle attachments and enrolment in phacopids.

Within the frame of a science project of the DFG "Biomineralisation" headed by H. K. Erben, Winfried Haas changed his field of study to the molluscs and here their ultrastructure. 1971 he presented his habilitation based on the shell structure and functional morphologyy of the Polyplacophora. The Bonn laboratory was equipped with one of the first stereoscan electronic microscopes in Germany and with this Winfried Haas studied the shell structure and functional morphology of the chitons.

Regrettably, this work took an age before it was published mainly because Winfried was a perfectionist and he never really considered any of his work completed and this was true of his research with cephalopods including *Sepia* and other coleoids. He built up large collections of these animals both fossil and living, the latter of which he collected while on a visit to Japan and visits to various marine biological centres, among them repeatedly the Laboratoire Arago in Banyuls-sur-Mer in France. His Bonn laboratory was equipped for studies of biomineralization of which he became an authority and included serial sectioning of shell material, microtoming and staining of soft tissue and photographing often using pieces of self built apparatus. He was a bioengineer and optical instruments fascinated him.

The few publications he produced on the cephalopoda are today major contributions to the understanding the origin of the Octopoda and evolutionary history of *Sepia* and *Spirula* among the coleoids of the Late Cretaceous and Tertiary. At the time of his death he had a large unfinished manuscript on fossil decapods which regrettably lacks the necessary illustrations for its completion.

Winfried Haas was appointed Professor in Bonn in 1983 where he became an active and teacher in invertebrate palaeontology until his retirement. He was politically conservative and held strong views on the future of Europe, its economic role in the world and its history. He was well read and had many hobbies including church architecture, botany and good wine. At the institute he was held in high respect by staff and students and his often formal style could suddenly disappear as he became one of the boys enjoying a beer and a cigarette. He adopted both Raimund Feist and the author when we were students at Bonn and protected us from the Godfather (Erben). His close friend and colleague, David Bruton, University of Oslo, remembers many enjoyable hours discussing trilobites and politics, about which they seldom agreed, but it was all good fun. He and his Oslo students remember Winfried as an inspiring leader on field trips to Germany and France, well dressed with tie and hat and equipped with a whistle which he blew and shouted "hop hop" when he required attention.

In his scientific work Winfried Haas was extremely careful in his interpretation and went into very useful, descriptive details so that due to his unsuspected early death he left some unfinished work on the ten armed inkfish. If we are successful in finding the intended illustrations to that unfinished work, we will try to make it available to the scientific audience.

With Winfried we lost a passionate and inspiring collegue and good friend who we will keep in our good memory.

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It's all in the cues: octopuses (*Enteroctopus dofleini*) learn to open jars

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Abstract

Previous researchers (Fiorito, von Planta & Scotto 1990) were not able to prove that an octopus can learn to open a jar in order to obtain crustacean prey inside. We studied that situation with a variety of cues about the prey: visual only (closed jar), minimal chemical cues (jar with holes) or maximal ones (jar smeared on the outside with mucus from herring). *Enteroctopus dofleini* seldom opened the jar lid within the 60-minute time limit in the first two conditions (mean 1/10 trials in Condition One and 2/10 in Condition Two) but did so in 9.5/10 of Condition Three, although they spent longer in contact with the jars (24 minutes) in Condition Two than in Condition One (8 minutes). These three Conditions were presented in a varying order, different for each octopus. Over the five days of presentation, octopuses in Condition Three significantly improved their latency to open the jar, from mean 41 minutes in Day One to 15 minutes in Day Three. Thus octopuses learned to open jars (decrease the time taken to do so) if non-visual cues were also available when the jar was out of sight under the arm web.

Introduction

Perhaps inevitably, we humans are anthropocentric in our evaluation of behavior and motivations of non-human animals (Eddy, Gallup & Povinelli 1993; Rivas & Burghardt 2002). This focus on the world as humans see it can lead to assumptions that bias research results or lead us to devalue the abilities of other animals. This is most true for the assessment and use of sensory information: a common text on human perception (Matlin & Foley 1997) devotes 8 chapters to vision and only 6 to all the other senses. Despite von Uexkull's (1934) clear exposition of the different Umwelts, or perceptual worlds, of other animals (see Rivas & Burghardt 2002 for "crotalomorphism") we often persist in expecting that animals with excellent ability in other senses will concentrate their evaluation of the environment on the use of visual information. This expectation is particularly challenging for animals that are as much unlike us as the octopuses, and can lead, as we will see, to an incorrect assessment of their learning capacity.

There seems to be a justification for expecting cephalopods to rely on visual information to understand the world around them, as their eyes are very similar structurally to those of "higher" vertebrates (Hanlon & Messenger 1996), and a long series of studies of their learning capacity has followed this assumption. Early researchers (e.g. Wells 1978) used a standard paradigm in which octopuses were rewarded for approaching a positive visual stimulus and punished with a small shock for touching a negative one. From this they learned about octopus assessment of visual information, learning and its control by different areas of the brain. Field research

(Forsythe & Hanlon 1997; Mather 1991a; Yarnall 1969) subsequently showed that octopuses use not vision but chemotactile search in prey finding. However, evaluations of learning (Papini & Bitterman 1991) and habituation (Kuba et al. 2006; Mather & Anderson 1999) have continued to use visual stimuli.

One situation in which octopuses should have been able to learn on the basis of visual cues, that of seeing a crab or other crustacean prey in a glass jar and subsequently learning to remove the lid more quickly and capture the crab inside, remained problematical. Using the manipulative capacity of their eight arms (Mather 1998), *Octopus vulgaris* (Cuvier, 1797) were able to remove the lid, but they did not decrease their time to do so and thus were not learning the task (Fiorito, von Planta & Scotto 1990). Even a later evaluation which included stimulus pre-exposure so that octopuses might be better acquainted with the task and the stimuli (Fiorito et al. 1998) did not allow them to solve the removal problem in shorter times. This situation was widely seen as a failure of learning in these animals.

Although the octopuses had not been able to learn to remove the cork from glass jars to capture the crab inside in less time, the task was still a favorite of film crews and aquarium keepers of octopuses. In the latter case, this was partly because it was of interest to the general public and partly as it was seen as a way of enriching the environment of captive octopuses (Anderson &Wood 2001) by making them have to work to capture prey. Anderson & Blustein (2006) did such a demonstration with *Enteroctopus dofleini* (Wülker, 1910), the giant Pacific octopus, in the Seattle aquarium, using not live crabs but pieces of herring in perforated jars as a reward. Although the laboratory evaluations of learning were not carefully spaced and were of different durations and the presentation method was not carefully controlled, the time until four octopuses opened the glass jar lid and took out the herring decreased in the linear sequence indicating learning (Anderson & Blustein 2006). As this was highly suggestive that octopuses could learn to open the jar lid more quickly in this situation, the present study was designed to investigate further and see what aspects of the methods might have led to learning where it had not been demonstrated before.

Materials & Methods

Subjects

The subjects were 12 giant Pacific octopuses (*E. dofleini*) held at the Seattle Aquarium off exhibit (Anderson 1997). They had not been given prey in bottles before this experiment took place, although this is a common practice in public aquariums (Anderson & Wood 2001). Octopuses (mean weight 8 kg, range 3-16 kg) were collected from the wild and held in tanks with opaque sides and either a clear lid that could be lifted for feeding or with clear glass on one side for viewing. Water was supplied by the Aquarium's running sea water system and was constantly drawn from Elliott Bay. Before testing with the bottles, the octopuses were hand-fed raw seafood (herring, squid, or fish fillets), approximately 1% of their body weight per day. One octopus became senescent (Anderson, Wood & Byrne 2002) during the trial period and refused all food; hence its data were not used.

Materials

Octopuses were presented live fresh water crayfish inside clear plastic 680 cc screw top jars with wide mouths and plastic lids. Jars were emptied, washed, and the paper labels removed. Crayfish (*Pacifasticus leniusculus*) were obtained from nearby Lake Washington by trapping, kept in the Aquarium's fresh water holding tanks until used and fed chopped herring *ad libitum*. Fresh water crayfish (mean carapace length 24 mm) were used as food to stimulate octopuses to open jars because crabs, a favorite food of *E. dofleini* (Hartwick, Tulloch & MacDonald 1981) and used by Fiorito, von Planta & Scotto (1990) could not be procured in a suitable size to fit inside the jars. While not a normal prey of them, fresh water crayfish are quickly taken and eaten by octopuses (Toll & Strain 1988; Anderson & Wood 2001).

Methods

Crayfish were presented to each octopus once in the morning between 0800 and 1000 and once in the afternoon between 1300 and 1500 for five days. In Condition One, the crayfish was placed in the water-filled jar and the cap loosely screwed on (one complete revolution of the jar lid) until it stopped turning easily. It was then positioned in the octopus tank so it touched the suckers and the animal reacted to the touch. If it was dropped or pushed away by the octopus within the first five minutes, it was again touched to the animal. After the first five minutes, if the octopus did not grasp or open the jar it was left in the tank for one hour before removal and the response was recorded as Did Not Open (DNO). As the jar was often covered by the arm web of the octopus, often the time to open a jar was calculated by the sight of the lid being removed and separated from the jar, a view of octopus arms within the jar, or by movements of the arms indicating non-visible prey consumption. In Condition Two six 1 cm diameter holes were drilled in each jar. A crayfish was added, and the five day testing was repeated with the same procedure. After these trials were completed, in Condition Three jars with holes and crayfish inside were smeared all over on the outside with herring "slime" - a small herring was rubbed on the jar - and the five day process was repeated as before. The sequence and beginning Condition varied for each octopus in a Latin Square arrangement so octopuses would not simply "learn" to open jars.

When all trials of the three Conditions were completed, for Condition Four a single re-trial of Condition One was conducted. A separate set of four octopuses was tested in Condition Five, when they were presented with crayfish in jars painted black to eliminate visual cues but with the same holes and coating of herring juice. Thus a total of 12 octopuses were tested, although one animal became senescent during the course of study and his results were not used for statistical analysis.

Results

The octopuses did not open the jars (DNO) a mean of 9 times in the 10 trials of Condition One, 8 times in Condition Two, and 0.5 times in Condition Three. These success rates were significantly different using a Kruskal-Wallis test ($\chi^2_{(2)} = 109.643$, $p<0.001$); essentially the octopuses did not open jars that did not have chemical cues on the outside. They maintained contact longer in the DNO trials of Condition Two (24 minutes) than in Condition One (8 minutes, however. Data for Condition Three after all three conditions were completed were submitted to an Analysis of Variance looking at the average time to open the jar with variables the Time of Day (2) and Day of Testing (5), where 60 minutes was assumed as the duration in the three DNO trials. There was no significant difference across time of day (morning vs afternoon). However, the day of testing during Condition Three was highly significant ($F_{(4, 40)} = 15.605$, $p < 0.001$; see Figure 1). Post-hoc Tukey HSD tests showed that Days One & Two were significantly different from Days Three, Four, & Five at the $p < 0.005$ level, obvious evidence of learning. No other comparisons were significantly different at the $p<0.05$ level. When the duration to open in the re-trial of Condition Four at the end of the study was compared to that of the very first trial of the sequence (again with DNO taken as 60 minutes), the difference was significant in a paired t-test, $t_{(5)} = -2.6956$, $p < 0.043$. This strongly suggests that learning had transferred to the previously unlearned situation. The octopuses in Condition Five had a DNO of 8.5/10 trials and held the jar for mean 19 min.

Discussion

Clearly, octopuses can learn to open jars, despite the failure of *O. vulgaris* (Fiorito, von Planta & Scotto 1990), if they are given chemical cues on the surface of the jars. In the context of the octopuses' ecology, it is probably not surprising that they needed these cues to learn that opening the lid of a jar could gain access to the food that had been earlier seen inside. The suckers of an octopus have touch and chemical receptors (Graziadei 1971) and in many cases prey is never seen but rather recognized on the basis of this non-visual information (Forsythe & Hanlon 1997; Mather 1991a; Yarnall 1969). In the laboratory octopuses often reach out for the discarded shells of previous molluscan prey, pull them under the arm web, evaluate them while they are in the grip of the suckers, and then reject them (Mather personal observation). Researchers attempting to study techniques that octopuses use for penetration of mollusk shells (Anderson & Mather 2007; Wodinsky 1973, 1978) have found that they simply rejected empty shells whose valves have

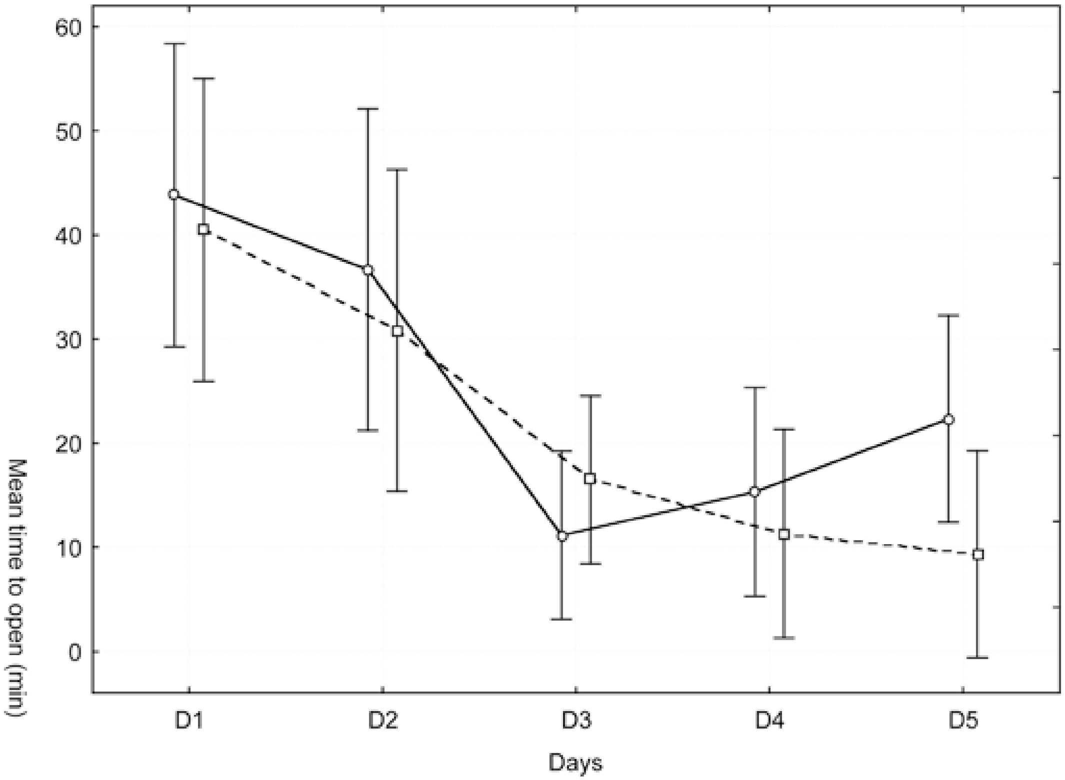

Fig. 1: The mean time (morning: o and afternoon: □) six giant Pacific octopuses (*E. dofleini*) took to open jars under Condition Three (jars with crayfish inside with holes and herring smeared on the outside). Vertical bars are the 95% confidence intervals.

been glued together, presumably because no chemical cues about the prey were present. The situation Fiorito, von Planta & Scotto (1990) created for octopuses must have been similar to this. Without confirming chemical cues, octopuses made no linkage to the sight of the crab in the jar, though the highly exploratory animals eventually removed the jar lid anyway.

Octopuses generally find hidden prey by touch (Forsythe & Hanlon 1997; Mather 1991a; Yarnall 1969). If octopuses then do not generally use their visual ability for locating prey, why do they have such well-developed eyes and an excellent visual system? One possibility is that it is useful for recognizing predators, as the shell-less octopus is vulnerable to a wide variety of fish and mammal predators (Hanlon & Messenger 1996). Yet the octopus is often hidden in a protective "home" and ventures out to hunt for prey less than 25% of the time (Hanlon & Messenger 1996). And when it is out, the excellent background-matching camouflage is not simply the result of evaluation by the octopus's visual system, as it is color blind (Messenger, Wilson & Hedge 1973).

Another more likely task for visual processing is navigation and spatial memory, an ability found widely across the animal kingdom (Shettleworth 1998). In the wild, *O. vulgaris* is a central place forager and has both location memory for its central home and procedural memory of where in its small home range it recently sought prey (Mather 1991b). As octopuses only occupy their home ranges for a period of approximately ten days to two weeks (Mather & O'Dor 1991), learning information about the new area may be an imperative for them. Wells (1978) believed that octopuses had no sense of their location in space and could not navigate through detours, but this was in large part because their brain is highly lateralized (see Byrne, Kuba & Griebel 2002) and visual guidance in the laboratory was mainly unilateral. In a larger area there was no evidence of this limitation, and subsequent researchers (Boal et al. 2000; Moriyama & Gunji 1997) have found that octopuses can use visual cues to make directional choices and navigate mazes. In addition, Leite (personal communication) has found that octopuses use visual guidance to make decisions about likely areas to search prey.

Of course one species does not represent all octopuses and species differences in ecology might result in differences in learning. Octopuses are highly exploratory (Mather & Anderson 1999) and the active warmer-water *O. vulgaris* evaluated jars even though they gave off no chemical cues when they were underneath the arm web out of sight, and did take the jar lids off (Fiorito, von Planta & Scotto 1990). In contrast, the larger and slower cold-water *E. dofleini* simply refused them most of the time. It would be interesting to see how the mobile and diurnal *O. cyanea* would do at jar opening. It explores with its arms during foraging (Forsythe & Hanlon 1997), takes crab prey nearly exclusively (Yarnall 1969; van Heukelem 1983), and sometimes aims moving skin displays called Passing Clouds to startle crabs which have "frozen" to evade capture (Mather & Mather 2004). Cuttlefish, *Sepia officinalis*, are predominantly visual predators whose attack sequence was documented decades ago (Messenger 1968) and whose prey selection is highly dependent on visual learning. But while comparisons across the family and even the sub-class would be interesting, the fact that octopuses can learn to remove prey from a closed jar given the right set of cues should remind researchers of our anthropomorphism. Our finding reinforces Bekoff, Allen & Burghardt's (2002) approach to understanding animal cognition in terms of what each particular species can do and not in terms of what we think we might be able to do if we were them.

Acknowledgements

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The 'yolk organ' of cephalopod embryos: on transient functions from crawling substratum to provisional knapsack

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Abstract

Cephalopod eggs are characterized by a large yolk mass, the volume of which is much (indeed several orders of magnitude) greater than the volume of the ovular cytoplasm containing the nucleus. The nutritive role of the yolk in a developing embryo seems obvious, and the ultimate formation of a nutritive reserve (in the so-called inner yolk sac of the embryo) clearly represents the procurement of a provisional "knapsack" for the newly hatched animal. In contrast to the evident role of a (dwindling) yolk mass as a nutritive substratum for embryonic tissues, relatively little attention is drawn to another function of the yolk mass, which indeed provides also a mechanical substratum for the embryonic cells crawling over it (especially the leading edge cells of the blastopore lip) and for further cellular complexes. In this context the widely used term 'yolk mass' appears as a poor description of what has been more appropriately termed 'yolk organ'.

Introduction

A possible opening question is: what is yolk? 'Google' (*www.yourdictionary.com/yolk*) offers the following definition: "the protein and fat stored in the ovum, serving as nourishment for the growing embryo". This yolk definition is satisfactory as far as nutritive aspects are concerned. In contrast, physical aspects relating to a possible *mechanical role* of accumulated yolk are not taken into consideration.

Cephalopod embryos (Fig. 1) indeed demonstrate both the nutritive and mechanical functions of the yolk mass. The mechanical function relates to cell turgidity and cell surface (cortex) robustness, both of which warrant the overall cohesion of the zygote. Moreover, a robust cortex offers a firm substratum for adhering cells, in particular those making up the leading edge of the blastopore lip during gastrulation (Figs 1, 2). A steady progression of the blastopore lip is only possible if these cells can "crawl" forward on a sufficiently firm surface (Marthy 1985). Whereas the yolk surface offers an essentially "passive" substratum to crawling cells, the sub-surface parts of the yolk do not necessarily form an inactive mass. In embryos of *Octopus vulgaris*, during gastrular stages, Painlevé et al. (1958) and Orelli (1960) observed that the entire yolk mass performs slow, alternating, spiral twisting movements, which are particularly distinct in the "free" part of the yolk that is not yet covered by the bell-shaped embryo proper (Fig. 3). These observations suggest co-ordinated contractions of the cell cortex and/or the fibrils of cytoplasm extending from the cortex and traversing the entire yolk mass, passing between the soft yolk bags (i. e. membrane-bound ampullae that appear as "platelets" [cf. Fig. 6] only in sections of fixed material; Sacarrão 1968).

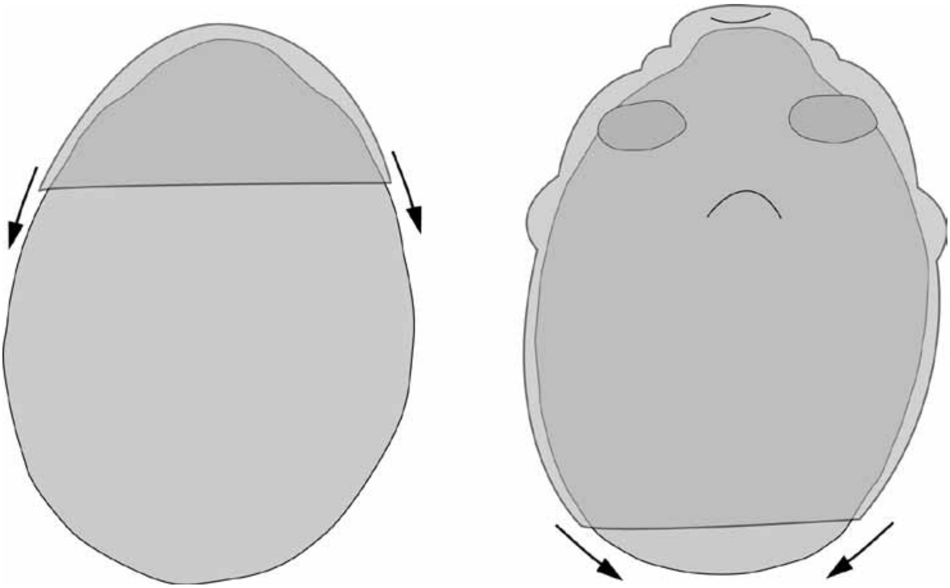

Fig. 1: Schematic representation of cephalopod embryos at early (left) and advanced (right) gastrular stages. The arrows indicate the steady progression of the blastopore lip.

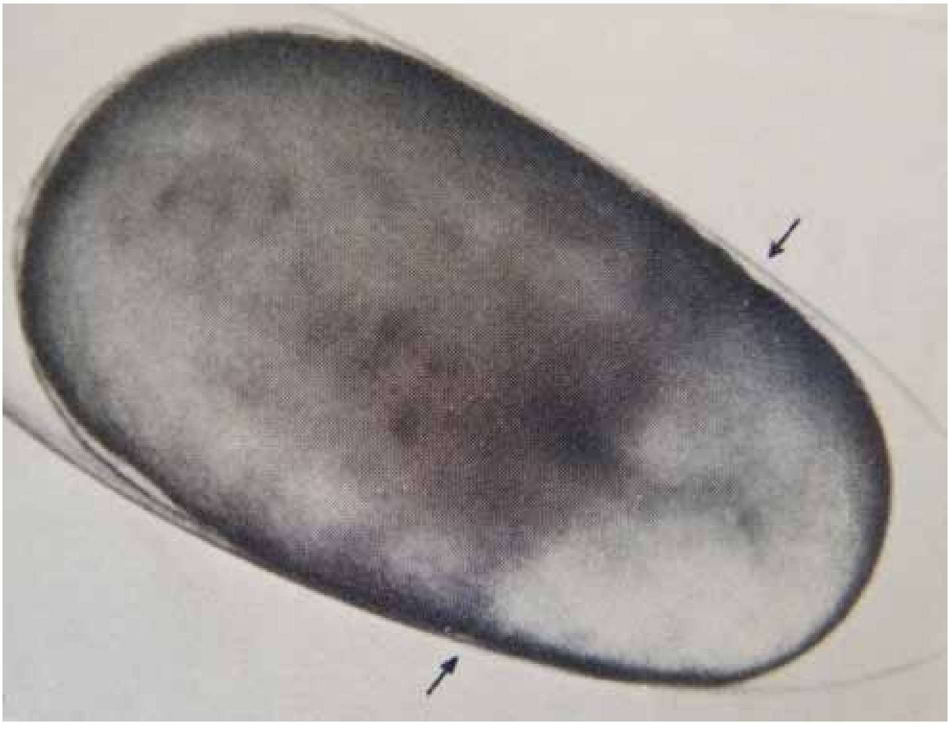

Fig. 2: A late gastrula (stage VI-VII), according to Naef (1928) of *Octopus vulgaris*, surrounded by the transparent chorion membrane. The arrows point at the actual position of the leading edge of the blastopore lip; note the slight depression of the yolk surface visible below the upper and above the lower arrow. (after Boletzky 1988)

Embryogenesis: setting the stage for post-hatching life

Given sufficient protection from predators and adverse environmental conditions, cephalopod embryos develop normally if the surrounding temperature and oxygen supply correspond to their species specific range of adaptation (Boletzky 1994). Depending on the species, cephalopod ova measure between 0.8 and 35 mm. The largest egg sizes exist in both nautiloids and certain coleoids (Octopoda, Cirroctopoda), and the corresponding durations of embryonic development range from 1 year (*Nautilus*) to more than one, possibly several years (in certain cirroctopods). At the opposite end of the scale, the smallest eggs (ommastrephid squids, the pelagic octopod *Argonauta*) permit embryonic developmental times of only a few days or weeks. These differences – relating to different reproductive strategies (Boletzky 1986) – may imply complex adjustments *inside* the ostensible black box of the so-called 'egg stage'.

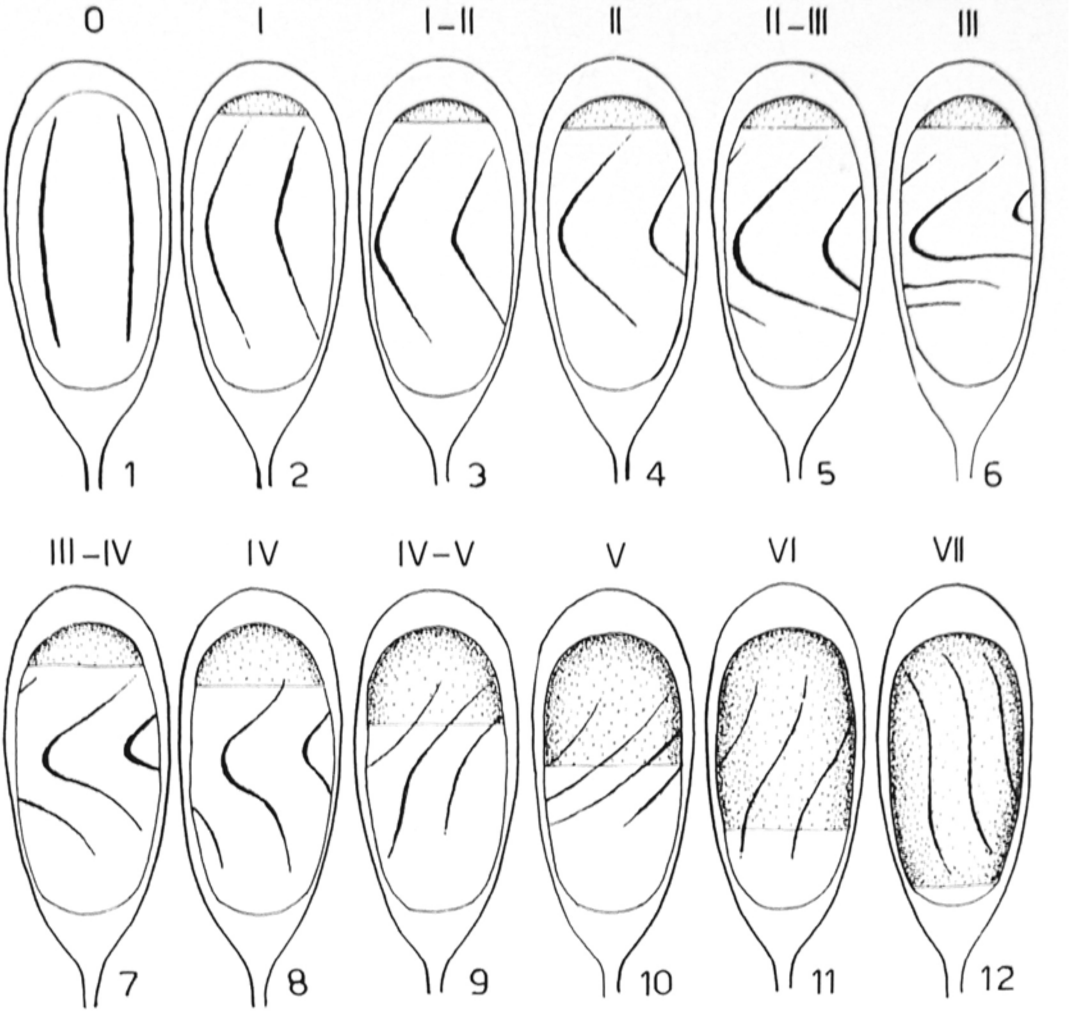

Fig. 3: A series of embryos (each surrounded by its chorion) of *Octopus vulgaris* showing opaque lines that evidence autonomous, slow twisting movements of the yolk complex during gastrulation. The roman numbers placed above the figures of individual embryos (1-12) are developmental stages according to Naef (1928). NB: The time-lapse film made by Painlevé et al. (1958) clearly demonstrated that the twisting, torsional movements are most intensive in the outer part of the yolk that is not yet covered by the gastrular cap. (after Orelli 1960)

The outset of embryogenesis in cephalopods may be summarised in a few lines: After fertilization, zygotes first undergo partial cleavage. The resulting syncytial 'blastocones', from the fourth cleavage onward, give off individualized blastomeres in the center of the cleavage area, at the animal pole (Naef 1928). The blastomeres and the surrounding blastocones together form a single-layered blastula (or discoblastula). Gastrulation begins with an apparent shearing movement of the sub-marginal part of the blastula reaching over and beyond the marginal ring, which comprises the remaining blastomeres and adjacent blastocones. What appears like a shearing movement in fact is a crawling movement of the cells forming the leading edge of the expanding central sheet, which now can be recognized as ectoderm (cf. below). This crawling movement continues until the opposite pole of the egg is reached. In the course of the strictly epibolic gastrulation, the originally disc- or cap-shaped blastula is transformed into a hollow, bell-shaped complex of cells arranged in a continuous outer layer (ectoderm) and an inner layer of limited thickness (mesendoderm). This complex finally envelopes the whole uncleaved yolk mass (Fig. 1). The cellular envelope remains very thin in the vegetal pole area, the ectoderm being associated only with a delicate network of thin mesodermal cells (derived from the margin of the mesendoderm). Once the blastopore is closed at the vegetal pole, these mesodermal cells differentiate rapidly to form a muscular network around the yolk mass. This early musculature is functional (as can be recognized from the peristaltic waves of contraction running over the ectodermic surface) long before the organ systems of the embryo proper are differentiated (Fig. 4). The so-called 'extra-embryonic' complex containing this musculature can now be called 'the outer yolk sac'. Its contents form a continuum with the yolk mass lying inside the embryo proper (the so-called inner yolk sac).

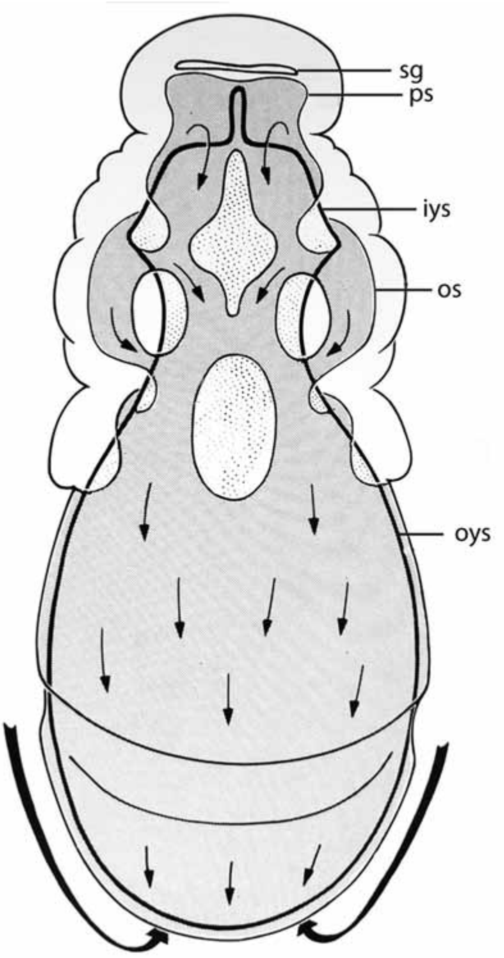

Fig. 4: Semi-schematic representation of a Naef stage XII embryo of *Octopus vulgaris*, in dorsal view, showing the outline of the yolk organ (thick line) and the blood circulation around it (small arrows). The two broad arrows indicate the peristaltic wave of surface contraction running over the outer yolk sac. sg = shell gland, ps = posterior (blood) sinus, iys = inner yolk sac, os = ophthalmic sinus, oys = outer yolk sac. (after Budelmann, Schipp & Boletzky 1997)

Definition of the yolk organ

In his Cephalopod Embryology Naef (1928: 98) stated: "*Above (pp. 93, 95) the yolk mass has been considered, in terms of cell morphology, as belonging to the yolk cells, and the whole from now on will be termed the 'yolk organ'. The latter will be considered a specially differentiated part of the endoderm that can be compared (ill-defined limits notwithstanding) with the macromeres of a typical molluscan gastrula after production of a number of more normal endoderm cells (Textfig. 7 f). At any rate the yolk organ belongs to the endoderm complex and indeed continues to suggest that relationship. Its subsequent development allows one to provide a more detailed justification of this view: I will later demonstrate that the yolk organ can be viewed as an embryonic organ sorted specially* *from the material of the embryonic liver. This suggests that the organogenetic relationship of the yolk is the same in cephalopods as it is in other molluscs, namely lamellibranchs, gastropods and chitons. In any case, this organ remains for a long time in connection with the midgut anlage, the latter being constituted only after its separation from the yolk organ.*"

According to Naef (1928) the yolk organ thus is the entire complex comprising the yolk mass proper (including also the syncytial sub-surface elements such as the nuclei and the accompanying cytoplasm that forms a three-dimensinal network of filaments running across the yolk mass). On the other hand, the blood-filled space surrounding the yolk mass in the outer yolk sac, and the transient musculature of the outer yolk sac are not parts of the yolk organ. In terms of functional relationships, this limitation may appear artificial. A similar problem arises with regard to the lining of the blood vessels and sinuses surrounding the inner yolk sac (Fig. 7), since they become a part of the definitive venous system (Boletzky 1975). In functional terms, these prospective venous vessels and sinuses are derivatives of the outer yolk sac blood lacuna (Fig. 6), the blood circulation being generated by the peristaltic waves of contraction of the outer yolk sac envelope (Fig. 4). It is therefore advisable to distinguish a morphologically defined, totally transient yolk organ *sensu* Naef (1928) from a functionally defined yolk organ that includes immediate annex structures, some of which persist beyond embryonic development.

Sculpturing the yolk organ

In the course of organogenesis, the overall contraction of the embryo cap, especially in the prospective cephalic part, leads to a major constriction, by which the separation of the inner from the outer yolk sac becomes increasingly distinct (Fig. 4). By the end of organogenesis proper, most of the contents of the inner yolk sac have been extruded to the outer yolk sac. Soon after that stage (about XV), however, the inner yolk sac begins to increase in size, apparently due to an "influx" of yolk from the outer yolk sac. Towards the end of embryonic development, only an inner yolk sac remains containing no yolk (in some octopods), or a considerable volume of yolk stored in one (octopods, cirroctopods) or several lobes (Boletzky 2002; Fig. 5).

Concluding remarks

Long ago Portmann (1926: 406) emphasized the functional interactions and constraints that control a developing embryo: "*If one considers the developmental stages solely as avenues leading to a goal, as inevitable transition grades before the adult stage of the animal is reached, one tends to overlook that at any moment of development the embryo or larva is a living whole whose organs must remain in close functional relation to one another, no matter what the final form eventually achieved*".

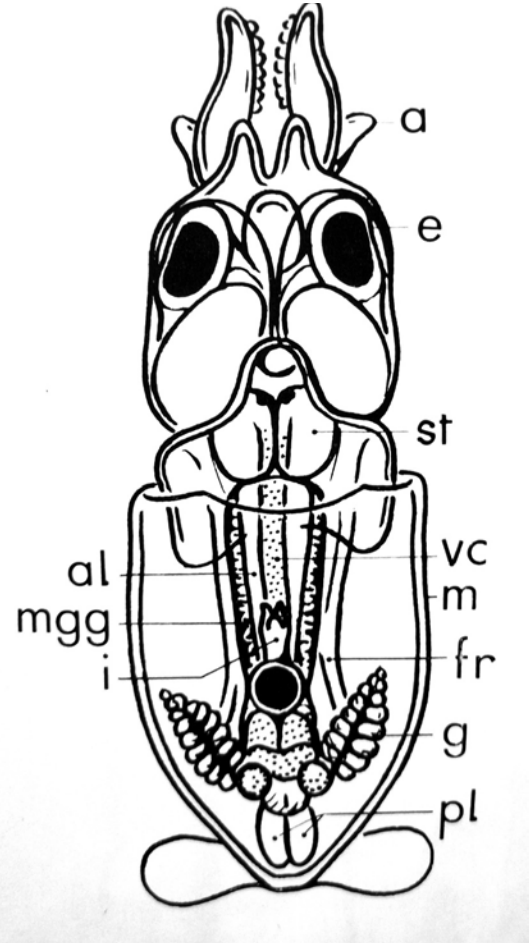

Fig. 5: Semi-schematic representation of a loliginid squid hatchling with a three-lobed inner yolk sac, in ventral view (al = anterior lobe, pl = posterior lobes). a = arms, e = eye, fr = funnel retractor, g = gill, i = intestine, m = mantle, mgg = midgut gland, st = statocyst, vc = vena cephalica. (after Boletzky 1975)

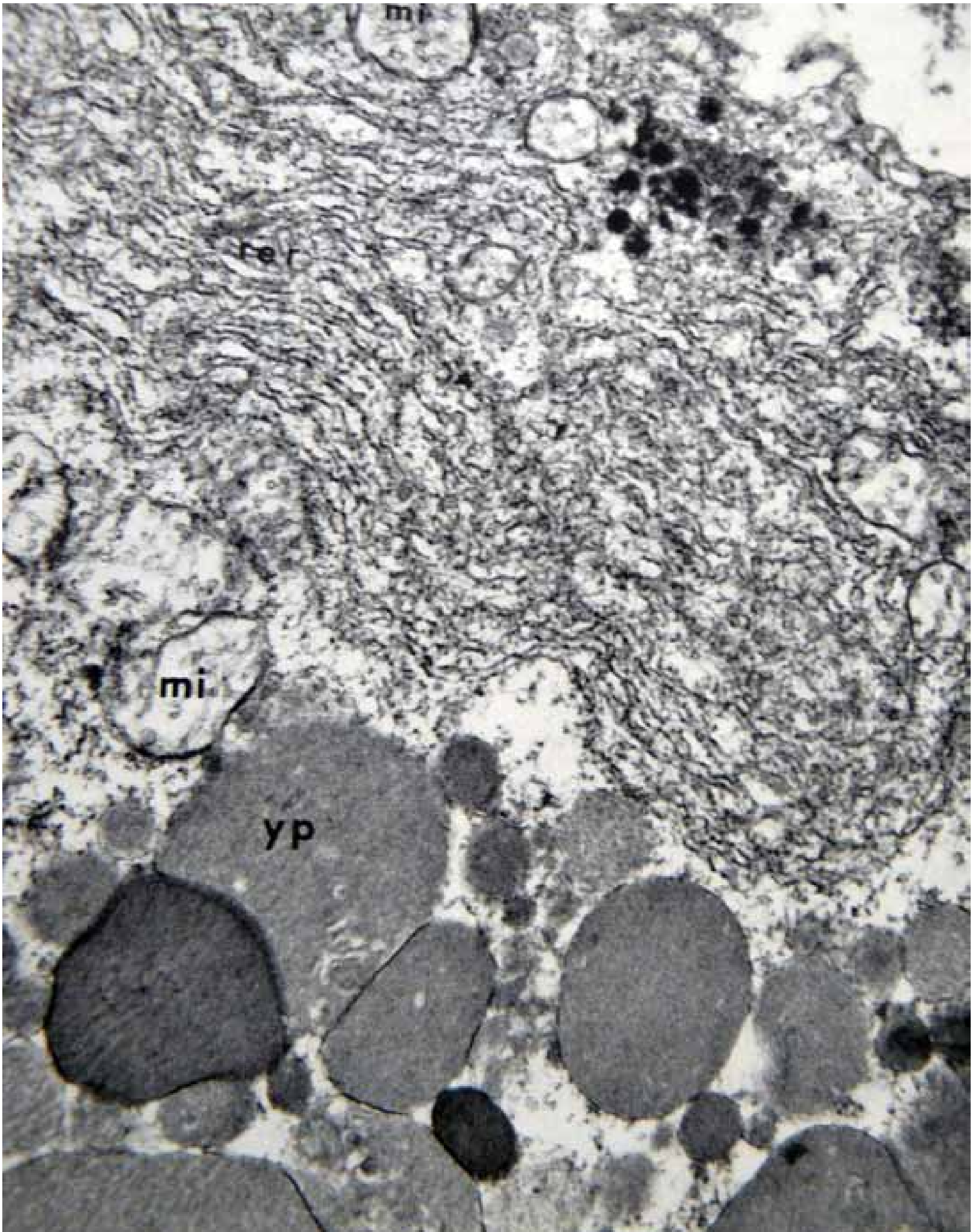

Fig. 6: Transmission electron micrograph (TEM) of a thin section through the yolk syncytium which is surrounded by a blood lacuna (upper right) of the outer yolk sac in an embryo of *Loligo vulgaris* at stage X of Naef. (mi = mitochondria, rer = rough endoplasmic reticulum, yp = yolk "platelet"; width of the figure is ca 6 µm; after Boletzky 1975).

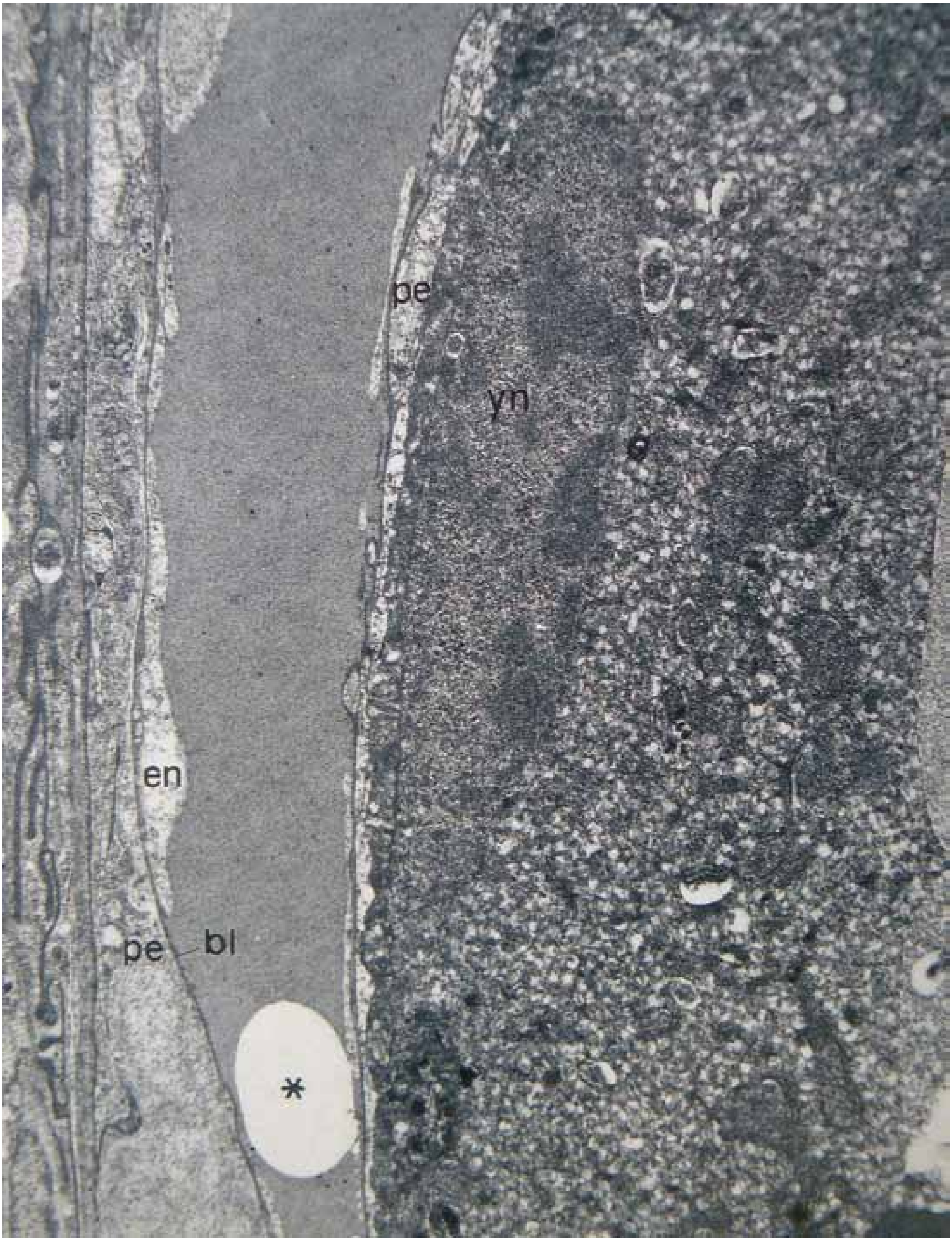

Fig. 7: TEM of a thin section through the posterior part of the inner yolk sac of a hatchling *Loligo vulgaris*, showing the cytoplasm of the yolk syncytium with a nucleus (yn); yolk "platelets" are not visible here (they are situated further to the right). In contrast to the outer yolk sac (cf. Fig. 5) the blood sinus of the inner yolk sac is surrounded by endocytes (en), a basal lamina (bl) and pericytes (pe). The space marked * is an artifact (width of the figure is ca 15 µm; after Budelmann, Schipp & Boletzky 1997).

The everyday requirements of this "living whole" – from zygote to hatchling – raise various physiological, ecological, and evolutionary questions, including those ultimately relating to gamete protection (Boletzky 2001). One should keep in mind that cephalopod ova are not viable if they are not properly protected. Primary (by itself insufficient) protection is provided by a special membrane called chorion, which is the ultimate product of the follicular tissue of the ovary (Fig. 2). Secondary protection is either based on a special female parent behaviour ("brooding" in octopuses), or on additional protective envelopes, which are produced by special glands of the spawning female.

Once the young animal hatches from the egg case, thus leaving this protective micro-environment, the food-supply of the hatchling is dual for several days, sometimes weeks. The inner yolk sac – being fully integrated in the circulatory system of the digestive gland – continues to provide energy to the organism. This final yolk absorption is achieved *independently* and in parallel to the onset of active foraging and digestion of captured prey. As visual predators, newly-hatched cephalopods depend on rapid learning to improve their "innate" abilities to catch appropriate living prey and escape being eaten by other predators (Boletzky 2003). The temporary overlap of the embryonic nutritive system with the post-hatching (essentially adult-like) predatory behaviour doubtless offers a safeguard of considerable adaptive value, allowing hatchlings to cope with a temporary food shortage that may be due either to limited availability of appropriate prey items or insufficient capacity to catch them.

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Non-invasive methods of identifying and tracking wild squid

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Keywords: Caribbean Reef Squid, *Sepiotheuthis sepioidea*, individual identification, male/female identification, field study, dot pattern.

Abstract

The ability to identify individual free-living animals in the field is an important method for studying their behavior. Apart from invasive external or internal tags, which may cause injury or abnormal behavior, most cephalopods cannot be tagged, as their skin is too soft and delicate for tag retention. Additionally, cephalopods remove many types of tags. However, body markings have been successfully used as a non invasive method to identify individuals of many different species of animals, including whale sharks, grey whales, seals, and zebras. We developed methods to sex and individually identify Caribbean reef squid, *Sepiotheuthis sepioidea*. Males showed distinct bright dots on their fins on a Basic Brown background and have a light line at the fin edge while the females had a gradual transition from Brown to Pale towards the edge of their fins without showing distinct fin-dots or lines. In the field we used four characters to distinguish individual *S. sepioidea* from each other – sex, relative size to each other, scars, and patterns of light-colored dots on their mantles and fins. These dot patterns are individually unique and constant in location through time. Observations in the field were backed up by an image database using illustrations and photography.

Introduction

Individual animals can be recognized by artificial marks e.g. tags, in mammals and fishes (Fedak, Lovell & Grant 2001; Willis et al. 1995), leg bands in birds (Wayne & Shamis 1977), spraying tiny color particles into fish skin (Jacobsen et al. 2001), injection of colored elastomers in squid (Replinger & Wood 2007), implantation of electronic tags under the skin of fish, dogs (Jefferts, Bergman& Fiscus 1963; Lord et al. 2007) or for species that exhibit sufficient phenotypic variation, by natural markings.

Artificially marking/tagging animals usually involves capturing and handling, which can stress individuals and may alter their behavior. Tagging often creates a wound, which is a potential site of infection. The use of natural features or markings to identify individuals within a population is non-invasive, and therefore does not pose the same risk as invasive artificial marking techniques. Individuals (or a particular region of their bodies) can be either drawn or photographed, and the resulting images compared with the images of previous observations. This technique has been employed most frequently in studies on mammals, both marine (e.g. humpback whales, *Megaptera novaeangliae,* Glockner & Venus 1983; southern right whales, *Eubalaena australis*, Payne et al. 1983; Mediterranean monk seals, *Monachus monachus*, Forcada & Aguilar 2000) and terrestrial (e.g. zebras, *Equus burchelli*, Petersen 1972; lions, *Panthera leo*, Schaller 1972 in Kelly 2001; chimpanzees, *Pan troglodytes*, Goodall 1986; badgers, *Meles meles*, Dixon 2003). It has also been applied to birds (e.g. Bewick's swan, *Cygnus columbianus*, Scott 1978; ospreys, *Pandion haliaetus*, Bretagnolle, Thibault & Dominici 1994; lesser white-fronted geese, *Anser erythropus*, Øien et al. 1996); reptiles (e.g. adders, *Vipera berus*, Sheldon & Bradley 1989; common garter snakes, *Thamnophis sirtalis* sirtalis, Hallmen 1999; five species of Central European lacertid lizards, Steinicke et al. 2000), amphibians (Archer's frogs *Leiopelma archery*, Bradfield, 2004*)* and fish (e.g. pipefish, *Corthoichthys intestinalis*, Gronell 1984; leafy seadragons, *Phycodurus eques*, Connolly, Melville & Keesing 2002).

As with any technique, field identification of individuals must be efficient. Individuals can be identified by eye in the field and this data can then be correlated with other behavioral variables at the time of observation. Additionally, subjects can be photographed or sketched in the field, and identifications made at a later stage in the laboratory. When the catalog of previous captures is relatively small, manual identification (i.e. identification entirely by eye) is rapid, but when the catalog is large, it can take substantially longer. Computer-assisted matching can be used if photo-matching entirely by eye is too time-consuming or difficult (e.g. Whitehead 1990; Kelly 2001). Dividing individuals into subgroups can facilitate rapid identification when there are a large number of previous captures, because the observer has to photo-match to a small subgroup rather than to all previous captures. For example, Gill (1978) was able to identify individual red-spotted newts, *Notophthalmus viridescens*, within 30 seconds, despite a catalog of over 8500 individuals, because individuals could be assigned to subgroups based on the number of spots on each side of the dorsal surface. Another advantage of this approach is that it results in a higher degree of accuracy, as the larger the catalog of photographs, the more likely it is that mis-identification will occur.

Image quality influences error rates, with poor images resulting in a higher number of incorrect identifications than high-quality images (e.g. Agler 1992; Forcada & Aguilar 2000; Gowans & Whitehead 2001; Stevick et al. 2001). Digital photographs have a number of advantages over traditional slide or print film images, even when photo-matching is conducted entirely by eye. Markowitz et al. (2003) compared digital and slide film images of New Zealand dusky dolphins (*Lagenorhynchus obscurus*), and reported a higher proportion of digital images were of suitable quality for use in photographic identification than slide film images taken by the same photographers. That is because digital images are available for inspection immediately after they are taken (i.e. directly in the field), and they can be archived, accessed, and printed easily and rapidly.

It is possible that two or more individuals in a population may have such similar natural markings that they cannot be distinguished from one another (Pennycuick 1978), resulting in false positive errors. The likelihood of this occurring increases with increasing population size, but decreases with increasing pattern complexity. The probability that a pattern will be repeated in a particular population was estimated (Pennycuick 1978). Variability in the degree of distinctiveness of individuals means that 'marked' individuals (i.e. those who have previously been photographed/sketched) do not necessarily all have the same probability of being recognized, and this can potentially have serious effects when estimating abundance (Hammond 1986 in Friday et al. 2000). Only individuals distinctive enough to have equal probabilities of recognition should be considered as "marked." An assumption common to capture-recapture methods is that marks do not change over time. However, natural markings do have this potential, which would also result in population overestimates.

Cephalopods are an interesting group with highly developed sense organs and a complex brain that rivals the complexity found in vertebrates (Hanlon & Messanger 1996). Their ability to change color and texture of their skin in fractions of seconds provides them with the means of visual communication with each other and their environment. The skin display of squid and octopuses is quite complex and subject to constant change (Messenger 2001). There are both expandable colored chromatophores in the skin surface and reflective leucophores and iridophores in deeper layers of the skin that reflect specific wavelengths of ambient colors when the chromatophores are contracted. Nevertheless, there are patterns of iridescent small spots and areas of few chromatophores on the skin and fins that can be used to identify individuals.

The Caribbean reef squid *Sepioteuthis speioidea* (Blainville, 1823) are a model species of cephalopod for generating a catalog of individuals because they live in easily accessible inshore, small, semi-permanent groups. Moynihan & Rodaniche (1982) observed that this species of squid has individual marks and can thereby be individually identified. A method to identify individuals opens the door to a much deeper understanding of their behavior.

The objectives of this study were to determine whether identification by eye in the field and photographic identification of naturally marked animals could be used to identify individual Caribbean reef squid *Sepioteuthis sepioidea* and if the markings used for identification are stable over time.

Methods

Field data for this project was collected during an eight-year observational study of *S. sepioidea* in a small near shore location off the west coast of the Caribbean island of Bonaire. The project was carried out in the months of May and June from 1998 to 2005. Total underwater observation time was over 1000 hours. This island is an ideal location for such a project because the waters around *Bonaire* are a marine park and the squid are habituated to recreational divers. A school of adult squid stays more or less in the same area and easily accessible groups can thereby be followed over periods of weeks. The main times for field observation were the early morning (0700 to 1000) and late afternoon (1500 to 1800) because the squid were most active during these times. Data were collected by snorkelers or divers who recorded notes and sketches on underwater slates and filed them shortly thereafter. For additional documentation squid were photographed with Sea&Sea, Nikonos, Fuji S2 and CoolPix cameras and filmed with a housed Sony Handycam. In the base camp a database for dot patterns of identified squid was drawn onto paper copies of the body outline of a squid and newly identified squid were named.

As verification that squid can be identified according to our method, two sets of 26 pictures each of different squid were shown to seven people. Each picture of set 1 had a match (= same squid) in set 2. Three of the seven volunteers worked on the project in Bonaire, and thus, had experience with squid in the water and four were naïve as they had never seen squid in the field before.

To answer the question if squid dot patterns, which we use for individual identification, have a stable location on the squid's body over time a lab experiment was conducted in 2004 on the island of Bermuda. A school of 10 *S. sepioidea* was caught in Whalebone Bay and brought to the wet lab of the Bermuda Biological Station for Research. There squid were housed in a flow-through system and fed with live silversides and live shrimp. As a control, subjects were tagged with Visible Implant Elastomers (VIE, Northwest Marine Technology) (Zeeh & Wood 2009). During a period of 56 days squid were measured and photographed six times. Six of the original 10 squid provided useful data for this experiment: their picture series were

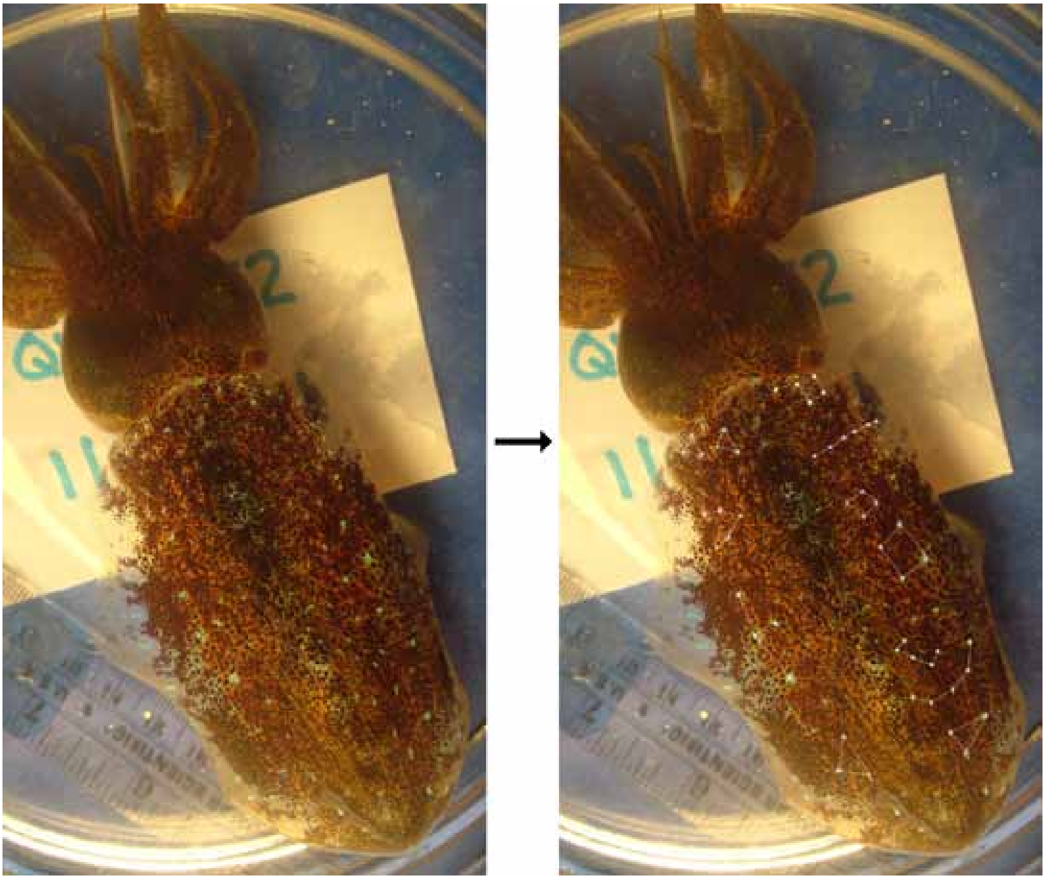

Fig. 1: To test if dot patterns on the dorsal mantle of a squid stay stable over time, we assigned 50 dots into 10 star-sign-like patterns. Photo: James Wood

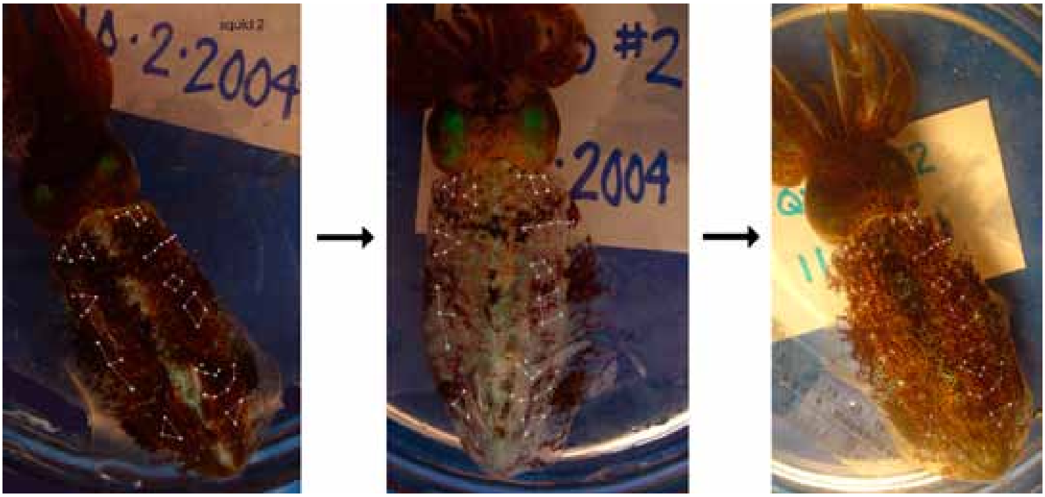

Fig. 2: Over the course of two months subjects were photographed six times. Picture 1, 3, and 6 of one subject's series show that the assigned dot patterns are stable over time. Photo: James Wood

analyzed for dot pattern retention by assigning 50 dots into 10 star-sign-like patterns (Fig. 1). These patterns were then followed on the subsequent five pictures of the six picture series (Fig. 2).

Results

Squid could best be identified when they were showing Basic Brown pattern which most often occurred during times of the day with high light intensity. Both scars and mantle and fin dots show most clearly against the dark brown background coloration. In the field we used four characters on how to distinguish individual *S. sepioidea* from each other, which are discussed in order of detail. They are sex, size, scars and dot patterns:

We were able to distinguish between adult male and female *S. sepioidea* in the field. The first observations of distinct sex dimorphism were made based on the squid's behavior. Instances of mating were observed with attention to which squid are involved and which one transferred spermatophores to the other one. During mating behavior male and female squid show very distinct sex-related displays (Moynihan & Rodaniche 1982; Hanlon & Messenger 1996; Griebel, Byrne & Mather 2002). However, after these activities, when they return to their basic coloration, the sexes can still be distinguished according to the following markings:

Males showed distinct bright dots on their fins on Basic Brown background as well as a light fin edge line (Fig. 3a), while the females had a gradual transition from Brown to Pale towards the edge of their fins without showing distinct fin dots. This does not mean that females do not have fin dots, but that the female fin dots are smaller in size and they cover them by opening the brown chromatophores above them (Fig. 3b).

Although *S. sepioidea* tend to school with squid of similar maturity stage (Boom, Byrne & Mather 2001), they can be distinguished by relative size towards each other. The groups consist of squid of different sizes and they often school in size sorted formations (Moynihan & Rodaniche 1982). The most common formation for squid groups including adults is a one-dimensional line formation (Boom, Byrne & Mather 2001). Here the largest squid tend to swim on either one or both

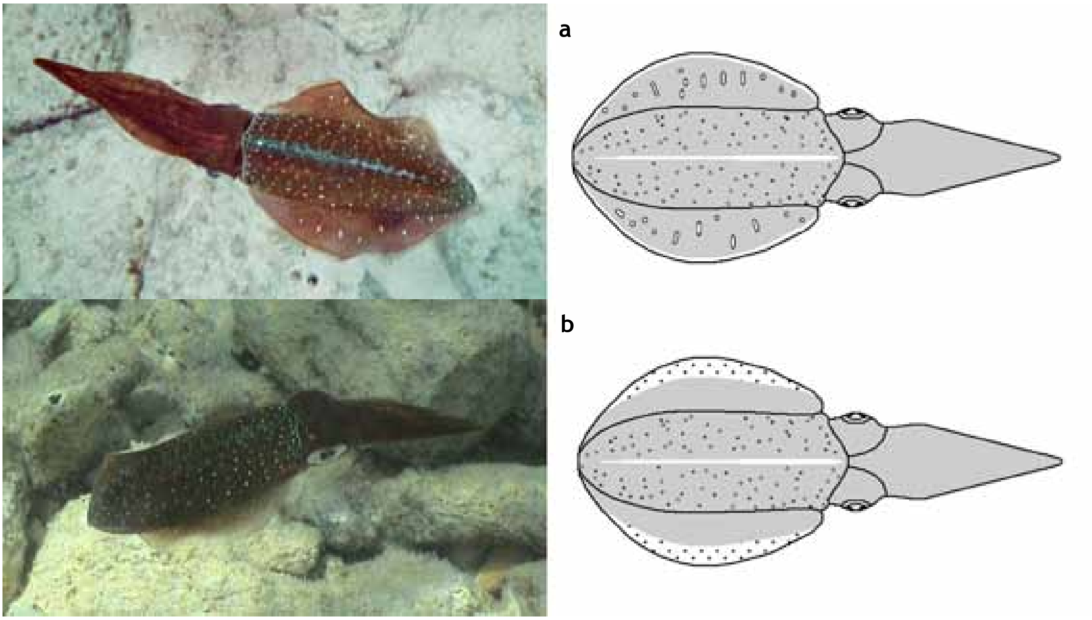

Fig. 3: Male and female sex dimorphism. a) Males can be recognized by looking at the pronounced fin dots as well as the light fin edge line. b) Females do not show clearly visible fin dots and have a transition from brown to translucent towards the fin edge. Photo: Ruth Byrne

ends of a line whereas the smaller ones are size sorted either towards the middle or to the other end of the line. Especially when identifying the larger squid in such a school knowledge of the most likely position of a specific squid within the group can be helpful.

Some squid have scars from predation attempts which can also be used as identification marks. Scars can be found in form of scratches on the mantle where the skin was hurt as there is no more pigmentation from the chromatophores visible. Such areas show up as pale or pink on a basic brown background. Fin scars include fins with missing portions or are visible as pale or lighter areas with no chromatophores (Fig. 3a). Also arms and tentacles can show marks of predation attempts as there are squid with missing or miss-built arms. To identify sub-adult and juvenile squid it is necessary to rely mostly on scars, as they do not show such distinct patterns of bright mantle and fin dots as the adults. Scars are good identification marks as long as they are fresh, but they have the disadvantage of healing and disappearing within a few weeks. This period of time has to be used to find other identification marks such as mantle and fin dots.

Mantle and fin dots are permanent marks that have an individual pattern on each squid's dorsal surface and most likely come about through iridophore reflection (Hanlon & Messenger, 1996). According to our data they do not change their location over longer periods of time in the squid's lifetime. Out of 300 dots (50 dots x 6 squid) we followed over the course of 6 time points for approximately 2 months we were able to track on average 99%. During this time frame the subjects grew on average 25 mm (Std = 4.4 mm) in mantle length. Only 10 dots were not found once or twice, but reoccurred in following images of the series. Reasons for missing dots were either the quality of

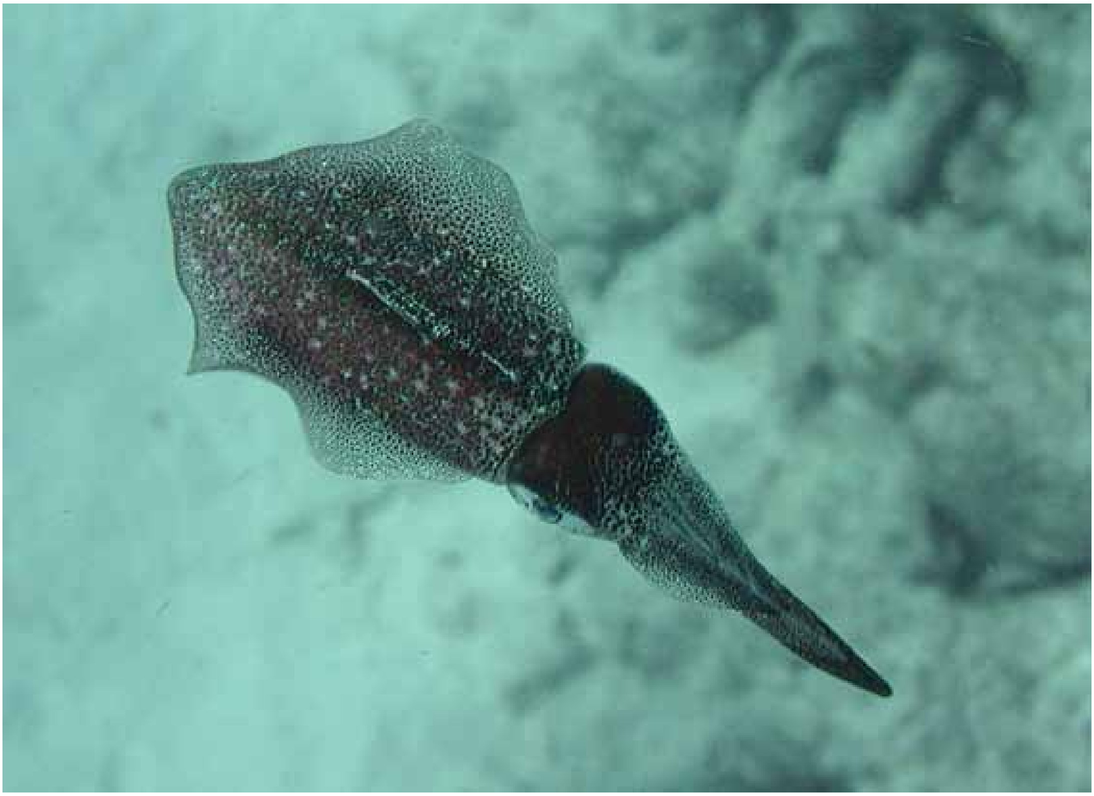

Fig. 4: Juvenile and sub-adult squid do not show clear dot patterns on the dorsal mantle. However, some of them have "freckles" that can be used for identification, as can be seen here on the anterior part of the mantle. Photo: Stephanie Bush

the image (i.e. the angle of picture (n = 4)), or the squids skin display (i.e. covered by a more intense Mid Dorsal Line (n = 1)). Five dots were missing without an obvious reason.

In the field the dots are clearly visible in the basic brown body coloration during the day, but less visible in mornings and evenings when the squid show basic intermediate or basic pale body coloration (Griebel, Byrne & Mather 2002). These dots show up in different intensities, some bright and clearly visible and some smaller, and thereby they form individual star-sign-like combinations on the dorsal surface of the squid. Although the location of fin and mantle dots does not change at least over the course of two months, if not much longer, the brightness of the dots can change, especially in the period of switching from being a sub-adult squid to an adult. A good example for this is that sub-adults do not show distinct fin dots, whereas adult males display bright and large fin dots. These very distinct dots are probably the best way to individually identify adult males. When fin dots are not distinct, as is the case in some still small males and especially in females, it has been useful to look for combinations of dots on the anterior and posterior tip of the mantle (Fig. 3b).

Sub-adult *S. sepioidea* are in general more difficult to identify than adults, as their dorsal surface is smaller and the patterns of bright fin-dots are less clearly visible. In contrast to adults, some sub-adult squid and juveniles show "freckles" (Fig. 4), different types of dots on their head, which can also be used for identification. Freckles are most likely areas of unevenly distributed chromatophores, most likely in location where mantle dots will appear later on, probably because of the skin stretching during this growth period. Unfortunately these freckles disappear in the transition to adulthood.

The three trained squid observers did really well in matching the two series of images of 26 different squid (3 x 100%). The four naïve observers reached on average 80% (100%, 81% and 2 x 69%).

Discussion

The ability to non-invasively identify individual squid is a useful tool for field and lab research on *S. sepioidea*. To track individual squid allows us to follow the development of their behavior patterns as they become mature, assess the mating success of different behaviors, assess mate fidelity, investigate group composition and stability, investigate group schooling behavior (Boom, Byrne & Mather 2002), and track them without altering their behavior over periods of up to several weeks at a time. Zeeh & Wood (2009) report that using VIE tags is one of the least stressful methods to artificially tag squid and that it did not affect the subjects growth rates. However, during their capture and tag process it became clear that this method influenced the subject's subsequent behavior. Squid tagged once were much harder to capture a second time compared to naïve conspecifics (Wood pers. com.). Thus, for the study of behavior a completely non-invasive technique, like the here presented dot pattern identification, seems to be the best choice.

Why should anyone care about identifying individual cephalopods? Cephalopods are an important and growing part of world's fisheries and they are an important prey for commercially and environmentally significant animals such as marine mammals (whales, seals, dolphins), fish (sharks, cod, etc), and birds (albatross, penguins). Yet little is known about their population dynamics and growth rates in the field. A number of invasive methods have been applied to answer these questions, some depending on dead caught animals, e.g. size analysis (Suguyama et al. 1989, tagging (Replinger & Wood 2007), and statolith work (Jereb, Ragonese & Boletzky 1991). These methods work well, but like all methods have their limits; i.e. assumptions about the subjects' size at time one in the case of statolith analysis. In addition, few species are validated with lab work. Individual identification would, at least theoretically and depending on the accessibility of the species, allow continuous observations of subjects living both in the field or in the lab. It also allowed us to make an ethogram of *S. sepioidea* (Griebel, Byrne & Mather 2002), similar to that of Jantzen & Havenhand (2003).

Contrary to Boal & Gonzales (1998) for *S. lessoniana*, not only could we discriminate the sex of adult S. *sepioidea* individuals, we could discriminate individuals over a period of weeks – a significant part of their rather short lifespan. We performed our investigations both in a natural setting as well as in the lab, while they did theirs in the lab only.

Boal & Gonzales (1998) describe the behavior of the squid as abnormal; according to their observations their subjects tried to mate with males and females indiscriminately. Perhaps because of our ability to individually identify the subjects, we never noted such behavior, either in the lab nor in the field. Or maybe in *S. sepioidea* there was an exchange of obvious visual sex-related signals among the squid that may have prevented such behaviors.

The identification of mature male squid by their dot patterns on the fins was relatively easy for observers, and this raises the question of whether such identification was being used by the squid themselves. The fin dot patterns were emphasized during male-male Zebra agonistic contests particularly during the stereotyped Formal Zebra display contest (Mather 2006) and this would be an opportunity to indicate one's identity to a rival (see Norman (2000), page 142-143; despite being mis-identified as a mating sequence, the photographs clearly show the dark background and pattern of dots). *S. sepioidea* and other loliginid squids are highly visual so a visual identification of others could be possible.

This unique identification of cephalopods, despite their underlying variability, gives hope that others of the group could also be individually identified. Research on the skin display system has focused on its variability (Messenger 2001), and its pattern production is certainly dazzling (Moynihan & Rodaniche 1982), but the observation that individuals can be identified nevertheless offers a new insight into this complex system, as well as gives us a tool to help understand the behavior, life history and population dynamics of these animals.

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Characterization of the adhesive organ of *Idiosepius biserialis* Voss, 1962 and *Idiosepius pygmaeus* Steenstrup, 1881 (Mollusca, Cephalopoda)

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Abstract

Chemical adhesives used for attachment are widely distributed in marine animals and also occur in cephalopods. The Idiosepiidae is one of the smallest cephalopod families (mantle length 6-30 mm), characterized by an epithelial adhesive organ on the posterior part of the dorsal mantle area. In adaptation on to their habitat in estuaries and tidal zones the organ is used to stick on several surfaces to avoid drifting, to capture prey and for camouflage. This study provides a description of the adhesive organ and improves the sparse knowledge about glandular structures in cephalopods. Ultrastructural analyses show that two secretory cell types (columnar and granular cells) and a ciliated sensory cell type (fusiform cells) are relevant for the adhesive organ. Both glandular cells contain granular material and have high and continuous synthesis rates of secretion. While the granular cells are solitary, the columnar cells always form aggregates of up to 15 cells, neighboured by at least one fusiform cell.

Introduction

Several cephalopod taxa possess adhesive mechanisms either for camouflage, prey capture, egg attachment or to avoid drifting (von Byern & Klepal 2006). Attachment is effected by mechanical mechanisms, e.g. reduced-pressure systems in suckers on the arms and tentacles (Kier & Smith 1990, 2002; Smith 1991, 1996; Pennisi 2002) or by chemical substances produced in special glandular cells. The localisation and morphology of the adhesive systems vary strongly according to the species. Chemical adhesives are supposed for species of the genera *Nautilus* (Fukuda 1980; Kier 1987; Muntz & Wentworth 1995), *Sepia* sp. (Roeleveld 1972; von Boletzky & Roeleveld 2000), *Euprymna* (Singley 1982), and *Idiosepius* (Steenstrup 1881; Sasaki 1921; von Byern et al. 2008).

The adhesive organ (AO) of Idiosepiidae was first described by Sasaki (1921). It is clearly restricted to the posterior area of the dorsal mantle surface and parts of the fins (Cyran, Klepal & von Byern 2008). The AO is a modification of the outer mantle epithelium. According to Sasaki (1921) two glandular cell types occur in the AO of *Idiosepius*: columnar cells with globular granules (Ø 1 µm) and granular cells containing closely packed polygonal granules (2-5 µm). Furthermore, cell types without secretory material are described as goblet cells, decayed columnar cells and sensory fusiform cells near the surface. Basal cells align along the basal membrane.

Histological and histochemical studies (von Byern et al. 2005; von Byern et al. 2008) indicate that, in addition to the granular and columnar cells, also the goblet cells are secretory cells and moreover all three glandular cell types contain neutral polysaccharides and basic proteins.

This study helps to understand the morphology of glandular cells and allows a more detailed comparison with adhesive systems in cephalopods as well as other molluscs. The complete, detailed paper of this study is in press in JMBA (Cyran, Klepal & von Byern).

This manuscript contains only the essential information regarding those parts of the AO, which are directly involved in the adhesion process.

Materials & Methods

Two species (*I. pygmaeus* and *I. biserialis*) were investigated. Collection, cultivation and preparation were carried out according to von Byern et al. (2008). The mantle was pre-fixed in toto for 5 min at room temperature (RT) (29-33°C) using 2.5% glutaraldehyde in 0.1 M Na-Cacodylate buffer (pH 7.3, including 10 % sucrose). Main fixation was made at RT for 5-8 h. Subsequently, the samples were washed three times for 15 min each with 0.1 M buffer solution and stored for further processing. For post-fixation the samples were immersed for 1.5 h in 1% osmium tetroxide with 0.1 M buffer solution and dehydrated in a graded series of ethanol.

For transmission electron microscopy the samples were embedded in Epon resin; ultrathin sections (40-80 nm) were mounted on copper slot grids coated with formvar in dioxane, stained with uranyl acetate and lead citrate (Reynolds 1963) and examined in a TEM Zeiss EM 9S-2 and Zeiss EM 902.

In addition to the standard procedure the following modifications were made:

1.) During the first fixation calcium chloride was added to the buffer solution until the saturation limit to improve the membrane preservation. 2.) For contrast enhancement 1% potassium ferricyanide was added to the osmium tetroxide fixative. 3.) After post-fixation samples were immersed for 40 min in 1% tannic acid and 45 min in 1.5% uranyl acetate to enhance visualisation of lipids and proteins.

For scanning electron microscopy the samples were washed several times in 100% acetone, immersed in HMDS (hexamethyldisilazane), dried in air over night, mounted on stubs, coated with gold in a Polaron 5800 sputter coater and viewed in the SEM Philips XL 20.

Results

While the surface of the regular mantle epithelium in *Idiosepius pygmaeus* and *I. biserialis* is smooth, the adhesive epithelium has deep furrows. The height of the adhesive organ (60 µm in *I. biserialis* and 80 µm in *I. pygmaeus*) is considerably higher than the regular outer mantle epithelium (30 and 40 µm, respectively). Otherwise no morphological differences can be found between the two species.

Characterization of the AO cell types

The outer mantle epithelium including the AO is generally composed of two cell layers, whereby the proximal layer exclusively consists of basal cells. In the distal cell layer three glandular (columnar, granular and goblet cells) and three non-secretory cell types (interstitial cells, saccular cells and small ciliated fusiform cells) can be distinguished morphologically.

The AO is characterized by presence of columnar, granular and fusiform cells. All other cell types occur likewise in the regular mantle epithelium.

The columnar cells are elongated pear-shaped. From a diameter of 10 µm at their base, the cells taper to 4 µm in the middle region and remain constant in diameter up to the epithelium surface. Around the nucleus, extensive rough endoplasmatic reticulum (rER) is present, interspersed with mitochondria. Along the lateral periphery of the columnar cells are several strands of filaments. They are anchored laterally in the zonula adhaerens near the apical cell pole and are restricted to the upper third of the cell. The cytoplasm is loosely filled with globular, membrane-bound secretion granules (Ø 1µm). Frequently, membrane residues are visible between the granules.

The granular cells are cylindrical (10 µm in diameter on average). The synthesis of the secretory material is similar to that found in columnar cells but the granules are larger (2-5 µm). In the loose stage they are also globular but when tightly packed they appear polygonal.

The present study shows that in the columnar as well as in the granular cells only parts of the entire cell content is released during secretion. In contact with the outer medium the released granules fuse immediately to a uniform mass.

The pear-shaped fusiform cells are about 15 µm long and always in the surface layer of the epithelium. The cytoplasm contains many vesicles, dictyosomes, lysosomes, mitochondria and some cytoskeletal filaments. One or more cilia (250 nm in cross section and 2 µm long) are positioned on the apical pole of the cells; their centrioles are 1 µm below the cell surface. The cilia arrangement seems to be random. In some cases the distal end of the cilia appears to be widened. No ciliary root could be detected. A single neurons is joined to the base of each fusiform cell.

Distribution of the cell types

Columnar cells always occur in aggregates of 5-15 cells. Between them are narrow interstitial cells, forming a hump with their elongated microvilli. One or more fusiform cells are positioned at the periphery of the aggregates. These aggregates are regularly distributed in the adhesive organ. The granular cells are also regularly distributed.

Discussion

A predominantly mechanical adhesion or release can be excluded for *Idiosepius*. The lack of mantle musculature in the dorsal mantle area prevents movement and thus participation in releasing from the substrate. Nevertheless, an influence of dermal musculature on detachment is conceivable, as suggested for *Euprymna* (Singley, 1982). The furrows in the epithelium may enhance the strength of attachment by increasing the ability for deformation and generating a sucker like effect (Gay 2002; Gay & Leibler 1999).

While basal, interstitial, goblet and saccular cells occur also in the non-adhesive epithelium, the columnar, granular and fusiform cells are restricted to the AO of *Idiosepius*. This implies their involvement in adhesion. The following attributes of the glandular cells support this diagnosis: Both the columnar and granular cells are distributed regularly in the adhesive organ. This does not apply to the goblet cells. Columnar and granular cells secrete only part of their contents during each attachment and new secretory material is produced continuously. This is necessary because the animals can attach and detach several times per minute (Suwanmala, von Byern & Nabhitabhata 2006).

In contrast, the goblet cells apparently stop synthesis as soon as the cells are completely filled up with secretory material. This would not appear to benefit a frequently used adhesive system.

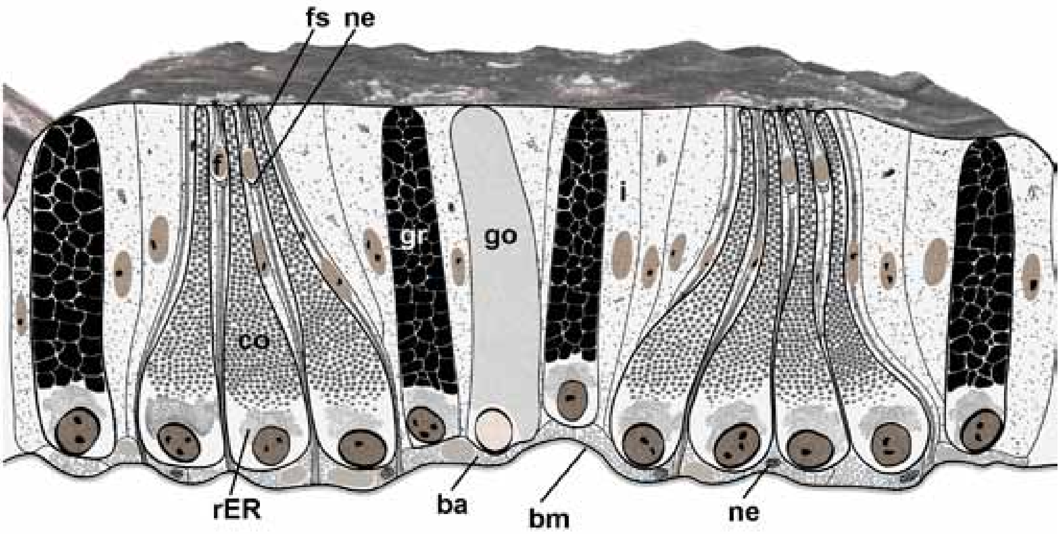

Fig. 1: Schematic drawing of the cell arrangement in the adhesive organ (after Cyran, Klepal & von Byern 2008). Ba, basal cell; bm, basal membrane; co, columnar cell; f, fusiform cell; fs, filament strands; go, goblet cell; gr, granular cell; i, interstitial cell; ne, nerve process; rER, rough endoplasmatic reticulum.

The cilia in the fusiform cells and the adjacent nerve process indicate a function as receptor cells for the perception of chemical or mechanical stimuli. The sometimes widened ends, the lack of a defined arrangement of cilia as well as the absence of a ciliary root are characters of a chemoreceptor (Thurm et al. 1983). Hence, in *Idiosepius* the fusiform cells presumably provide information about the chemical substrate properties. Ciliated cells comparable to the fusiform cells were also found in *Nautilus* (Muntz & Wentworth 1995), whereby their association with the adhesive mechanism is not clear.

The configuration of rER and dictyosomes in the columnar and granular cells indicates synthesis and packaging of proteins and modifying with carbohydrates. In goblet cells the proteins, produced by rER are apparently not modified by the Golgi apparatus. This is evidence for a lower level of carbohydrates in the secretory material. These findings correlate with the histochemical results (von Byern et al. 2008).

Further analyses of the secretory product, including its biochemical characterization, are necessary to clarify the mode of adhesion (duo-gland or two component system).

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A radula and associated cephalic remains of a Late Carboniferous coleoid from Oklahoma, USA

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Abstract

A Late Carboniferous coleoid radula and associated arm hooks together with the cartilaginous cephalic remains are described and illustrated in detail. The specimen comes from the Missourian (=Kasimovian; Late Carboniferous) Eudora Shale in Oklahoma. The radula has 11 elements in each transverse row: one central, two lateral, and one marginal tooth, and a pair of marginal plates beyond the marginal tooth on each side. A small piece of a flattened structure that is partly exposed near the radula might have belonged to a mandible. This is the second recovered radula having a pair of marginal plates beyond the marginal tooth. The earlier recovered radula belonged to the Late Carboniferous *Saundersites* from the Mazon Creek *Lagerstätte* in Illinois. The coleoid origin of the radula is based on its association with horny arm hooks that have a typical morphology for similar structures in belemnoids. The radula and the arm hooks are surrounded by abundant debris of cartilaginous tissue that is distinguished by its honeycomb-like structure due to the tesserate calcification. This pattern is typical in chondrichthyan fishes, and our study demonstrates that it also occurs in the cranial capsule of fossil coleoids.The organic remains were buried in a phosphate concretion that is 29 mm long, 15 mm broad and 11 mm thick. This concretion might be a shark coprolite as shark remains often occur in the Eudora Shale in Oklahoma. No other organic remains were found in the concretion. These remains probably represent a cephalic part of an unknown coleoid individual.

Introduction

Preservation of non-mineralized cephalic remains in coleoids requires special conditions for fossilization. First of all, fast mineralization of soft tissue is required to prevent total disintegration of organic structures. For example, preservation of the radula required rapid mineralization of the organic ribbon bearing numerous transitional rows of teeth. Otherwise the radula teeth would not keep their arrangement in transitional rows.

The fossil radulae are preserved either separately (Solem & Richardson 1975), or in association with any other fossilized part of the mollusc, such as a shell (Mehl 1984; Gabbott 1999), mandibles (Saunders & Richardson1979; Doguzhaeva & Mutvei 1992) or arm hooks (Johnson & Richardson 1968; Doguzhaeva et al. 2007). In modern cephalopods, there are two types of radula structure: one type with 11-13 elements in every transverse row as in the *Nautilus* type (Saunders et al.1978), and a second type with 7-9 elements in each row (Fig. 1A-D) as in the coleoid type (Nixon 1988). These two types of radula structure also occur in fossil cephalopods. A radula with 13 elements in a transverse row (*Nautilus* type) was described as *Paleocadmus* Solem & Richardson, 1975. In ammonoids, the radula structure is known in about 10 Mesozoic and Paleozoic genera. It is similar to that of the coleoid type by having 7-9 elements in a transverse row (Lehmann 1967, 1971, 1979; Closs 1967; Closs & Gordon 1966, 1968; Doguzhaeva & Mutvei 1992; Tanabe & Mapes 1996; Doguzhaeva et al. 1997). Based on a similar radula structure in coleoids and ammonoids, Lehmann (1967) introduced the taxon Angusteradulata to emphasize their close phylogenetic relationship. The taxon Lateradulata was introduced by Lehmann (1967) for Recent *Nautilus* and nautiloids. The hitherto

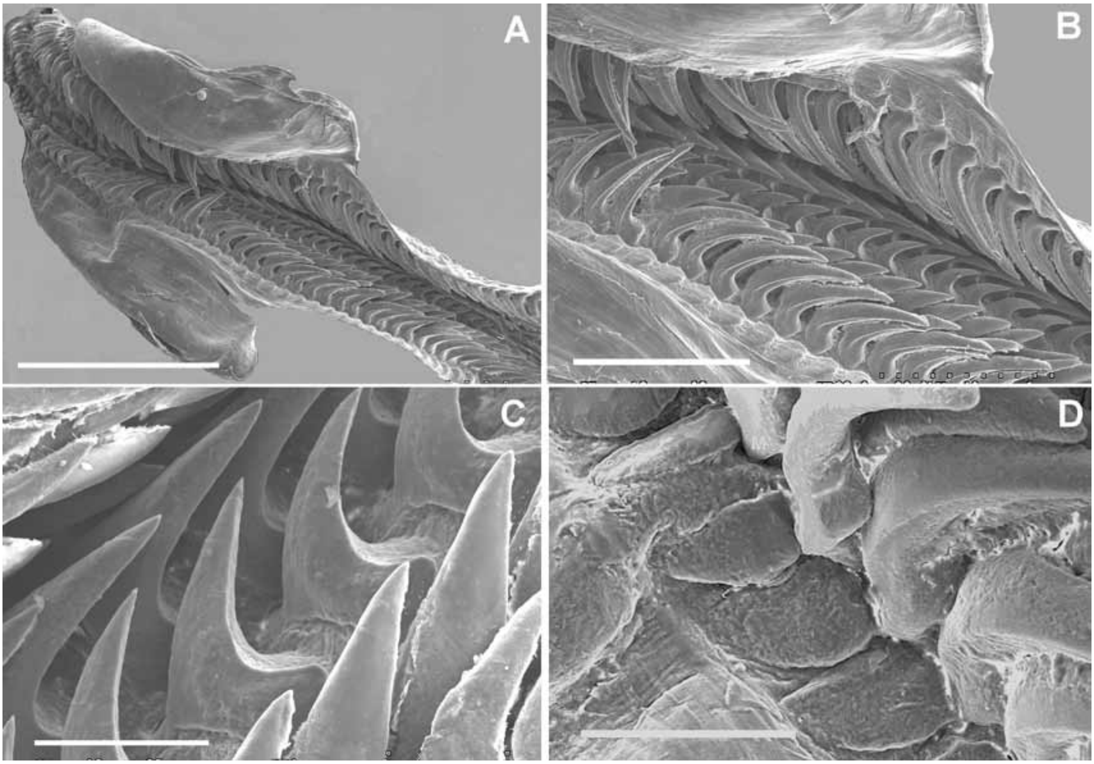

Fig. 1: Radula in *Loligo* sp.; North Sea, Skagerrak, Sweden. A) General view on the radula; scale bar = 2. 5 mm. B) Enlarged detail of A showing a longitudinal row of marginal plates beyond a row of marginal teeth; scale bar =1 mm. C) The pointed and curved central and lateral teeth, the central tooth is shorter and has a triangular shape and a broad basal part; scale bar = 200 µm. D) Enlarged view of B to show a semicircular shape of the marginal plates, scale bar = 250 µm.

known radula in coleoid cephalopod was collected from the Mazon Creek *Lagerstätte* in Illinois. It was first described and attributed to the Late Carboniferous teuthid coleoid *Jeletzkya douglassae* Johnson & Richardson, 1968 (Saunders & Richardson 1979). Later, it was restudied and redescribed as *Saundersites illinoisiensis* Doguzhaeva, Mapes & Mutvei, 2007. This radula is preserved in front of the body chamber in association with the arm hooks and reveals a unique structure. The radula has 11 elements in a transverse row with three teeth and two marginal plates on each side of the central tooth; the two marginal plates are located lateral to the marginal tooth (Doguzhaeva, et al. 2007: Figs. 6.3, 6.4). In other molluscs, a radula with 11 elements in each transverse row and two marginal plates located lateral to the marginal teeth is known in monoplacophorans (Starobogatov 1990a, b) that are believed to have given rise to cephalopods (Yochelson, Flower & Webbers 1973). The arm hooks indicate that *Saundersites* belonged to coleoid cephalopods. The presence of the breviconic shell with a long body chamber, a short pro-ostracum like structure, a short phragmocone, and a mostly organic, probably weakly calcified rostrum made it possible to refer the genus to the order Donovaniconida Doguzhaeva, Mapes & Mutvei, 2007.

In the present paper, the second discovered coleoid radula that is slightly younger than the radula of *Saundersites* (collected from Missourian and Desmoinesian, respectively), is illustrated in detail. A preliminarily description of the specimen was made by Doguzhaeva, Mapes & Mutvei (in press). The coleoid origin of the radula is indicated by the presence of the arm hooks that have the typical morphology for similar structures in coleoids. The radula and arm hooks are associated with debris of cartilaginous tissue and a supposed fragment of the mandible. All listed organic remains are described and their fossilization is analyzed.

Material, status of preservation and methods of study

The concretion that yielded the assemblage of organic remains was collected from the Eudora Shale in Oklahoma. The Shale is Missourian age (=Kasimovian; Upper Carboniferous). The concretion is small, 30 mm in length, 15 mm in width and 11 mm in thickness, and has an ovoid shape. During preparation, the concretion was split longitudinally into two halves of about equal size. The remains of the cartilaginous tissue are the most abundant animal fragments that are dispersed along the exposed surface of the concretion. The radula and the supposed fragment of the mandible lie in the center of the concretion. The four arm hooks can be recognized because they are oriented parallel to the fracture surface of the concretion and show a typical coleoid hook shape in the lateral view. Two of them are located in the central part, not far from the radula; the other two are situated on the concretion periphery. In addition, the fracture surface shows numerous dispersed circular holes, the diameter of which corresponds to the diameter of the arm hooks in orientations. These holes are interpreted to be dispersed arm hooks that are obliquely oriented to the fracture surface.

After examination with the light microscope, the fractured surfaces of the concretion were studied with the scanning electron microscope Hitachi S-4300. The chemical composition of the organic remains and the concretion matrix was analyzed with the energy dispersive spectrometer (EDS) at the Swedish Museum of Natural History, Stockholm.

The described specimen (OUZC 5723) is housed at the Department of Geological Sciences, Ohio University, Athens, USA.

Observations

1. Radula (Figs 2B, D; 3; 4A-E; 5; 6A; 8A, B)

Several transitional rows of the radula teeth together with disintegrated teeth are exposed on the fractured surfaces of the concretion (Figs 3; 6A). Together, these teeth give an overall idea of the radula structure (Fig. 5). Additionally, there are numerous holes in the concretion matrix, close to the radula teeth, that may be the tips of radula teeth not completely exposed on the fracture plane. The central tooth (C) is distinguished by its symmetrical shape and central position in a transverse row of the radula (Figs 3, 8A). This tooth is approximately 2.5 times shorter than the marginal teeth. Its basal portion is broad and stout, and its

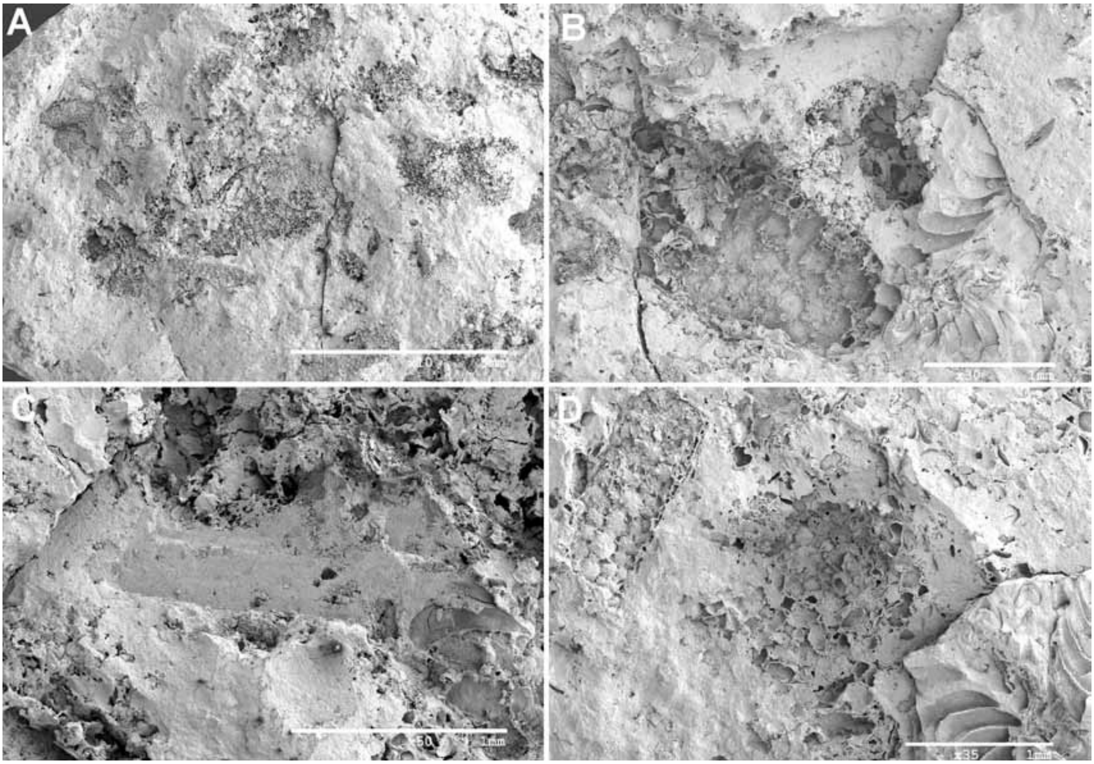

Fig. 2: A) Fractured surface of the concretion showing an assemblage of organic remains; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian; Oklahoma; scale bar = 5 mm. B) Another area of the same surface as in A to show a radula (right bottom) and a large fragment of the cartilaginous tissue with a honeycomb structure (center) situated below the radula; scale bar = 1 mm. C) Enlarged detail of B to show a skeletal fragment that might have belonged to a cephalopod mandible; scale bar = 1 mm. D) Another area of the same surface as in A to show a fragment with cartilaginous structure (right upper corner); the "cells" form parallel rows, and they are smaller at the periphery than centrally; scale bar = 1 mm.

upper portion is triangular with an angle of 35 degrees and with a pointed tip. At approximately one third of the distance above the base, the sides of the central tooth curve inward, forming a sinuous symmetrical outline. The marginal teeth (M) are long, asymmetrical, and strongly curved with pointed tips (Fig. 3). The lateral teeth (L1, L2) are supposedly shorter than the marginal teeth, but longer that the central tooth. There are two marginal plates outside of the marginal teeth on each side of the radula (Figs 3, 4A, C). The total number of teeth in each transverse row can be estimated to be 11.

The radula elements – teeth and plates- are preserved as internal moulds of the central cavities that were apparently filled with an organic material in life-time (Figs 1E, 8A, B). The internal moulds have a micro-granular ultrastructure, with the globules being aggregated in micro-spheres, the size of which is less than 1 mm (Figs 4D, E; 8A, B).

2. Arm hooks (Figs 6A-F; 8C, D)

The arm hooks are of two sizes: small (about 0.6 mm in length) and large (about 1.8 mm in length). The small arm hooks are similar in size with the marginal teeth of the radula (compare Figs 6B and 6F). The small and large arm hooks are uniform in the shape and composition. They are shiny, curved, hollow structures composed of black horny material. Their shaft is comparatively long and thick. Their distal part (from the maximum curvature to the tip) is long and well developed. The angle between the shaft and the distal part is about 90º. The arm hooks are ornamented with a delicate longitudinal striation. The original material that has built the wall of the arm hooks (supposedly chitin) is preserved in an altered form that has a typical blocky, fracture structure (Figs 6B, C, F). The micro-globular ultrastructure of the arm hook material (Fig. 9D) is similar to that described in the arm hooks in the Upper Triassic coleoid *Phragmoteuthis* (Doguzhaevaet al. 2007: Fig. 5A-C).

3. Mandible (Fig. 2B, C)
A small piece of a plate-like skeletal part that is partially exposed near the radula might have been a fragment of the coleoid mandible.

4. Cartilaginous tissue (Figs 2A-D; 6A, C, E, F; 7A-H)
The specimen exhibits numerous fragments of a material with a honeycomb or cell-like structure (Figs 2A-D; 6C, E, F; 7A-F) that is typical of fossilized cartilage in chondrichthyan fishes (Dean & Summers 2006). The fragments are dispersed on the fracture surfaces of the concretion and clustered around the radula and arm hooks. The compartments ("cells") of the cartilaginous tissue are less than 200 µm in diameter. Several fragments show that in their periphery the compartments are smaller, approximately 30-70 µm in diameter (Fig. 7B, C). The compartments are separated by walls, which are double in places. The walls have numerous rounded holes of about 5 µm in diameter (Fig. 7E-H). The holes appear to be the places where the collagenous fibres crossed the compartments. The association between the cartilage and the radula indicates that the cartilaginous tissue belonged to a cartilaginous cranial capsule.

5. The EDS data on chemical composition of the cephalic remains (Fig. 8A-F)
The matrix of the concretion (Fig. 8D, F), the radula elements (Fig. 8E), and the cartilaginous tissue debris (Fig. 8A, B) contain P, Ca, O and C. Additionally, the matrix and the cartilage show in places Fe. The arm hooks (Fig. 8C) are composed of Ca, Si, O, and C but lack P.

Fig. 3: Radula of an unknown coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A symmetrical, triangular central tooth; two poorly exposed lateral teeth on each side of the central tooth; long, strongly curved marginal teeth; and marginal plates beyond the marginal teeth (right top); scale bar = 0. 5 mm.

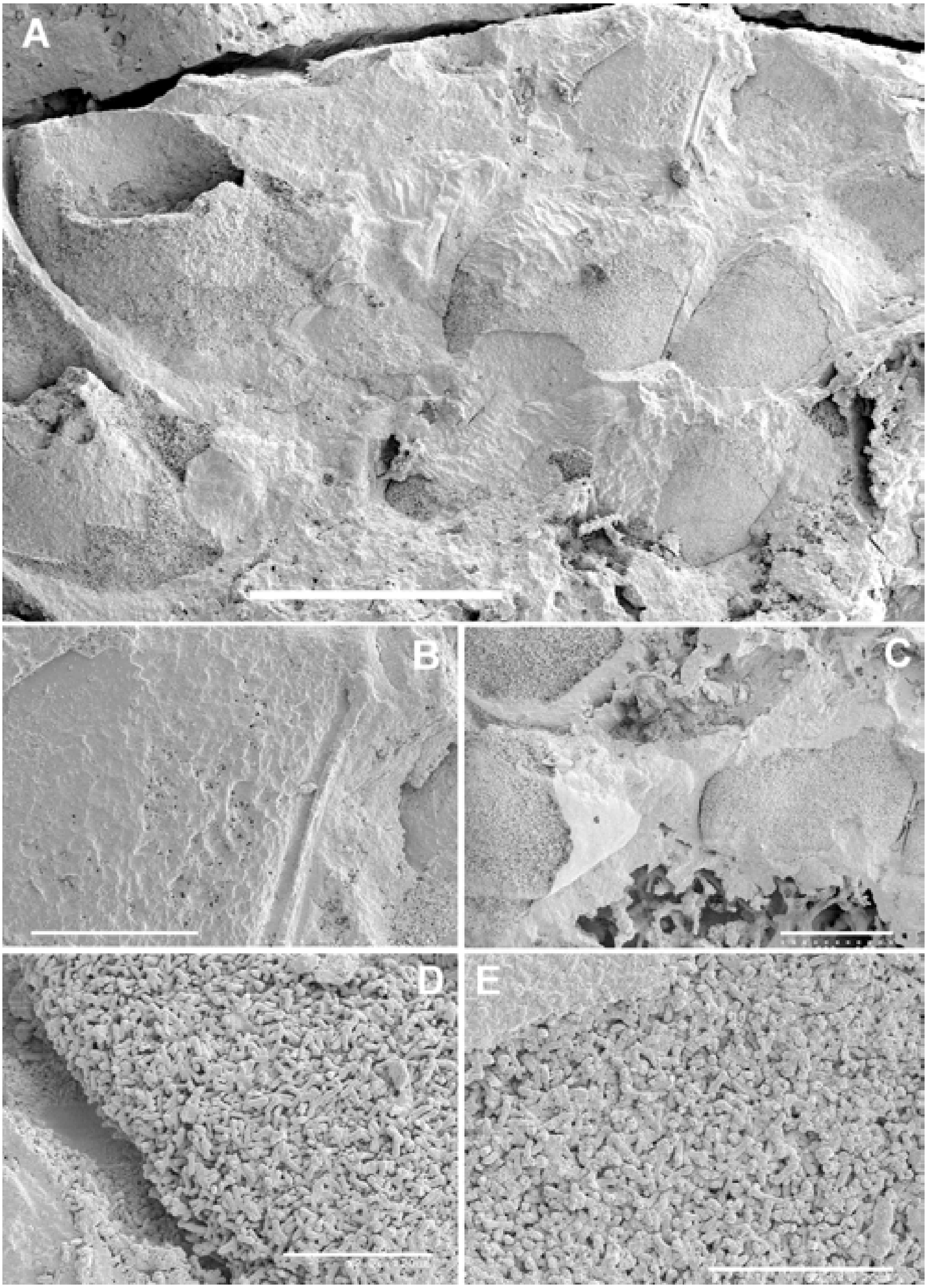

Fig. 4: Radula of unknown coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A) Enlarged detail of Fig. 3 to show two marginal plates beyond the marginal tooth in each raw; scale bar = 200 µm. B) Enlarged detail of A showing a curved edge of the inner side of a marginal plate and straight edge of the external side; scale bar = 50 µm. C) Partly exposed marginal plate (right) and basal parts of two marginal teeth; scale bar = 100 µm. D) Enlarged detail of A to show the granular filling of a marginal tooth; scale bar = 20 µm. E) Enlarged detail of C to show a granular filling of a marginal plate; scale bar = 20 µm.

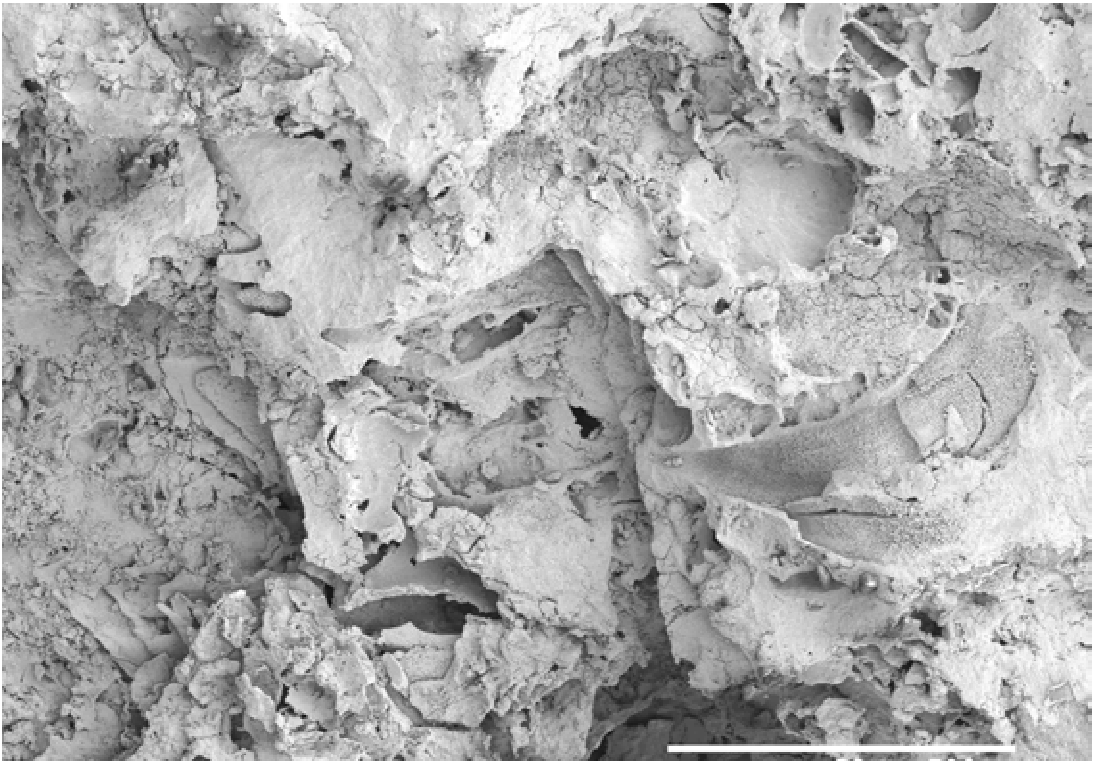

Fig. 5: Radula of unknown coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. The dispersed radular teeth are exposed at different levels on the fractured surface of the concretion: on the right side an impression of a long marginal tooth, on the left, upper side three conical tips of teeth, and on the left, lower side an internal cast of the central tooth; scale bar = 0. 5 mm.

Discussion

1. Comparison of the radulae of the Oklahoma coleoid and Saundersites from Illinois, with notes on possible scenarios for the evolutionary development of the radula in coleoids.

SEM examination reveals that in the unknown coleoid from Oklahoma, several transitional rows of the radula are exposed within the cartilaginous debris (Figs 3, 4A-E, 5). They give an overall idea of the radula structure. The radula formula is C L1 L2 M MP1 MP2 (11 elements in total), where C is the central tooth, L1 and L2 the first and the second lateral tooth, M the marginal tooth, and MP1 and MP2 the first and the second marginal plate (Fig. 10C). The specific character of the radula, namely, two marginal plates on each side of the marginal teeth, and totally 11 elements, has been previously observed in the Late Carboniferous coleoid *Saundersites* (Doguzhaeva et al. 2007). In the Oklahoma coleoid the marginal plates are elongated and about two times longer than broad. In *Saundersites* the marginal plates are more isometric. In both genera the marginal tooth is comparatively long and has a broad basal portion. It is pointed and curved backwards. The central tooth in the Oklahoma coleoid has a symmetrical, triangular, pointed shape. It is about two times shorter than the marginal tooth. In *Saundersites* the central and lateral teeth are poorly preserved and cannot be compared.

Doguzhaeva, Mapes & Mutvei (2007) suggested two possible scenarios for the evolutionary development of the radula in coleoids. The radula possessing 11 elements in each transverse row, like that in the studied specimen and in the Early Carboniferous *Saundersites* (Fig. 10D), might have been inherited from the monoplacophoran radula, and that of the radula with 11 elements is

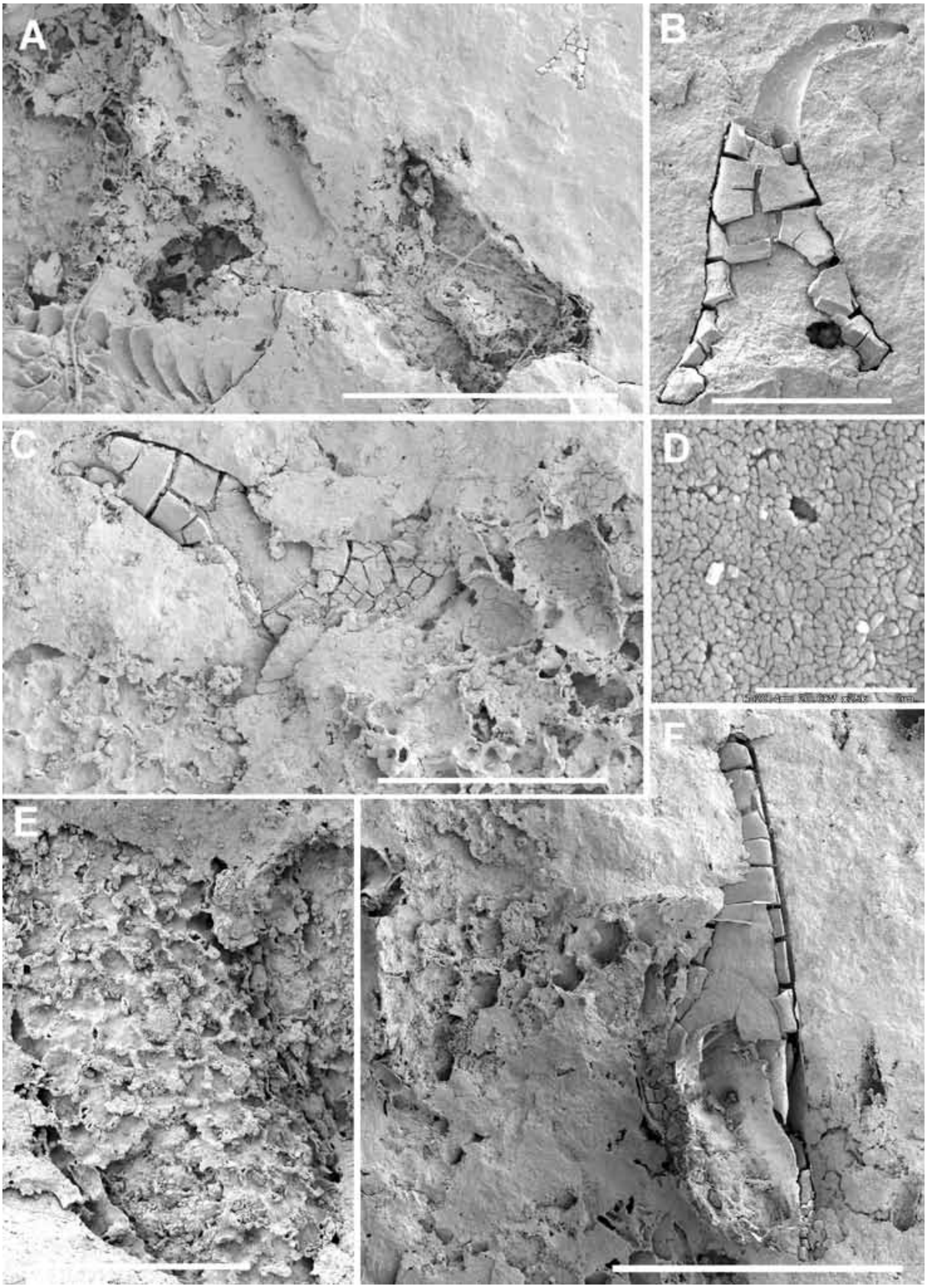

Fig. 6: Arm hooks and cartilaginous debris associated with a radula of an unknown coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A) General view to show the position of an arm hook (right top corner) at a short distance from the radula (left bottom corner); scale bar = 2. 5 mm. B) Enlarged detail of A to show a small-sized arm hook; scale bar = 0. 25 mm. C) Upper part of an arm hook surrounded by cartilaginous tissue that has a honeycomb-like structure; scale bar = 0. 5 mm. D) Enlarged view of B to show granular structure on the arm hook surface; scale bar = 2 µm. E) An elongated piece of cartilage with a honeycomb-like structure; scale bar = 0. 5 mm. F) Large-sized arm hook and cartilaginous debris close to it; scale bar = 1 mm.

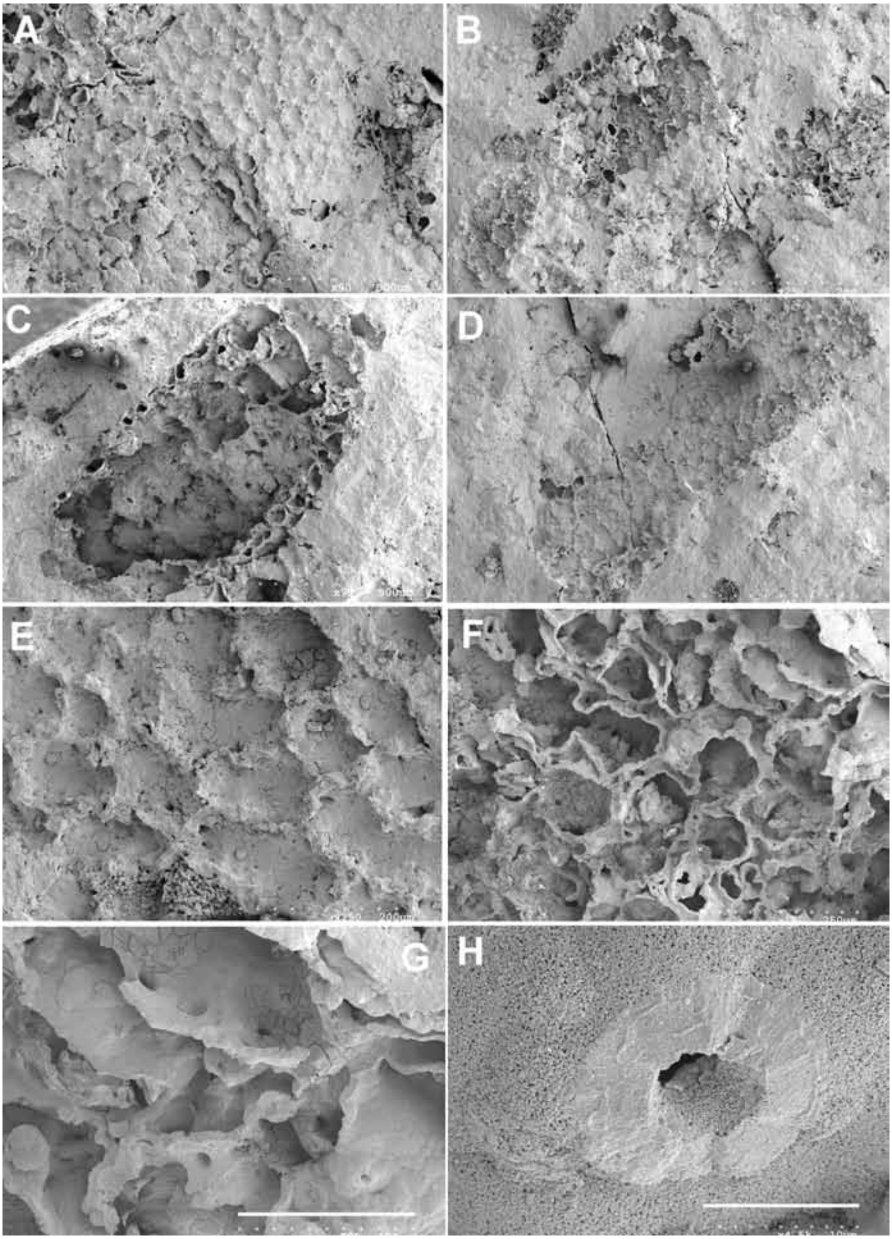

Fig. 7: Cartilaginous remains associated with a radula of unknown coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A) Honeycomb-pattern of cartilaginous remains; scale bar = 0. 5 mm. B) Fragment of a cartilaginous tissue showing rows of compartments; scale bar = 2 mm. C) Enlarged detail of B to show that the compartments in the periphery of the cartilaginous remains are of uniform size and smaller that those in the center; scale bar = 0. 5 mm. D) Another piece of cartilaginous tissue with a honeycomb-pattern; scale bar = 1 mm. E) Enlargement of D to show the uniform size and shape of the compartments of the cartilaginous tissue, scale bar = 200 µm. F) "Walls" between the compartments of the cartilaginous tissue; scale bar = 250 µm. G) Enlargement of F to show the holes in the wall between the compartments; scale bar = 100 µm. H) Enlarged detail of G showing a hole in the wall between the compartments; scale bar = 10 µm.

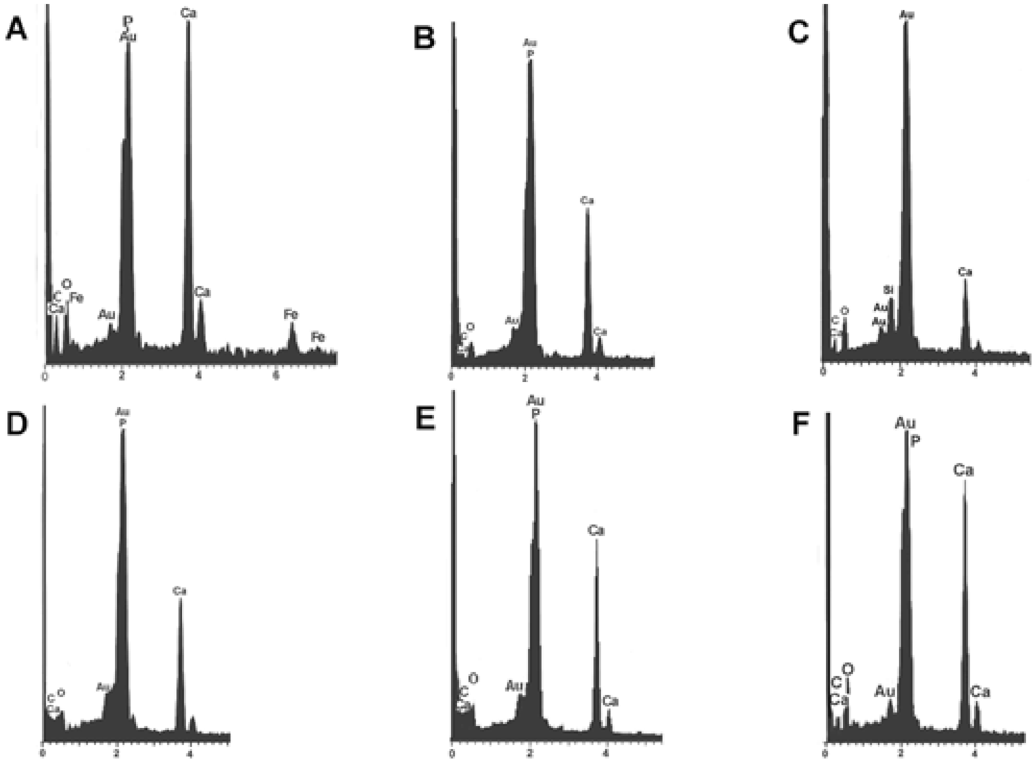

Fig. 8: EDS data on the organic remains of the studied coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A-B, cartilage. C, large arm hook. D, matrix of the concretion close to the radula. E, filling of the central cavity of the radula tooth. F, matrix of the concretion.

similar to the archaic radula of early cephalopods. The nautiloid type of radula, with 13 elements in each row (Fig. 10B), might have arisen from a radula with 11 elements by adding an additional marginal tooth between the two marginal plates on each side. The second scenario is as follows: the radula with 11 elements (seven teeth and four plates) was derived from the *Nautilus*-type radula with 13 elements (Saunders et al. 1978) by the elimination of the second marginal tooth. That resulted in an adjacent position of the two marginal plates, which is the case with the radula in the Late Carboniferous *Saundersites* and in the herein studied specimen. The next evolutionary step might lead to fusion of the two marginal plates into one plate, and as a consequence, the appearance of the radula that had 9 elements per transverse row (Fig. 10A, E, F). Finally, the marginal plates might have been become entirely eliminated, and a radula with 7 elements in each transverse row was formed.

2. Comparison of the arm hooks in the studied coleoid from Oklahoma and those in Saundersites from Illinois.

The arm hooks of the studied specimen from Oklahoma and those in *Saundersites* show significant morphological differences. In the studied specimen, the arm hooks are of two sizes, about 0.6 mm and about 1.8 mm in length. In *Saundersites* they are about 0.2 – 0.3 mm long, and this is about 2-3 times smaller than the smallest arm hooks in the studied specimen. Also, in the studied specimen, the arm hooks have a comparatively long shaft while in *Saundersites* the shaft is short. In the studied specimen the cross section of the arm hooks is rounded while in *Saundersites* it is triangular. A similarity between the compared arm hooks is the approximate angle of about 90º between the shaft and the distal part.

3. Pathway in fossilization of the cephalic remains in the new coleoid from Oklahoma (Figs 2A-D; 4D, F; 5; 6A-F; 7A-H; 8A-F; 9A-D).

The assemblage containing the radula, arm hooks and cartilage was preserved in an ovoid concretion that is about 30 mm long and 15 mm broad. The concretion evidently represents a coprolite of a small shark or other fish as their remnants are relatively common in the phosphate concretions from the Eudora Shale (Pennsylvanian; Missourian stage) in Oklahoma.

The radula elements (teeth and plates) are preserved as internal moulds of the central cavities that were apparently filled with organic material in the life-time. Their external surfaces were preserved as impressions in the matrix of the concretion. The internal moulds have a micro-granular ultrastructure, with the globules being aggregated in micro-spheres, the size of which is about 1.5 μm and micro-sticks about 2.2 μm long (Figs 3D, E; 9A, B). The organic material inside the radula teeth and marginal plates were probably reworked and/ or altered by bacteria. Hence the granular filling that forms the internal mould of the radula elements is probably the product of bacterial activity.

The material of the arm hooks (originally chitin) is dense and has a typical blocky fracture structure and a micro-globular ultrastructure (Figs 6A-D, F; 9C, D). Similar structure has been observed earlier in the arm hooks of the Late Triassic *Phragmoteuthis* (Doguzhaeva et al. 2007), and in the mandible of the Late Triassic *Lunzoteuthis* (Doguzhaeva et al. 2006). The arm hooks, like the teeth and plates of the radula, had a central cavity. The radula elements have been preserved as internal moulds of this cavity, whereas the arm hooks have the diagenetically mineralized, outer wall preserved. This wall shows a micro-globular ultrastructure with the size of the globules less than 0.1 μm. Contrary to the concretion matrix, radula and

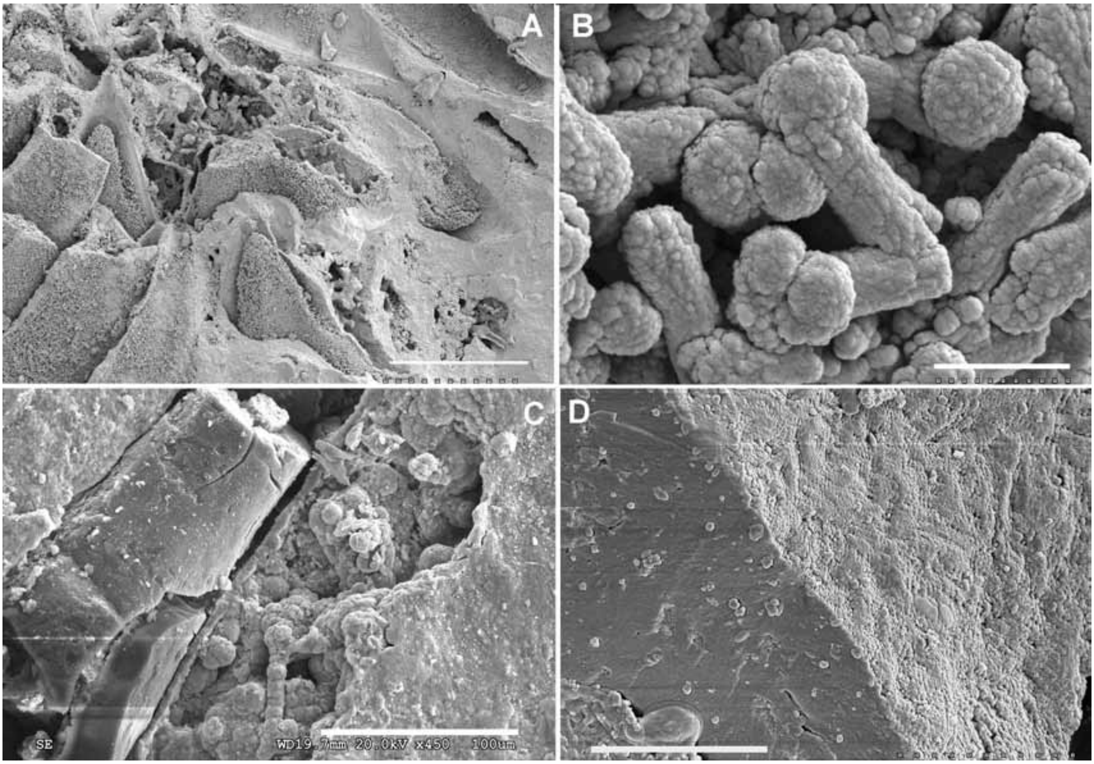

Fig. 9: Preservation of the radula (A, B) and the arm-hooks (C, D) in the new coleoid from Oklahoma; Eudora Shale; Missourian, Upper Carboniferous, Pennsylvanian. A) Central, lateral and marginal teeth preserved as internal moulds; scale bar = 200 μm. B) Enlargement of A to show globular and stick-like aggregates of smaller particles; scale bar = 2 μm. C) Arm hook showing the wall and the central cavity (top); scale bar = 100 μm. D) Enlargement of C to show micro-globular ultrastructure of the wall of the arm hook; scale bar = 10 μm.

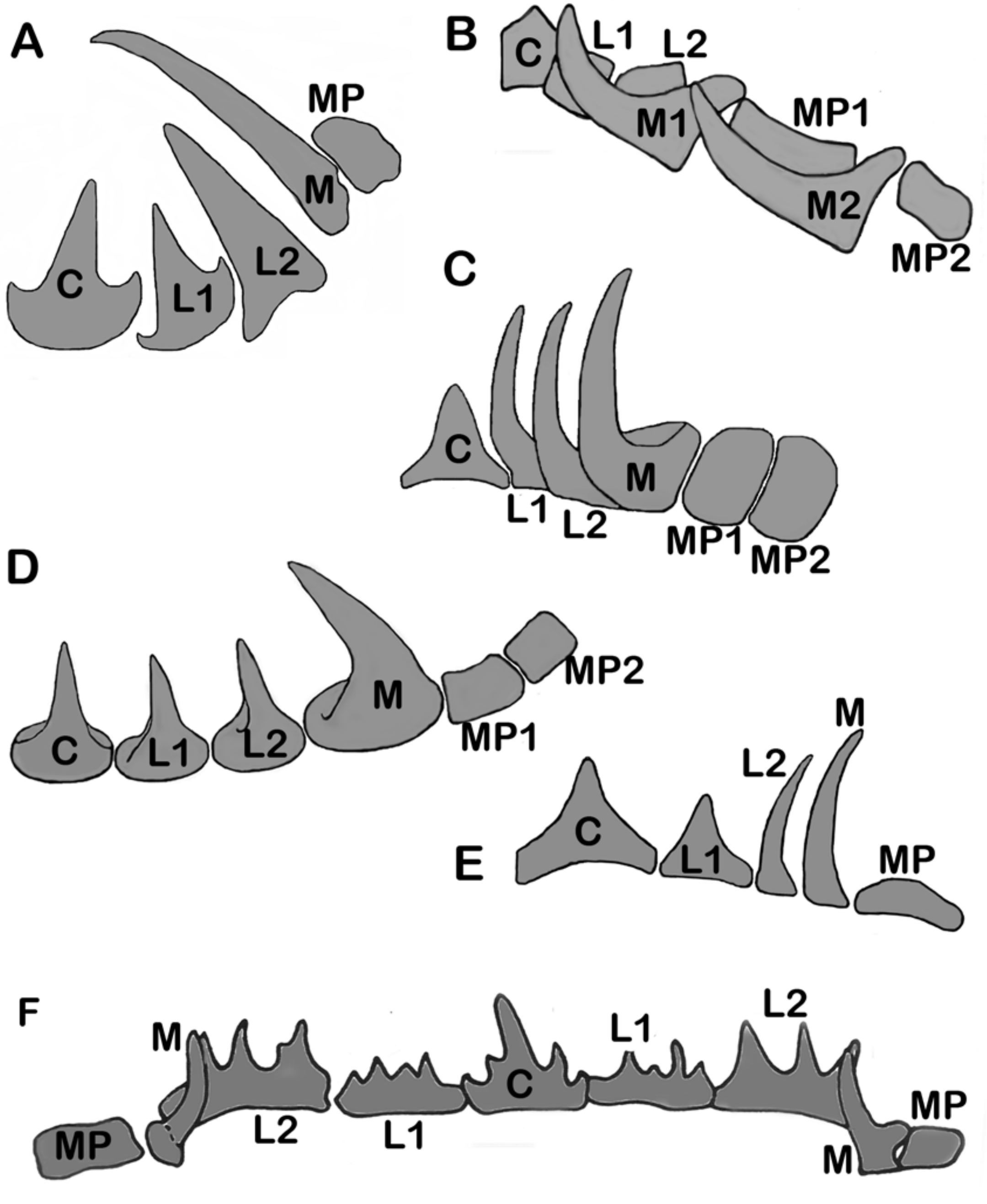

Fig. 10: Radula structure in recent (A, B, F) and fossil (C-E) cephalopods: A) *Octopus*. B) *Nautilus* (modified from Saunders et al. 1978). C) unnamed coleoid, U. Carboniferous (modified from Doguzhaeva et al. 2009). D) *Saundersites*, U. Carboniferous (modified from Doguzhaeva et al. 2007). E) Early Carboniferous ammonoid *Gertyoceras* (modified from Doguzhaeva et al. 1997). F) *Japetella* (modified from Nixon 1988). C, central tooth; L1 and L2, first and second lateral teeth; M, marginal tooth; M1 and M2, first and second marginal teeth; MP, marginal plate; MP1 and MP2, first and second marginal plate.

cartilaginous tissue, the wall of the arm hooks lacks P, although P is found in the internal mould of the cavity of the arm hooks. This phenomenon can be only explained by a selective phosphatization of organic remains different from chitin.

The cartilage is preserved due to mineralization (phosphatization) of the walls between the compartments. This observation is in agreement with the experimental data on phosphatization of the cranial cartilage in invertebrates, including the living squid *Loligo* (Eilberg, Zuckerberg & Person 1975), and on the analysis of the mineralized cartilage in the skeleton of chondrichthyan fishes (Dean & Summers 2006) and rodents (Yotsuyanagi et al. 1999). In the chondrichthyan fishes, the endoskeleton is surronded by a perichondra that is calcified and forms a mineralized, "honeycomb-like" prismatic layer over the surface of the endoskeleton. This phenomenon is known as a tesserate calcification. The prismatic pattern of mineralization, observed in chondrichthyans, was thought to be unique (Dean & Summers 2006). Our SEM study of the cephalic remains in the Late Carboniferous coleoid from Oklahoma reveals a tesserate calcification in ancient coleoid cephalopods.

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The first diplobelid coleoid from the Cenomanian of south-eastern India

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Keywords: Diplobelida, *Conoteuthis*, Cenomanian, Karai Formation, south-eastern India.

Abstract

A single breviconic phragmocone from an outcrop near Odiyam (Ariyalur district, Tamil Nadu, south-eastern India) has proofed to represent a previously unknown species of *Conoteuthis,* a well-known diplobelid genus from the Lower Cretaceous of Europe. *Conoteuthis azizi* n. sp. has been found in deposits that belong to the Cenomanian Karai Formation. A morphological comparison has shown that *Conoteuthis azizi* n. sp. slightly differs from the type species *Conoteuthis dupiniana* from the Aptian of Europe through a slightly larger apical angle and septa that are lesser inclined. *Conoteuthis azizi* n. sp. is the first unambiguous evidence of the Diplobelida in Upper Cretaceous rocks and the second diplobelid species from the southern hemisphere.

Introduction

The Diplobelida is a small aberrant group of belemnoid coleoids that is presently known from the early Jurassic to the early Cretaceous. With one exception in the southern Tethys, its distribution seems to be restricted to the European Tethys. Diplobelids resemble belemnoteuthids in having a short, investment-like sheath around a longiconic or breviconic phragmocone. However, characters such as a remarkably narrow proostracum and sutures with dorsal saddles clearly distinguish diplobelids from belemnoteuthids.

Diplobelida is a highly interesting group, because it might play a key role in the evolutionary history of modern coleoids as various authorities regarded it as the root stock of the Spirulida and the Sepiida. Already d'Orbigny (1842) and Zittel (1868) compared the shells of diplobelids with those of Cenozoic sepiids and spirulids. Naef (1922: 94) assumed that tertiary forms might have evolved from *Diplobelus*-like belemnoids. Dauphin (1984) derived shell ultrastructures of *Belosepia* and *Sepia* from diplobelids and Hewitt & Jagt (1999: 323) concluded that "…separate origin of the Sepiida and Spirulids within the Cretaceous diplobelid belemnites is still the most attractive hypothesis, until the case for pre-Aptian fossil record of spirulids become overwhelming." Nevertheless, this hypothesis is unpopular among other authors (e.g. Jeletzky 1966, 1981; Doyle, Donovan & Nixon. 1994). Recently, Fuchs (2006: 29) did not want to reject the "diplobelid pathway", but pointed out its improbability because presumed "pre-Aptian spirulids" are meanwhile known (Doguzhaeva, Mapes & Mutvei 1999; Doguzhaeva 2000).

In the light of this debate, each record of a diplobelid phragmocone appears worthwhile to be published. It is therefore the aim of the present article to describe the first diplobelid from the Cenomanian of south-eastern India.

Geological setting

The single phragmocone was collected by V. A. and K. G. in 1989 from an outcrop 0.5 km west of the village Odiyam (Odiyam: 11°13'00"N, 78°59'30"E; c. 14 km northwest of Ariyalur, Ariyalur district, Tamil Nadu; Fig. 1). The Ariyalur area is situated in the western part of the so-called Cauvery Basin, a well known fossiliferous area in south-eastern India that exposes a continuous sequence ranging from Lower Cretaceous to Recent deposits (e.g. Sundaram et al. 2001). Lithologically, the carbonate is the most dominant facies in the basin. Blanford (1862) for the first time divided these deposits into three distinct groups namely the lower Uttattur, the Trichinopoly and the upper Ariyalur group. Many workers later modified this classification but the basic frame work of this classification remained unchanged until today. In the present work the authors followed the latest stratigraphic classification proposed by Sundaram et al. (2001). Accordingly, the Uttattur Group, from where the studied specimen comes from, consists of four major formations, the Terani, the Arogyapuram, the Dalmiapuram and the uppermost Karai Formation (Fig. 2). Sundaram et al. (2001) suggested a Late Albian to Early Turonian age for the Karai Formation and further subdivided the formation into two major members, the Odiyam Member (argillaceous) and the Kunnam Member (arenaceous). The specimen described herein is from the Odiyam Member (in contrast, *Naefia* sp. described by Vartak, Fuchs & Ghare in this volume originates from the slightly younger Kunnam Member). The thickness of the Odiyam Member is about 235 m and consists of sandstone, shale, siltstone and gypsiferous mudstone and some lenticular beds of coquinite. The presence of *Scaphites obliquus* indicates an Early to Middle Turonian age. Sundaram et al. (2001) reconstructed open marine conditions of deposition of the Karai Formation.

Systematic Palaeontology

Subclass COLEOIDEA Bather, 1888
Superorder BELEMNOIDEA Hyatt, 1884
Order DIPLOBELIDA Jeletzky, 1965
Family DIPLOBELIDAE Naef, 1926

Type genus: *Diplobelus* Naef, 1926

Genera included: *Chondroteuthis* Bode, 1933; *Quiricobelus* Combémorel & Mariotti, 1986; *Diplobelus* Naef, 1926; *Pavloviteuthis* Shimansky, 1957; *Tauriconites* Kabanov, 1984 (see Drushtchits, Kabanov & Nerodenko, 1984); *Chalalabelus* Jeletzky, 1981; *Vectibelus* Jeletzky, 1981; *Conoteuthis* d'Orbigny, 1842;

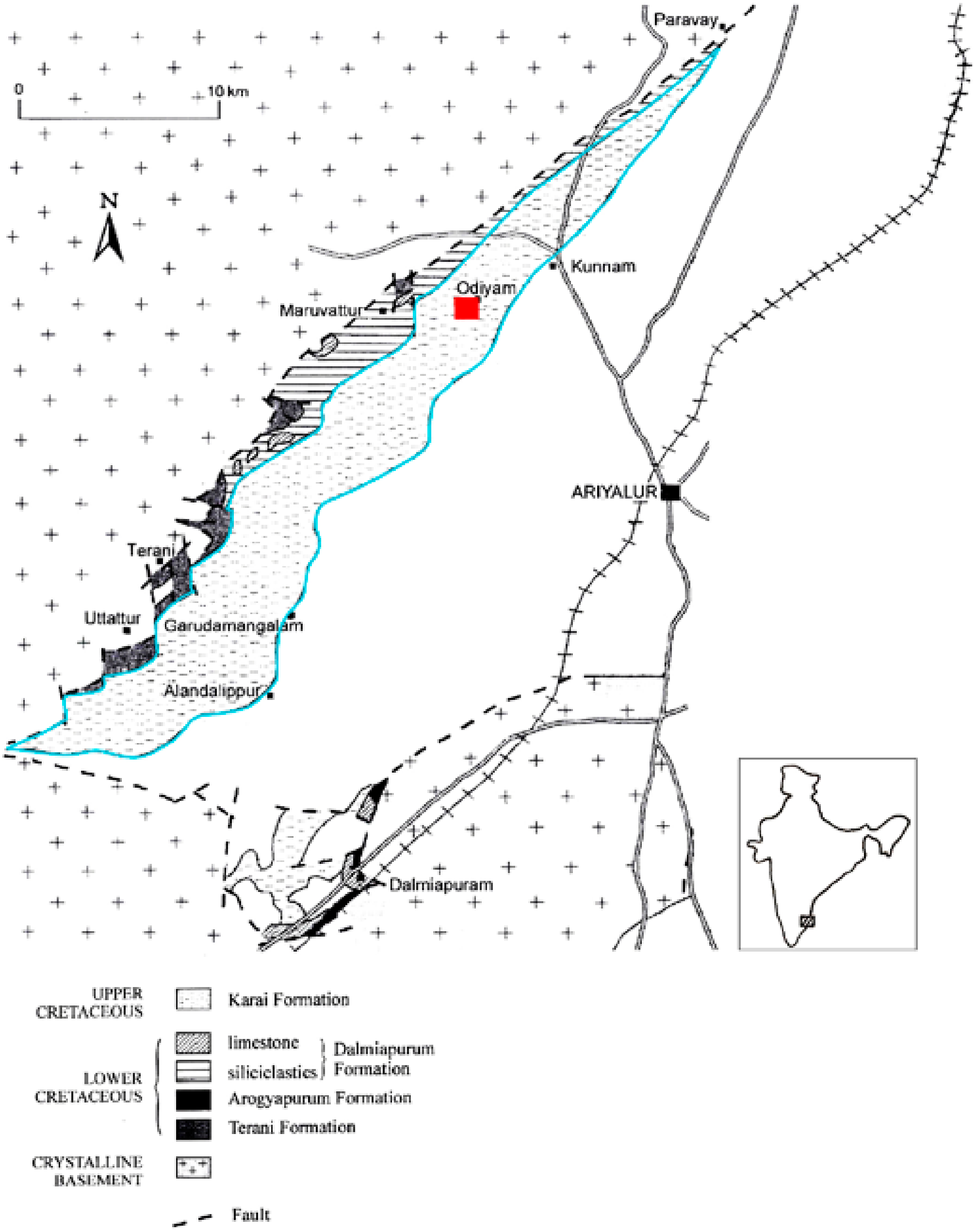

Fig. 1: Map of India with south-eastern India in inset (slightly modefied after Sunaram et al. 2001). The outcrop area of the Karai Formation is highlighted in blue.

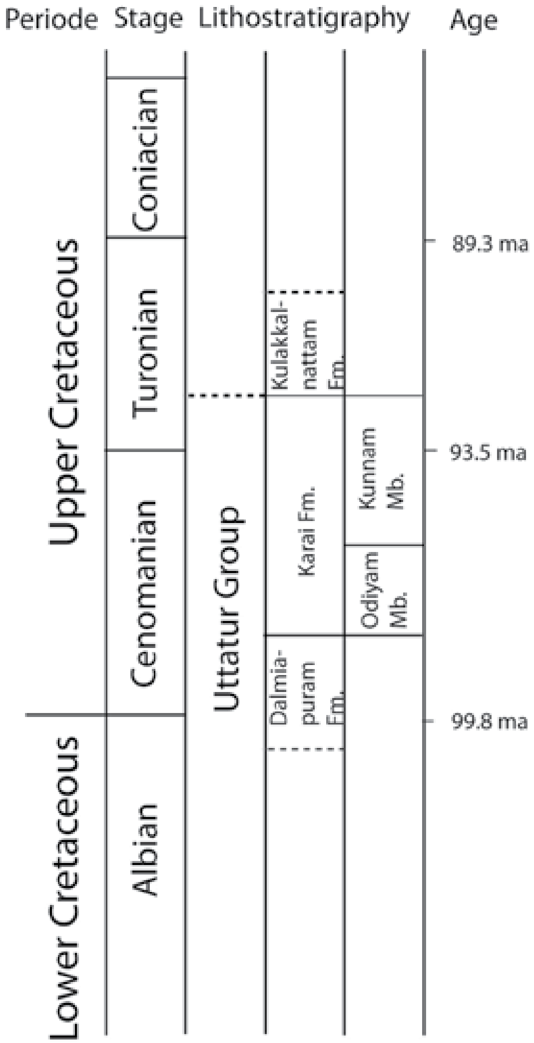

Fig. 2: Stratigraphic position of the Karai Formation.

Genus *Conoteuthis* d'Orbigny, 1842

Type species: *Conoteuthis dupiniana* d'Orbigny, 1842

Diagnosis (after Jeletzky 1981: 122): "Diplobelidae combining feebly to markedly but always regularly endogastrically incurved phragmocone with paper-thin, sheath-like guard, which only thickens slightly in the proximity of the protoconch; longitudinal mediodorsal keel of the phragmocone begins in a close proximity of shell's apex and extends to its oral end gradually increasing in prominence oralward; this keel is superimposed on the tops of dorsal saddles of the suture lines which are broadly rounded initially but become sharp-topped and angular further adorally; the keel is also expressed on the guard's surface where it is ornamented by a median furrow and flanking ridges."

Species included: *Conoteuthis dupiniana* d'Orbigny, 1842 from the Hauterivian – Lower Aptian of UK and France and *Conoteuthis azizi* n. sp. from the Cenomanian of Southeastern India.

Remarks: *Conoteuthis woodwardi* Spath, 1939 is a valid taxon that is according to Spath (1939: 3) based on a single "rapidly decomposing" specimen (BMNH C.7849) of Albian age (see also Riegraf et al. 1998), but a morphological comparison is impossible as the specimen is nowadays completely decomposed (pers. observation) and Spath (1939: 3, fig. 2d, e) gave only a poor characterization of "*Conoteuthis woodwardi*".

Jeletzky (1981: 120) tentatively assigned the Cenomanian *Acanthoteuthis syriaca* Roger, 1944 along with *Conoteuthis*. This act is somewhat doubtful, because, the original specimen of Roger (1944: pl. 2) shows only soft parts, i.e. a calcified phragmocone that would allow morphological comparisons is not preserved in that specimen (Engeser 1995, pers. observation).

Geographic and stratigraphic range: Hauterivian of Speeton (UK), Lower Aptian of the Anglo-Paris basin (Isle of Wight, Auxerre), Cenomanian of South India, middle Turonian of Japan (see Takahashi & Hayakawa 1999).

Conoteuthis azizi n. sp.

Derivation of name: In memory of the late Dr. S. A. Aziz for his contribution in South Indian Brachiopods and Echinoids.

Holotype: MACS G5153, deposited in the Agharkar Research Institute (ARI), Pune, India.

Type locality: Odiyam, northwest of Ariyalur, Tamil Nadu district, south-eastern India

Type horizon: Uttatur group, Karai Formation, Odiyam Member (Late Cretaceous, Cenomanian)

Other localities: Known only from the type locality.

Description: *Phragmocone.* The holotype preserves a distinctly breviconic phragmocone of 9 mm

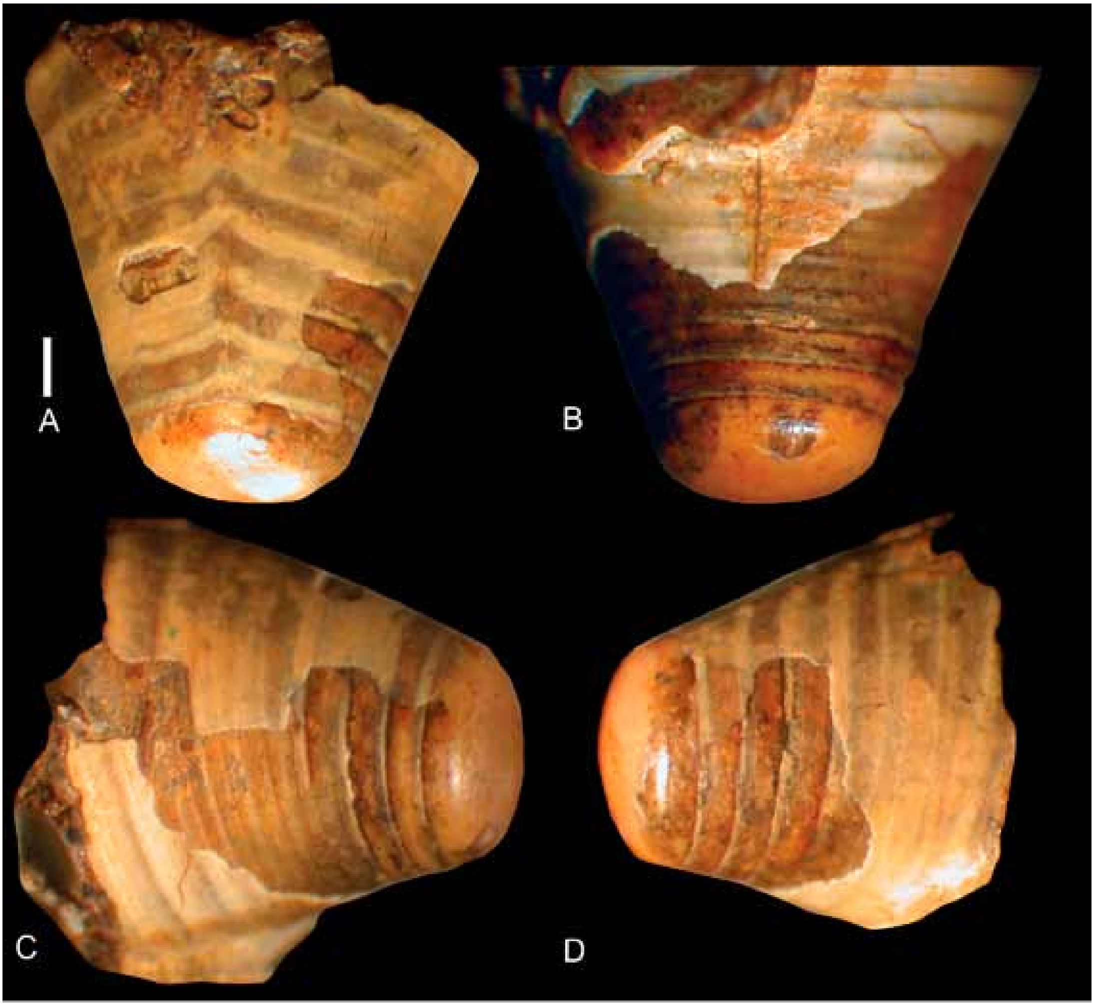

Fig. 3: Holotype (specimen MACS G5153) of *Conoteuthis agazizi* n. sp. from the Cenomanian of Odiyam, south-eastern India. A) Dorsal view. B) Ventral view. C) Left lateral view. D) right lateral view; note that a guard-like sheath is not preserved why mural parts of the septa are visible through a whitish semi-transparent conotheca; scale bar = 1 mm.

length (Fig. 3A-C). The phragmocone includes 8 chambers, which probably represent the most anterior (youngest) chambers. Posterior (older) chambers including the embryonic chamber (protoconch) are missing. The maximum diameter of the youngest preserved chamber is 9.5 mm wide; that of the oldest 4.3 mm. The cross section is slightly oval with a longer dorsoventral diameter. The ratio chamber height/diameter of the smallest preserved chamber is 0.3; the same ratio of the largest preserved chamber is 0.15. The apical angle is 36 in dorsoventral and 43° in lateral direction. The venter is straight to slightly concave; the dorsum is distinctly convex. The dorsum bears a weakly elevated roof-shaped mid-dorsal keel with a maximum width of 1 mm. The keel itself bears two ridges. Chambers are filled with sediment. The siphuncle is ventral (internal structures of the siphuncular system are not visible).

Sheath and conotheca. A sheath (an investment-like guard in other terminologies) that usually covers the phragmocone from outside is whether absent or (more likely) not preserved. Except in some places, the chambers are enveloped by very thin, whitish and semi-transparent layers (Fig. 3A-C, Fig. 4A-D). These layers are here interpreted to represent the phragmocone wall, the conotheca (ultrastructural analyses will be subject to a forthcoming study). Mural parts of the septa are visible through the conotheca (a sheath would be most likely non-transparent). Apart from weak longitudinal ornamentations, forward bended growth lines are visible on the outer surface of the conotheca, but not on the internal mould (steinkern).

The ventral conotheca bears a peculiar fissure (Fig. 4C). As the latter structure is not situated in the mid-ventral axis, a preservational artifact can not be excluded.

Proostracum. Although the phragmocone does not preserve an anterior projection, the forward projected growth increments visible on the dorsolateral and dorsal surface clearly indicate the existence of a remarkably narrow and pointed proostracum (Fig. Fig. 4A, D, E). Dorsolaterally, growth lines meet the lateral margins of the keel in a smooth arch. The maximum width of the proostracum coincides well with the lateral margins of the mid-dorsal keel (Fig. 4B, E). The maximum length of the proostracum must remain uncertain.

Septa and suture lines. Posterior septa appear to be more or less straight; anterior septa, in contrast, are distinctly inclined towards the aperture. Suture lines show mid-dorsal saddles that are sharply arcuated (Fig. 3). Lateral, ventrolateral or ventral lobes are not present (Fig. 4C).

Comparisons: The new form can be easily distinguished from most presumed diplobelid genera by their remarkably large apical angle. The phragmocone of *Conoteuthis azizi* n. sp. is distinctly breviconic compared to the more longiconic phragmocones of early Jurassic *Chondroteuthis wunnenbergi* Bode, 1933, late Jurassic *Diplobelus belemnitoides* (Zittel, 1886); *Quricobelus italicus* Combémorel & Mariotti, 1986, *Pavloviteuthis kapitzkei* Engeser, 1995, early Cretaceous *Pavloviteuthis cantiana* (Spath, 1939), *Pavloviteuthis kabanovi* Shimansky, 1957 and *Tauriconites nikolai* Kabanov, 1980.

In contrast to *Conoteuthis azizi* n. sp., *Chalalabelus renniei* (Spath, 1939) is characterized by a well-developed thick guard and a more incurved phragmocone (Jeletzky 1981: text-fig. 12).

At least the anterior part of the phragmocone of *Vectibelus vectensis* (Spath, 1939) is similar to *Conoteuthis azizi* n. sp., but differs from our specimen in the lack of a pronounced mid-dorsal keel and a larger apical angle.

According to the description given above, the holotype of *Conoteuthis azizi* n. sp. performs typical character as Jeletzky (1981: 11) required in his diagnosis for the genus *Conoteuthis*.

Conoteuthis azizi n. sp. differs from *Conoteuthis dupiniana* through a slightly larger apical angle. Furthermore, septa are lesser inclined in *Conoteuthis azizi* n. sp. than in *Conoteuthis dupiniana* (compare d'Orbigny (1845: pl. 32); Jeletzky (1981: pl. 21) and Mutterlose (1984: Fig. 2)). Finally, lateral lobes, ventrolateral saddles and ventral lobes as described by Jeletzky (1981: 12) for *Conoteuthis dupiniana* are absent in *Conoteuthis azizi* n. sp.

Discussion

Since "*Conoteuthis*" *(Acanthoteuthis) syriaca* is here considered to be not related to *Conoteuthis,* Cenomanian *Conoteuthis azizi* n. sp. is the first unambiguous evidence of the order Diplobelida from the Upper Cretaceous. A presumed *Conoteuthis*-like diplobelid from the middle Turonian of Japan confirms the existence of diplobelids during this period (Takahashi & Hayakawa 1999). Furthermore, *Conoteuthis azizi* n. sp. represents the first record of the genus *Conoteuthis* and the second diplobelid species at all from the southern hemisphere. So far, the only diplobelid from the southern hemisphere was *Chalabelus renniei* from the Aptian of Mozambique.

Mutterlose (1984) and Engeser (1995: 6, fig. 2) discussed phylogenetic relationships among diplobelids and both concluded that *Conoteuthis* is closest to *Vectibelus* owing to the presence of a mid-dorsal keel. Since *Conoteuthis azizi* n. sp. revealed no new relevant characters, further phylogenetic implications are hampered. New characters with a high phylogenetical impact can be expected only from ultrastructural analyses.

Nevertheless, the genus *Conoteuthis* seems to show a remarkable morphological stability as Hauterivian, Aptian and Cenomanian forms show only very faint phragmocone modifications.

Concerning the life habitat of *Conoteuthis azizi* n. sp., the lithology of the Karai Formation indicated an offshore, but shallow marine paleoenvironment.

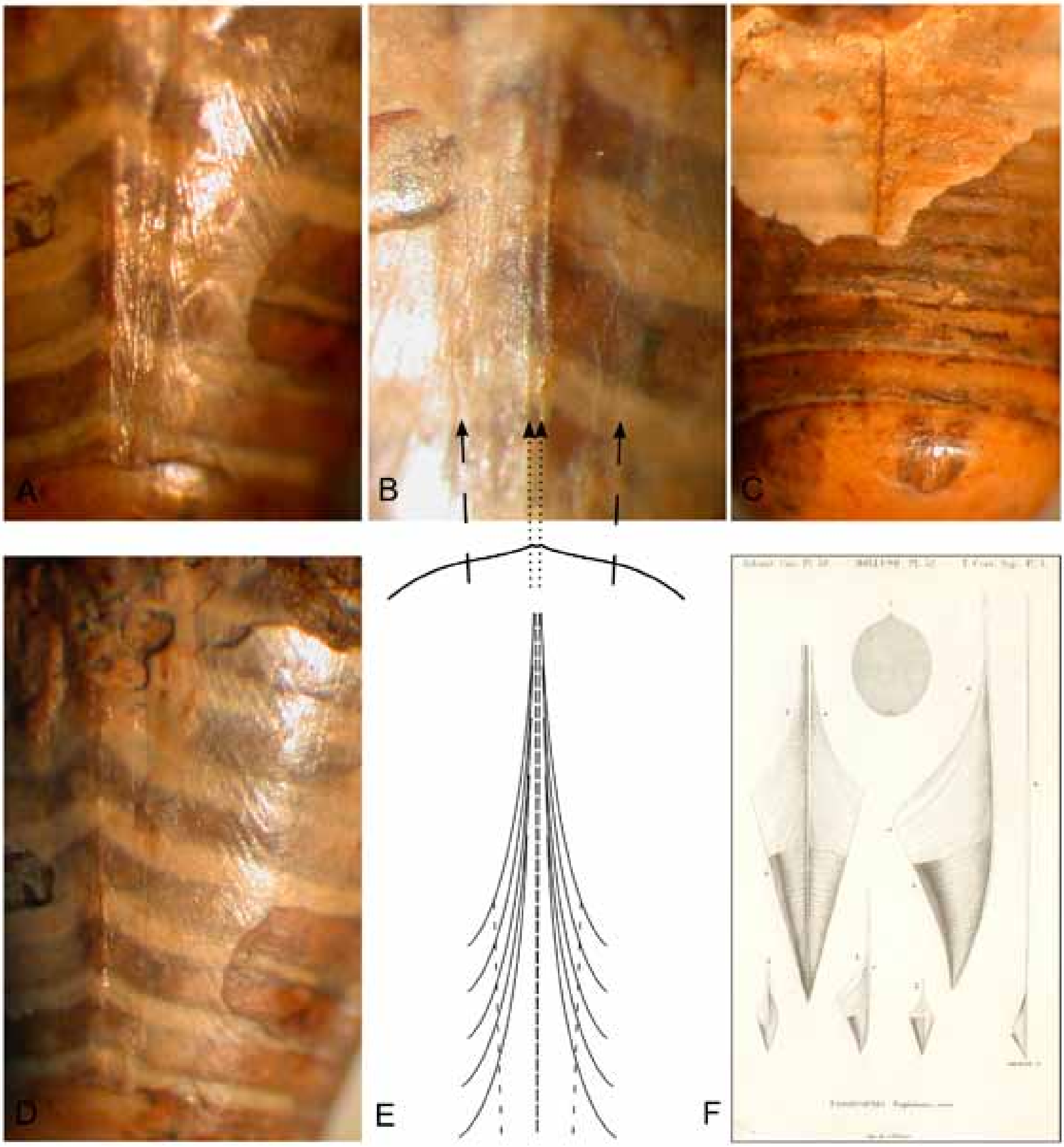

Fig. 4: Details of *Conoteuthis agazizi* n. sp.: A) Close-up of the dorsal phragmocone surface to show anteriorly projecting growth lines that cross the lateral margins of the keel. B) Close-up of the mid-dorsal keel to show the slight elevation with a pair of ridges on the top (dashed lines = lateral margins of the keel; dotted lines = pair of slightly diverging ridges). C) Detail of the venter to show the peculiar fissure on the outer surface of the conotheca (white layer), which do not represent the longitudinal axis. D) Dlose-up of the dorsolateral phragmocone surface to show anteriorly projecting growth lines. E) Reconstruction of the growth lines indicates a pointed proostracum. F) D'Orbignys reconstructions of *Conoteuthis dupiniana* to show enormous similarities in proostracum shape with *Conoteuthis agazizi* n. sp. (reproduction of D'Orbigny 1845: pl. 32).

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A rare and unusual teudopseid coleoid from the Upper Cretaceous of Hâqel (Lebanon)

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Keywords: *Teudopsinia* n. gen. *haasi* n. sp., Hâqel, Lebanon, Teudopseina, gladius morphology, phylogeny.

Abstract

A new vampyropod coleoid from the late Cenomanian limestones of Hâqel. (north-west Lebanon) is described. *Teudopsinia* n. gen. *haasi* n. sp. preserves a complete gladius. Owing to a sharply pointed anterior gladius extremity, *Teudopsinia* n. gen. *haasi* n. sp. is classified as a representative of the Teudopseina. A morphological comparison with other teudopseid groups shows that lower level attributions are, however, problematic since the general gladius outline is unique. The most unusual character is the relative length of the hyperbolar zones. Whereas teudopseid families are normally typified by hyperbolar zones not longer than the half gladius length, those of *Teudopsinia* n. gen. *haasi* n. sp. occupy c. 55% of the total gladius length. This observation indicates that the comparatively narrow anterior median field of *Teudopsinia* n. gen. *haasi* n. sp. is not only reduced in width but also in length. A gladius with a strongly reduced median field is presumed for the (hypothetical) last common ancestor of the Octopoda and Cirroctopoda. A side branch of the Teudopsidae leading from early Jurassic genus *Teudopsis* to late Cretaceous *Teudopsinia* n. gen. *haasi* n. sp. is therefore considered to be a possible root-stock of the Octobrachia.

Introduction

The Teudopseina is a common group of gladius-bearing coleoids that is known to appear during the Early Jurassic (Toarcian) and become extinct after the Late Cretaceous (Maastrichtian). As can be judged from the current fossil record, teudopseids show the highest diversity, the highest abundance and the widest distribution of all Mesozoic groups (Fuchs, Keupp & Engeser 2003; Fuchs 2006a + b; Fuchs, Engeser & Keupp 2007; Fuchs, Klinghammer & Keupp 2007; Fuchs & Schultze 2008; Fuchs & Weis 2008, 2009a + b; Fuchs et al. in press). Their gladius can be easily identified by an anteriorly pointed gladius end. Soft parts preserved particularly in trachyteuthidids strongly suggest octobrachian affiliations of the Teudopseina (see Fuchs 2006a +b; Fuchs, Engeser & Keupp 2007). Haas (2002) first proposed the idea whereupon the Octopoda/Cirroctopoda clade originated within a teudopseid root-stock. Haas (2002) and later Bizikov (2004, 2008) considered the comparatively wide posterior median field as present in the teudopseid gladius to be the ideal pre-requisite to reduce the median field and to evolve a U-shaped gladius vestige similar to modern cirroctopods. Although Fuchs, Bracchi & Weis (2009) recently proposed arguments for a loligosepiid origin, the search for the direct ancestors of the Octobrachia has been mainly focused on the Teudopseina (see Fuchs 2009 for an overview of different ideas).

During morphological studies on trachyteuthidid gladii, the author has found a peculiar gladius from the Cenomanian of Hâqel that has been preliminarily determined as "*Glyphiteuthis* sp." (Fuchs 2006b). However, after careful comparisons, the specimen is different from *Glyphiteuthis* and without doubt represents a previously unknown genus and species. Since the gladius yielded a strongly reduced median field, it might moreover play a key role in the evolution of the Octobrachia.

It is the aim of the present work to describe the specimen and to discuss its phylogenetic position within the Teudopseina.

Materials & Methods

The examined specimen has been found in the palaeontological collection of the Museo Civico di Storia Naturale in Milano and comes from the sub-lithographical Limestones of Hâqel in north-west Lebanon (Fig. 1). Hâqel is about 45 km northeast of Beirut and 15 km east of the coastal city of Jbail. Latest studies confirmed a late Cenomanian age for Hâqel, owing to the presence of the ammonite *Allocrioceras* cf. *annulatum* (Wippich & Lehmann 2004). *Allocrioceras annulatum* is a member of the lower late Cenomanian *Sciponoceras gracile* Zone in the Western Interior of USA and the *Metoicoceras geslinianum* Zone of the international standard (for more geological details about Hâqel see Patterson 1967; Hückel 1970; Hemleben 1977; Philip et al. 1993).

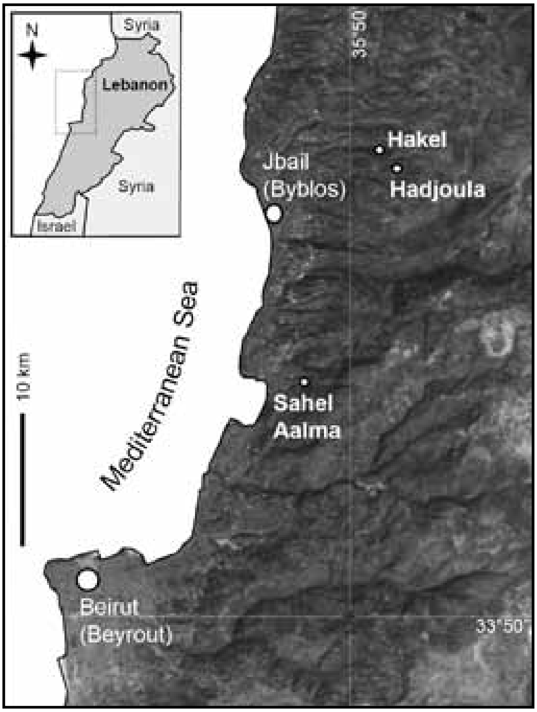

Fig. 1: Topographic map of north-western Lebanon showing Hâqel (adapted from Fuchs, Bracchi & Weis 2009).

The examined specimen shows a gladius, the sturdy but flexible chitinous structure within the dorsal mantle of many extinct and living coleoid groups. Terminology and measurements of the gladius used herein follow Fuchs (2006a), Fuchs, Engeser & Keupp (2007: 577, fig. 1) and Fuchs & Weis (2009, in press). Since the specimen preserves no soft parts, the systematic position is solely based on gladius characteristics

Institutional abbreviations: coll. Keupp, collection Keupp (Freie Universität Berlin); MNHN, Musée National D'Histoire Naturelle Paris; MNHNL, Musée National D'Histoire Naturelle Luxembourg; MSNM, Museo Civico di Storia Naturale di Milano; NMP, National Museum Prag; SMNS, Staatliches Museum für Naturkunde Stuttgart.

Systematic palaeontology

Subclass COLEOIDEA Bather, 1888
Superorder VAMPYROPODA Boletzky, 1992
Order OCTOBRACHIA Fioroni, 1981
Suborder TEUDOPSEINA Starobogatov, 1983

Assigned families: Teudopsidae Regteren Altena, 1949; Trachyteuthididae Naef ,1921; Palaeololiginidae Naef, 1921, and Muensterellidae Roger, 1952.

Family TEUDOPSIDAE Regteren Altena, 1949

Assigned genera: *Teudopsis* Eudes-Deslongchamps, 1835 and *Teudopsinia* n. gen.

Genus *Teudopsinia* n. gen.

Type species: *Teudopsinia haasi* n. sp.

Diagnosis: Anteriorly pointed gladius with a hyperbolar zone length that slightly exceeds the half gladius length.

Stratigraphical and geographical range: Cenomanian (late Cretaceous) of Lebanon.

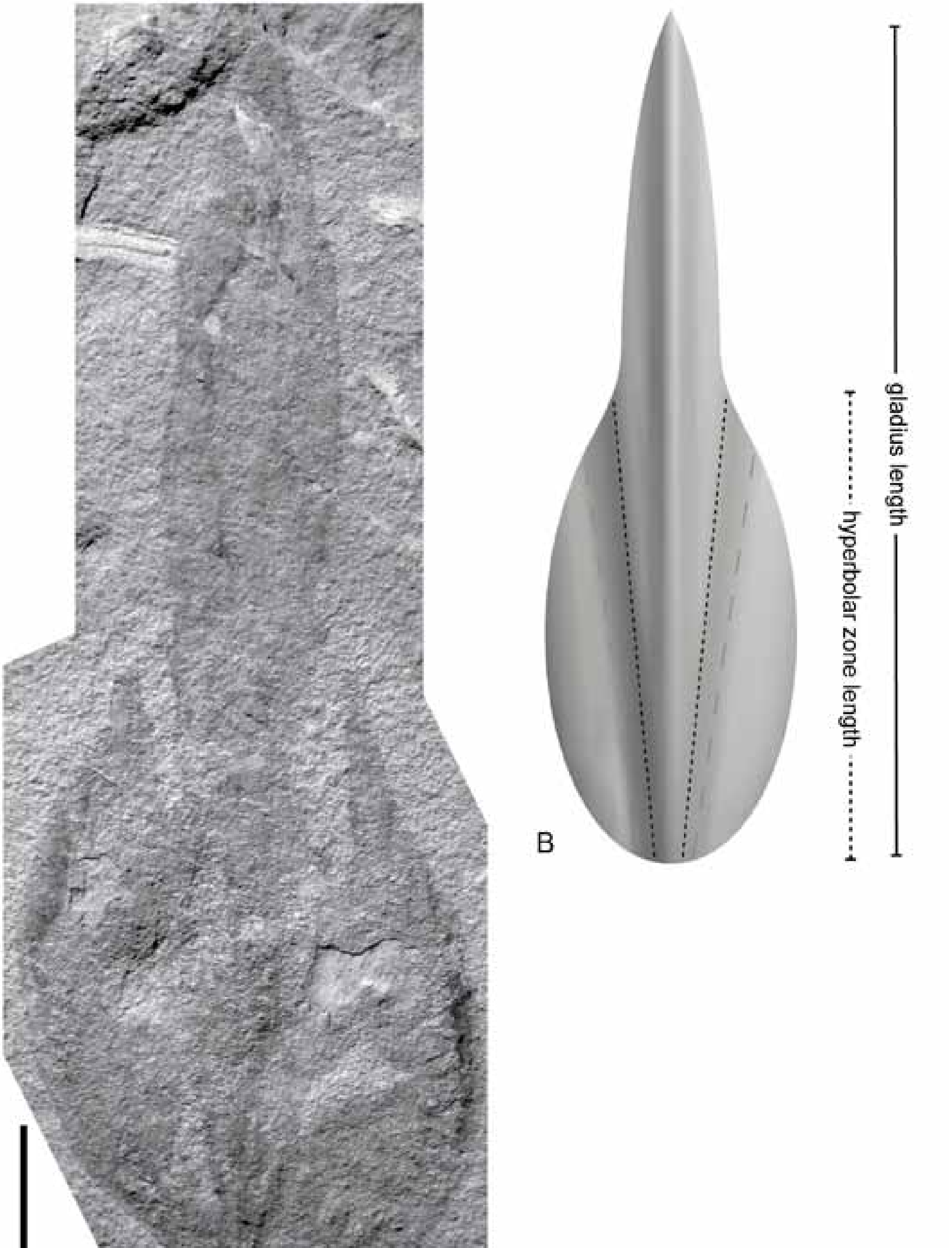

Fig. 2: *Teudopsinia haasi* n. sp. from the Upper Cenomanian of Hâqel, Lebanon. A) Holotype (MSNM i12627); scale bar = 10 mm. B) 3-dimensional gladius reconstruction (black dashed line = inner asymptote, grey dashed line = outer asymptote).

Teudopsinia haasi n. sp.

non v 1922 *Beloteuhis libanotica* n. sp. – Naef, p. 146, fig. 54b. (= *Palaeoctopus newboldi*)
? 1946 *Palaeololigo libanotica* (Naef). – Roger, p. 47, Fig. 11.
non 1966 *P. libanotica* (Naef, 1921). – Jeletzky, p. 45. (= *Palaeoctopus newboldi*)
? 1977 *Palaeololigo libanotica.* – Donovan, p. 37.
? 1984 *Palaeololigo libanotica* Naef. – Riou, p. 24.
? 1986 *Palaeololigo* n. sp. – Engeser & Reitner, p. 11.
non v 1986 *Parateudopsis libanotica* (Naef 1922) n. comb. – Engeser & Reitner, p. 10, text-fig. 3, pl. 2, fig. 3, 4, 5. (= *Palaeoctopus newboldi*)
? 1988 *Palaeololigo* n. sp. – Engeser, p. 76.
non v 1988 *Parateudopsis libanotica* (Naef, 1922). – Engeser, p. 89. (= *Palaeoctopus newboldi*)
v 2009 undescribed palaeololiginid – Fuchs, p. 185, Fig. 2M-N

Derivation of name: The name is dedicated to the late Winfried Haas, who first introduced the idea that the octobrachian gladius vestige evolved from a teudopseid gladius.

Holotype: MSNM i12627.

Type locality: Hâqel (northwestern Lebanon)

Type horizon: Lebanese Plattenkalks of Hâqel (*Metoicoceras geslinianum* Standard Zone; corresponds to *Sciponoceras gracile* Zone in Western Interior); early late Cenomanian.

Diagnosis: Anteriorly pointed gladius with a hyperbolar zone length that slightly exceeds the half gladius length; anterior median field comparatively slender, but not rachis-like.

Description: The holotype preserves a complete gladius in ventral aspect (Fig. 2A). The gladius has a total length of 76 mm and a maximum width of 27 mm. Since the gladius is dorsoventrally as well as laterally compressed, width measurements are problematic for morphometric comparisons. Apart from this, ruptures on both sides of the anterior hyperbolar zones strongly distort width indices. The original gladius must have been thus a bit more slender (Fig. 2B). The free margins of the anterior median field, which are almost straight (feebly curved towards outside), form a very low angle of only 16°. The anterior median field is therefore distinctly pointed. The posterior end, in contrast, is rounded. The length of the hyperbolar zones is 41 mm and occupies more than the half gladius length (approximately 55%). The inner asymptotes diverge at an angle of 12°. Growth increments are not visible. As can be judge from the general gladius outline, the hyperbolar zones seem to be comparatively wide and weakly curved; the transitions hyperbolar zones – lateral fields (i. e. the outer asymptotes) are therefore indistinct. In at least three places (mainly in the area of the hyperbolar zones and the lateral fields), the original gladius material is missing. In these spots, the dorsal gladius surface left imprints. The two larger imprints are smooth and do not show characteristic features such as e. g. granules. However, the tiny spot at the very posterior extremity exhibits imprints of two grooves with a ridge in between them. Although a median furrow that would clearly indicate a dorsal keel is not preserved, this latter observation let at least assume the presence of a median keel. Weak diverging structures next to the median field's midline support this assumption.

Comparisons: The pointed anterior gladius, the length of the hyperbolar zones and the presumed median keel are characters that legitimate placing *Teudopsinia haasi* n. sp. within the Teudopseina (Fig. 3-4). Affinities to the Loligosepiina and Prototeuthidina can be excluded owing to their triangular median field (Fuchs, Engeser & Keupp 2007; Fuchs & Weis 2008).

Although the gladius generally resembles those of most teudopseids, *Teudopsinia haasi* n. sp. is unique by its comparatively long hyperbolar zones (Fuchs 2006a; Fuchs, Engeser & Keupp 2007; Fuchs & Weis 2009, in press).

Teudopsinia haasi n. sp. differs from members of the Trachyteuthididae through its longer hyperbolar zones and its narrow median field (Fuchs, Engeser & Keupp 2007; Fig. 3A-B). Affinities of *Teudopsinia haasi* n. sp. with trachyteuthidids must be nevertheless considered in case new material of *Teudopsinia haasi* n. sp. yields evidence of dorsal granules.

The anterior median field is distinctly wider in *Teudopsinia haasi* n. sp. than in late Jurassic *Palaeololigo oblonga* (Engeser & Keupp 1999; Fuchs 2006a; Fig. 3C-D), where the anterior median field is clearly rachis-like. The relative width of the anterior median field is similar to co-existing *Rachiteuthis donovani* (Fuchs 2006b; Fig. 3E-F), but

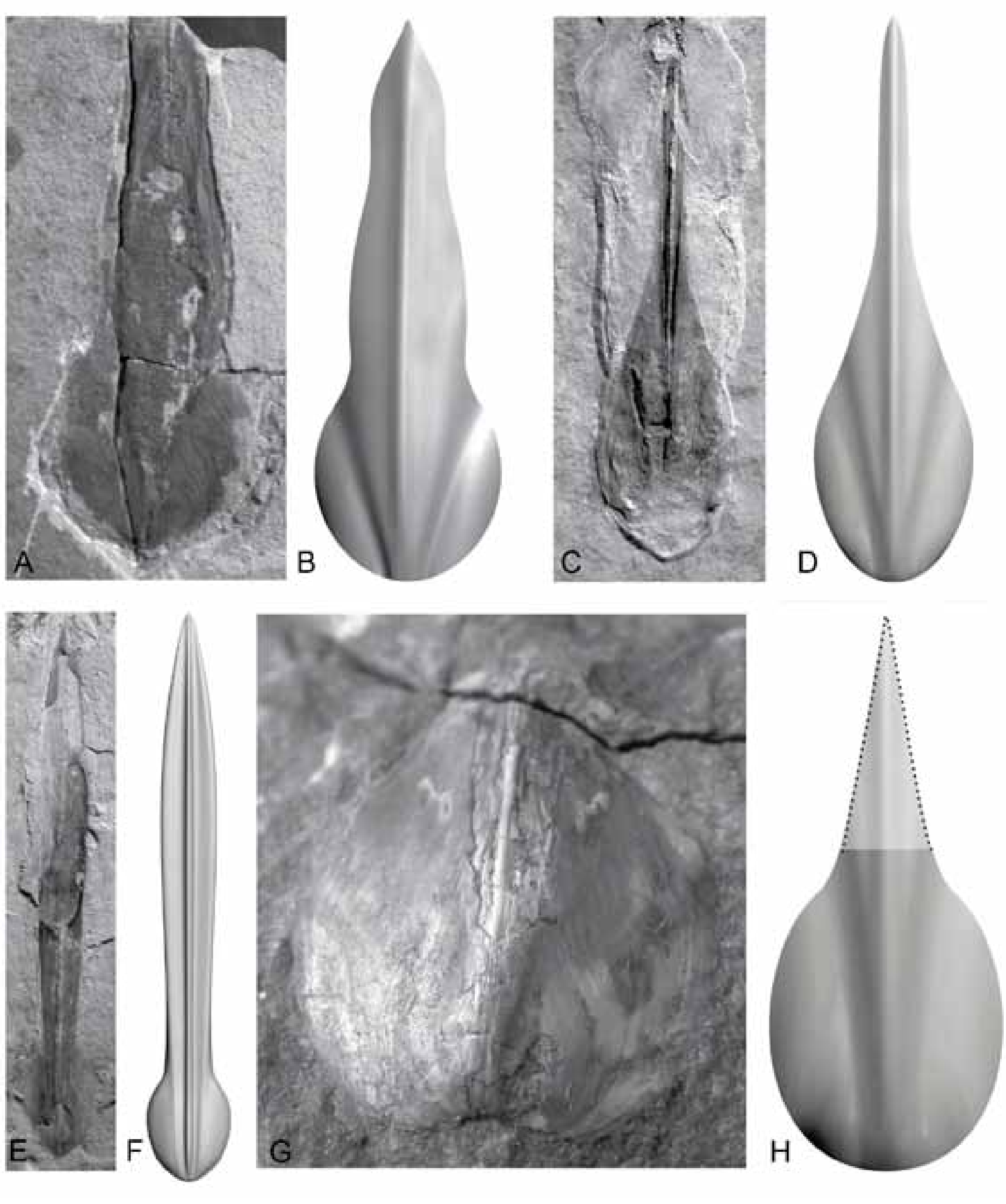

Fig. 3: A) *Glyphiteuthis libanotica* (Fraas, 1878) from the Upper Cenomanian of Hâqel, Lebanon (holotype, SMNS 26271). B) 3-dimensional gladius reconstruction of *Gl. libanotica*. C) *Palaeololigo oblonga* (Wagner, 1859) from the Lower Tithonian of Solnhofen, Germany (coll. Keupp MC-1). D) 3-dimensional gladius reconstruction of *P. oblonga*. E) *Rachiteuthis donovani* Fuchs, 2006 from the Upper Cenomanian of Hâqel, Lebanon (holotype, MSNM i25142). G) *Styloteuthis convexa* Fritsch, 1910 from the Turonian of Bohemia, Czech Republic (NMP O3221). H) 3-dimensional gladius reconstruction of *S. convexa*.

the "free rachis" is much longer in *Rachiteuthis donovani* than in *Teudopsinia haasi* n. sp. (i.e. the length of hyperbolar zones is much shorter in *Rachiteuthis donovani*). The overall gladius shape of *Teudopsinia haasi* n. sp. appears to be similar to poorly known *Styloteuthis convexa* from the Turonian of Bohemia (Fritsch 1910; Kostak 2002), but the posterior gladius is more rounded in *Styloteuthis convexa* (Fig. 3E-F) than in *Teudopsinia haasi* n. sp.

When compared to early Jurassic teudopsids (Fuchs & Weis in press), the gladius of *Teudopsinia haasi* n. sp. is surprisingly close to *Teudopsis bollensis* (Fig. 4A-B) and *Teudopsis bunelii* (Fig. 4C-D). *Teudopsinia haasi* n. sp. appears to be transitional between these two taxa. The three forms share a moderately reduced median field (Fig. 4). Compared to *Teudopsis bunelii* and *Teudopsis bollensis*, *Teudopsinia haasi* n. sp. exhibits longer hyperbolar zones.

Nomenclatural remarks: In order to prevent future mistakes, the taxonomic history of *Beloteuthis libanotica* Naef, 1922 needs to be clarified.

Beloteuthis Münster, 1843 is commonly regarded as a junior synonym of *Teudopsis* Eudes-Deslongchamps, 1835 (Engeser 1988; Riegraf, Janssen & Schmitt-Riegraf 1998; Fuchs & Weis in press).

Naef (1922: 146, Fig. 54b) introduced *Beloteuthis libanotica* n. sp. on the basis of a single laterally compressed gladius (Fig. 5A) from the Santonian Plattenkalks of Sâhel Aalma. Naef (1922) could not know, at that time, the bipartite gladius vestige of *Palaeoctopus newboldi* (Woodward, 1896), because it was first shown in Roger (1944: Fig. 1). As is visible in Fig. 5A-C, the gladius of *Beloteuhis libanotica* Naef, 1922 without doubt represents one half of an originally bipartite gladius vestige of *Palaeoctopus newboldi* (Woodward, 1896). *Beloteuthis libanotica* Naef, 1922 is therefore a junior subjective synonym of *Palaeoctopus newboldi* (Woodward, 1896).

Although he well presented the gladius vestige of *Palaeoctopus newboldi,* Roger (1946: p. 19, fig. 13) himself was not aware of Naef's misinterpretation

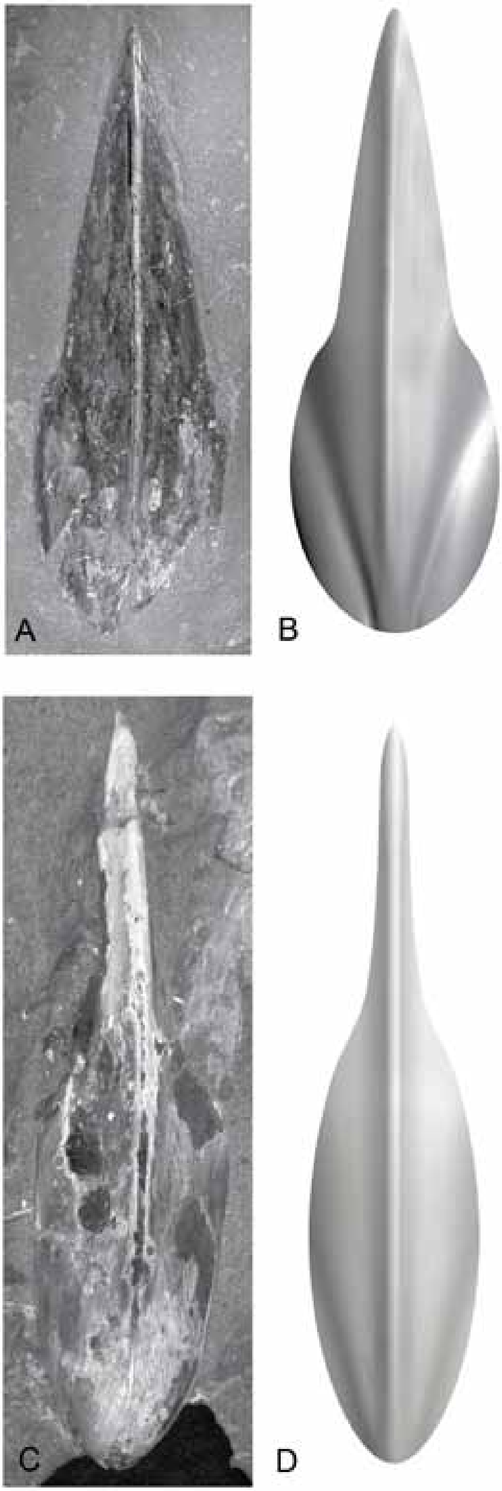

Fig. 4: A) *Teudopsis bollensis* Voltz, 1836 from the Toarcian of Germany (MNHN 2947). B) 3-dimensional gladius reconstruction of *T. bollensis*. C) *Teudopsis bunelii* Eudes-Deslongchamps, 1835 from the Toarcian of Luxembourg (MNHNL TV234). D) 3-dimensional gladius reconstruction of *T. bunelii*.

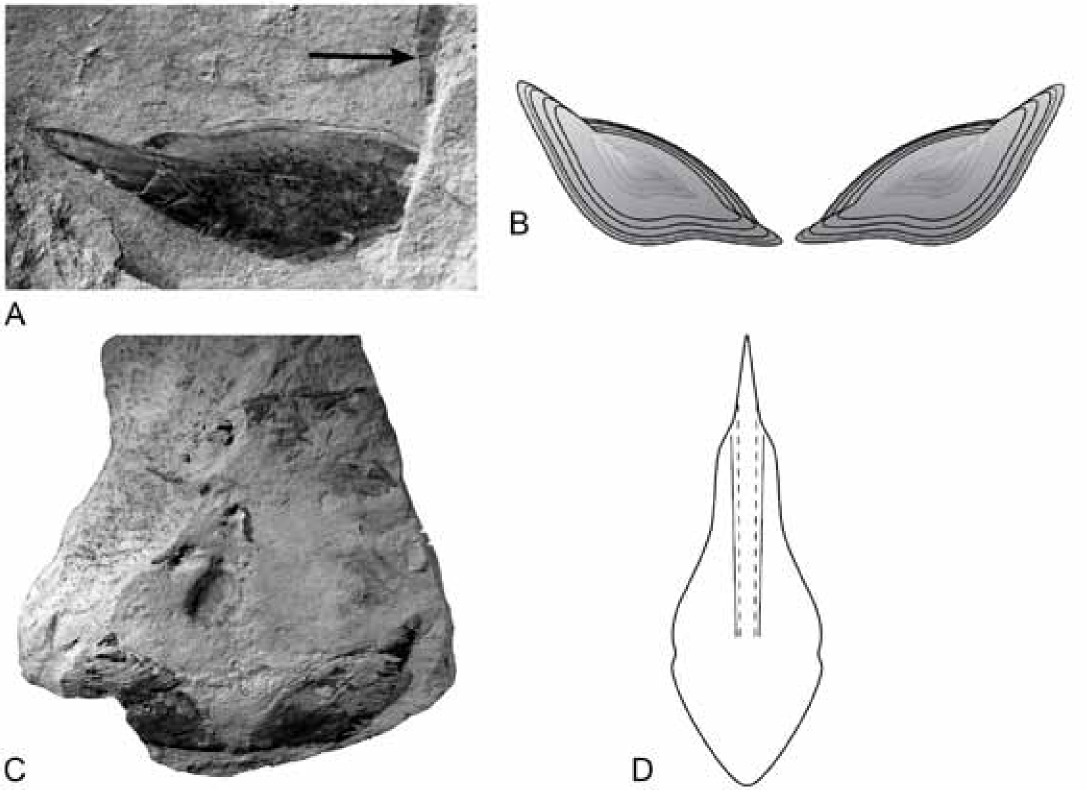

Fig. 5: A) Gladius vestige of *Palaeoctopus newboldi* (Woodward, 1896) from the Santonian of Sâhel Aalma, Lebanon (SMNS 26270); original of "*Beloteuthis libanotica* Naef, 1922" (arrow points at remains of the corresponding part). B) Reconstruction of *P. newboldi*. C) *Palaeoctopus newboldi* (original of Roger 1944, MNHN B18834a) showing the bipartite gladius vestige. D) Sketch of "*Palaeololigo (Beloteuthis) libanotica* (Naef, 1922)", adapted from Roger (1946: fig. 11).

and distinguished between *Palaeoctopus newboldi* (Woodward, 1896) and *Palaeololigo (Beloteuthis) libanotica* (Naef, 1922). On the basis of new specimens, Roger (1946) thought to provide additional information about *Palaeololigo (Beloteuthis) libanotica* (Naef, 1922), i.e. it was not his intention to erect a new species. Indeed, according to Rogers's re-description, the new gladii seem to differ from *Palaeoctopus newboldi* (Woodward, 1896). Unfortunately, Roger (1946: fig. 11) gave no photographs and only a vague sketch of the gladius. The sketch is reproduced in Fig. 5D. It shows a gladius with a peculiar outline that has never been confirmed in later publications. A re-study of Rogers's specimens is impossible since their repository is unknown. Both the description and the sketch are insufficient to compare Rogers's specimens with the holotype of *Teudopsinia haasi* n. sp., but it is worth to mention that some similarities exist.

Engeser & Reitner (1986) again overlooked that *Beloteuthis libanotica* Naef, 1922 is a junior subjective synonym of *Palaeoctopus newboldi* (Woodward, 1896) and transferred *Beloteuthis libanotica* Naef, 1922 to a new genus: *Parateudopsis* Engeser & Reitner, 1986. *Parateudopsis (Beloteuthis) libanotica* (Naef, 1922) is therefore a junior subjective synonym of *Palaeoctopus newboldi* (Woodward, 1896), too.

On the other side, Engeser & Reitner (1986) and Engeser (1988) correctly recognised that Roger's specimens assigned to *Palaeololigo (Beloteuthis) libanotica* (Naef, 1922) differ from *Parateudopsis (Beloteuthis) libanotica* (Naef, 1922) and considered *Palaeololigo (Beloteuthis) libanotica* (Naef, 1922) as *Palaeololigo* n. sp.

The taxonomic confusion concerning *Beloteuthis libanotica* Naef, 1922 is enhanced when considering

Glyphiteuthis libanotica (Fraas, 1878) (Fig. 3A-B), a well-know Cenomanian trachyteuthidid with a gladius outline that is quite similar to *Beloteuthis libanotica* Naef, 1922 (see Fuchs & Weis 2009).

To summarise, the author does not want to exclude the possibility that *Teudopsinia haasi* n. sp. might be conspecific with *Palaeololigo (Beloteuthis) libanotica* (Naef, 1922) *sensu* Roger (1946) and *Palaeololigo* n. sp. *sensu* Engeser & Reitner (1986) and Engeser (1988). However, since 1) a re-study of Roger's specimens is impossible, 2) *Beloteuthis libanotica* Naef, 1922 is a junior subjective synonym of *Palaeoctopus newboldi* (Woodward, 1896), and 3) Engeser & Reitner (1986) and Engeser (1988) did not propose a new name, there is no nomenclatural obstacle to erect the new taxon *Teudopsinia haasi* n. sp.

Phylogenetic implications

Belemnoid phragmoteuthidids with their three-lobed proostracum are commonly thought to represent the root-stock of Mesozoic gladius-bearing coleoids (Jeletzky 1966; Doyle, Donovan & Nixon 1994; Bizikov 2004; Donovan 2006; Fuchs 2006a). However, the direct ancestor of the Teudopseina is still uncertain (Fuchs & Weis in press). As a result, the search for an adequate out-group is somewhat problematic; polarisation of character states therefore mostly equivocal. Fuchs & Weis (in press) have found only a single unambiguous apomorphy for the Teudopseina: the presence of a pronounced median keel.

Despite of this phylogenetic difficulty, the systematic position of *Teudopsinia haasi* n. sp. within the Teudopseina is well-founded through the pointed anterior gladius end.

Compared to coeval trachyteuthidids, muensterellids or palaeololiginids, the described gladius features suggest that *Teudopsinia haasi* n. sp. belongs to an isolated lineage (Fig. 6). Significant differences prevent to place *Teudopsinia haasi* n. sp. into one of the latter groups. Late Cretaceous muensterellids (i.e. *Enchoteuthis*) as well as palaeololiginids (i.e. *Rachiteuthis*) show that the muensterellid/palaeololiginid clade developed a narrow and elongated free rachis (Fig. 6). In contrast, *Teudopsinia haasi* n. sp. pursues the opposite way, namely to shorten the anterior median field. The most important apomorphy of the trachyteuthidid clade, the presence of dorsal granules, seems to be absent in *Teudopsinia haasi* n. sp.

Phylogenetically, *Teudopsinia haasi* n. sp. seems to be closest to Toarcian *Teudopsis bunelii* or *Teudopsis bollensis.* The three gladii share a slender, but not rachis-like median field. The Teudopsidae has been long time thought to become extinct after the Early Jurassic, but it is quite possible that *Teudopsinia haasi* n. sp. (and possibly *Styloteuthis convexa* too) descended from teudopsids. In this scenario, *Teudopsinia haasi* n. sp. belongs to a comparatively old lineage within the Teudopsidae (Fig. 6). As an alternative scenario, Fuchs (2009) regarded the new gladius to be phylogenetically closer to *Palaeololigo*. However, there is no reasonable apomorphy for this sister-group relationship.

The most striking character that is responsible for the isolated position of *Teudopsinia haasi* n. sp. is the relative length of the hyperbolar zones. Teudopseids are actually typified by hyperbolar zones that are not longer than 50% of the total gladius length (Fuchs & Weis in press). The hyperbolar zones in *Teudopsinia haasi* n. sp., in contrast, clearly exceed the half gladius length (55%). The question arises whether the hyperbolar zones have been elongated or has the median field length been shortened. As noted above, there is a general trend towards more slender median fields within the Teudopseina (even within the Trachyteuthididae). This general reduction influences in a varying degree the anterior as well as the posterior median field while the relative length of the hyperbolar zone remains more or less constant (35-50%). This means that the median field reduction concerns exclusively the width. Hence, the median field of *Teudopsinia haasi* n. sp. seems to be reduced in both width and length.

This observation becomes important in the light of the origin of the Octopoda and the Cirroctopoda. Haas (2002) supposed that the wide gladius of the Trachyteuthididae is the initial source of a gradual reduction that leads to a U-shaped gladius vestige. Based on the fact that we could not find any evidence of a significant median field reduction, members of the Trachyteuthididae can be deleted from the list of possible ancestors of the Octobrachia (Fuchs 2009).

According to Fuchs (2009), the search should be rather focused on the families with a clearly reduced median field: the Muensterellidae

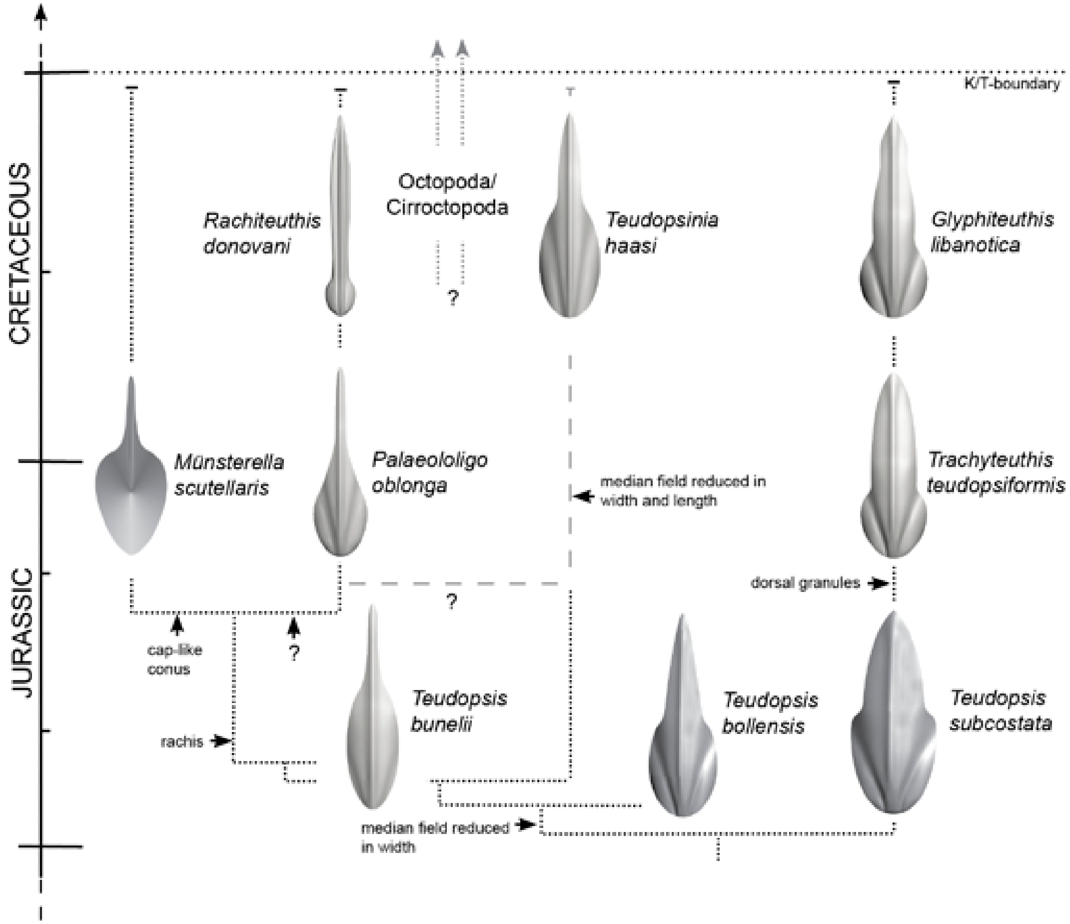

Fig. 6: Proposed phylogenetic tree of the Teudopseina showing the presumed position of *Teudopsinia haasi* n. sp.

and the Palaeololiginidae. He considered the Palaeololiginidae because he preliminarily included an "undescribed palaeololiginid" in his argumentation. Since this specimen - here described as *Teudopsinia haasi* n. sp. - belongs more likely to the teudopsid lineage, palaeololiginids can be also refused to be the precursors of the Octobrachia. Instead, *Teudopsinia haasi* n. sp. provides evidence that a side branch of the Teudopsidae with a distinctly reduced median field gave rise for the octobrachians.

Since soft parts are unfortunately not preserved, it remains unknown if the anterior gladius tip of *Teudopsinia haasi* n. sp. has reached the anterior mantle margin (a fundamental character that would definitely proof a significant median field reduction). Future records of *Teudopsinia haasi* n. sp. with preserved soft parts might therefore throw new light on this problem.

Conclusions

The length of the hyperbolar zones that occupy more than 50% of the total gladius length is the key character that separates *Teudopsinia haasi* n. sp. from other teudopseids. Owing to this unusual gladius shape, *Teudopsinia haasi* n. sp. considerably increases the morphological variability within the Teudopseina and therefore throws new light on their evolutionary pathways. It is likely that *Teudopsinia haasi* n. sp. originated from teudopsids with a comparatively slender gladius similar to Toarcian *Teudopsis bunelii* or *Teudopsis bollensis*. Accordingly, *Teudopsinia haasi* n. sp. belongs to an old lineage. In contrast to other teudopseids, the median field in *Teudopsinia haasi* n. sp. is reduced not only in width but also in length. This conspicuous gladius pattern turns the Teudopsidae into a potential stem-group of the Octobrachia.

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A new *Plesioteuthis* with beak from the Kimmeridgian of Nusplingen (Germany)

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Abstract

A new specimen of the common coleoid *Plesioteuthis prisca* was discovered in the Nusplingen Lithographic Limestone (Late Jurassic, Late Kimmeridgian, *Beckeri* Zone, *Ulmense* Subzone; SW Germany). Although heavily deformed remains of the beaks as well as isolated beaks which had been tentatively assigned to this taxon have already been described, this specimen is the first which allows linking a specific form of upper beak to this coleoid species which was largely based on the gladius. As documented for other coleoids from Nusplingen before, this specimen most likely represents remains of a specimen some predator or scavenger had fed on.

Introduction

For the knowledge of coleoid palaeobiology, southern German Jurassic Fossillagerstätten such as the Holzmaden region, Dotternhausen, the Solnhofen/ Eichstätt region, and Nusplingen play a central role. These localities yielded fossils preserving non-mineralised body parts either in phosphate or carbon. Concerning the Late Jurassic, the small quarry of Nusplingen produced a number of valuable cephalopod specimens which display previously poorly known or unknown structures (e.g. Dietl & Schweigert 1999; Schweigert & Dietl 1999, 2001, 2008, in press; Schweigert 1999; Klug et al. 2005, in press). Nusplingen is of special importance, because at this locality (Fig. 1), cephalopod fossils are often preserved with carbonised remains (Klug et al. 2005) of the unmineralised parts of the beaks such as the upper beaks of ammonoids, coleoid beaks and the non-calcitic lamellae of nautilids (same references as above). In the other Fossillagerstätten mentioned above, at least the coleoid mandibles sometimes are preserved, but usually only as dark spots (cf. Keupp 2006) rather than with fine morphological details.

Klug et al. (2005) described and reconstructed three different types of upper beaks of coleoids (p. 178, 179, figs. 8, 9, 12: "forms 5-7 "). These three types were assigned to *Trachyteuthis* based on two specimens where gladii were associated with this form (6), to *Leptotheuthis* because of the large size of form (7) and to *Plesioteuthis*, inferred from the abundance of both isolated gladii and isolated beaks of form (5).

In this article, we shortly describe the first specimen of *Plesioteuthis prisca* preserved with the upper beak *in situ*, we discuss its taphonomy and reconstruct its morphology.

Material

The only specimen described here is stored in the Staatliches Museum für Naturkunde in Stuttgart. Slab and counterslab carry the number SMNS 67543 and were excavated in the Nusplingen quarry by the excavation team of the Staatliches Museum für Naturkunde in Stuttgart in 2008. It was discovered 0-10 cm from the top of layer D, *Beckeri* Zone, *Ulmense* Subzone, Late Jurassic, Late Kimmeridgian (for section see Dietl et al. 1998).

Description of the new *Plesioteuthis*

The slab is 277 mm long and maximally 200 mm high (Fig. 2E). 98 mm of the crushed, phosphatised gladius is preserved. 90 mm anterior to it, the flattened and carbonised upper beak is situated (Fig. 2A, E). These two hard parts are surrounded by a grey shade which is 186 mm long and 132 mm high. The majority of its surface is light grey while an elongate darker field is visible in its centre. This darker field that measures about 100 x 20 mm is arranged roughly parallel to the presumed venter of the gladius, about 20 mm below it. Near the anterior end of the darker field, an irregular spot of a diameter of 10 mm is visible, which represents the darkest part of the darker field (for an

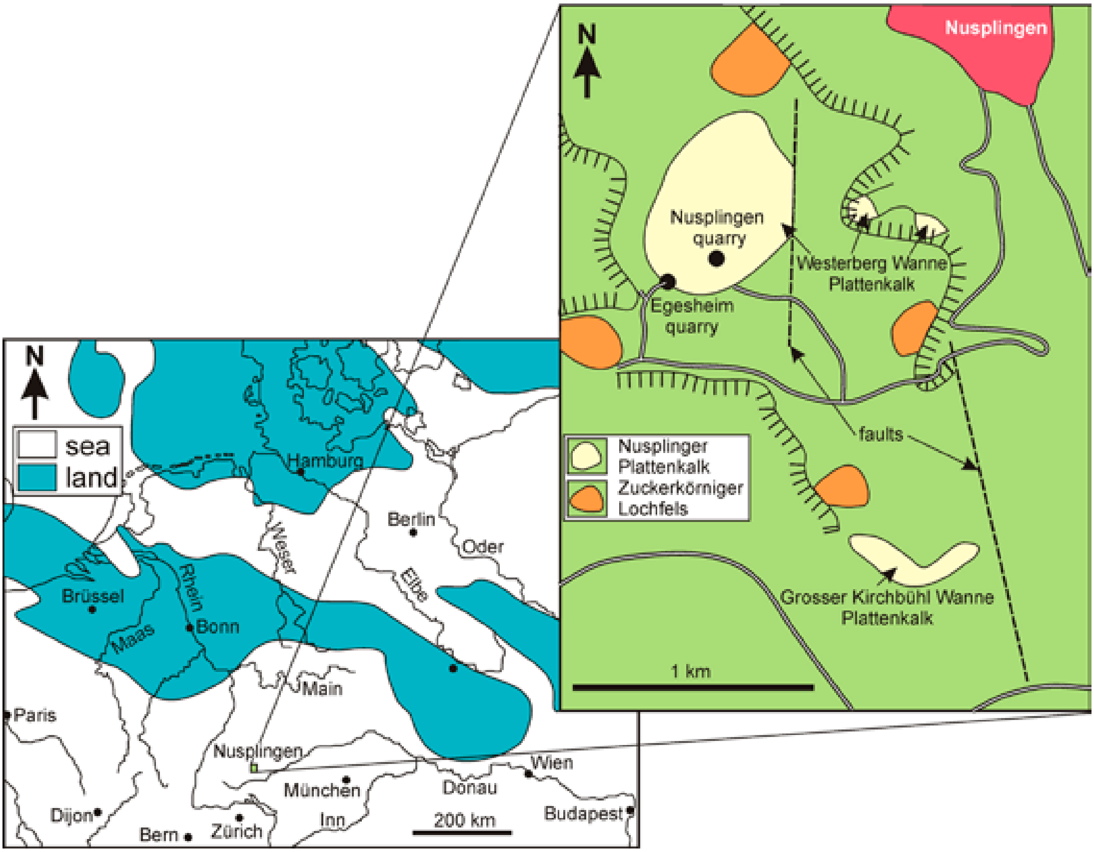

Fig. 1: Map with the location of the Nusplingen and Egesheim quarries, SW Germany, showing the distribution of land and sea during the Late Kimmeridgian (from Klug et al. 2005).

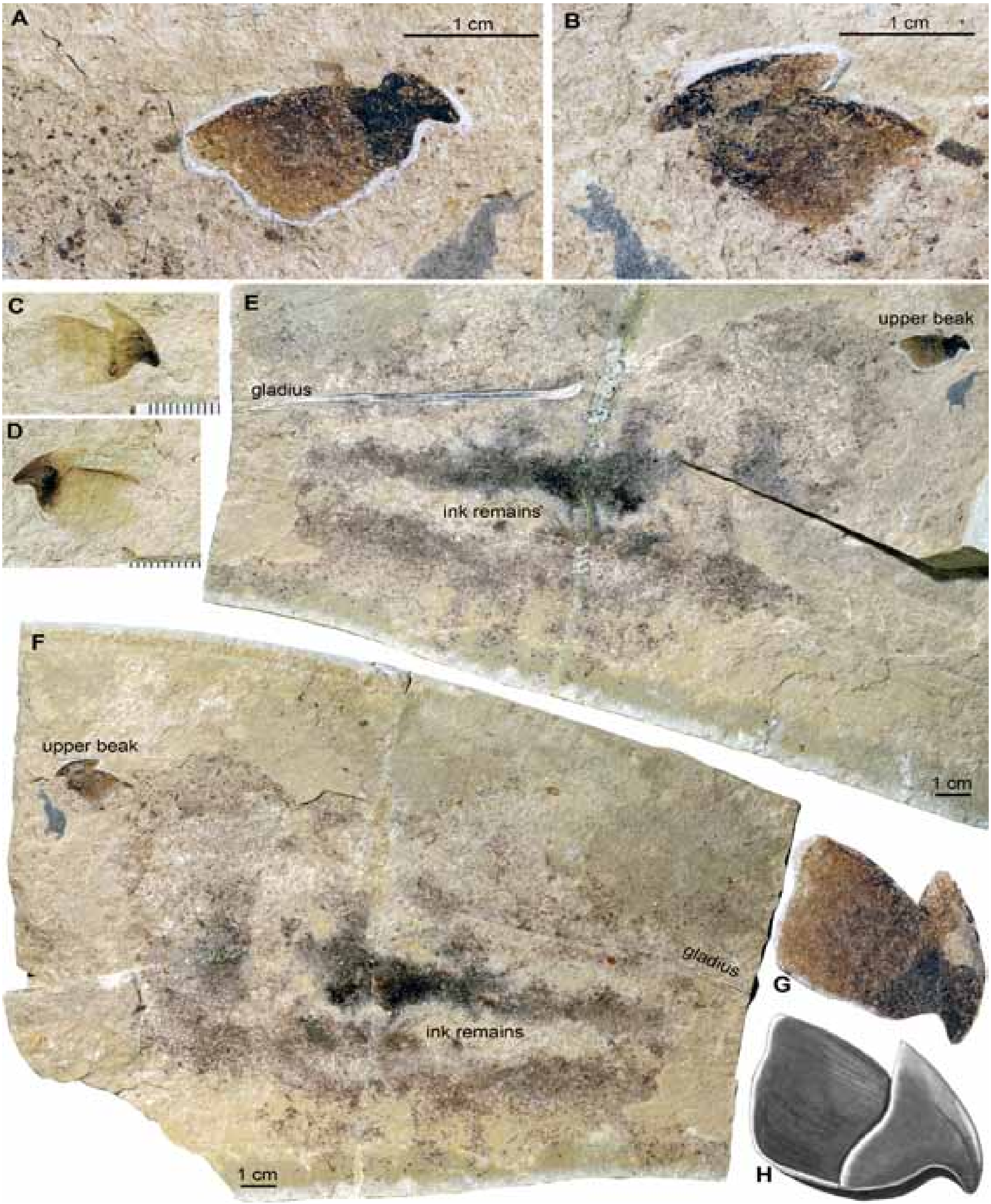

Fig. 2: *Plesioteuthis prisca*, Nusplingen, *Beckeri* Zone, *Ulmense* Subzone, Late Jurassic, Late Kimmeridgian. A-B, E-G) SMNS 67543; C-D) SMNS 64603. A) Upper beak of the plate, note the more distinct coloration of inner and outer lamella and the missing part of the hood. B) Upper beak of the counterplate. C-D) Upper beak, modified after Klug et al. (2005: fig. 8A, B). E) Plate, note the distribution of ink and the position of the gladius in relation to the upper beak. F) Counterplate. G) Superimposed images of A and B, B below A, and A displayed at 60 % opacity to show the complete outline; same scale as in A and B. H) Reconstruction of the upper beak of *Plesioteuthis*, based on Klug et al. (2005: fig. 12B); proportions and shape adapted to the new specimen; scale as in A and B.

explanation see the taphonomy chapter). The upper beak is 21.6 mm long (Fig. 2A), the inner lamella is 11.7 mm high and the maximum length of the wing of the outer lamella is 10.9 mm high (terminology according to Tanabe, Hikida & Iba 2006). The latter is clearly separated from the inner lamella by its darker colour. The hood of the outer lamella is slightly damaged. The rostrum is pointed to rounded with its ventral edge forming nearly a right (jaw) angle to the shoulder. The crest of the inner lamella is clearly vaulted and the coloration fades out from the crest both ventrally and posteriorly.

The counterplate measures 228 x 180 mm (Fig. 2F). Most of the gladius is preserved as external mould and only minor remains of the phosphatic gladius matter (90 mm long) still sticks to the counterplate. In most aspects, the counterplate displays the same pattern, only the beak shows some differences in preservation (Fig. 2B). Its outer lamella, especially the hood, is complete, but most of the carbon is on the other plate. Therefore, the beak on the counterplate has a lighter colour. The preservation of the inner lamella is worse. Thus, the beak remains are at 19 mm length 1.6 mm shorter than on the other plate. By contrast, wing and hood are more complete, displaying a length of 12.7 mm.

Beak morphology

Since the upper beak differs in outline and preservation on slab and counterslab, we combined images of the beaks from both sides in Adobe Photoshop CS3 (Fig. 2G). The specimen from the counterplate was put under that of the plate. To make the lower image partially visible, the layer containing the image of the beak from the plate was set to 60% opacity. Its outline was compared to the reconstruction of Klug et al. (2005, fig. 12B) and revealed some minor differences. By means of the "Liquify "-filter, we adapted this earlier reconstruction to the shape of the new specimen (Fig. 2H). Accordingly, the rostrum became longer, the crest less vaulted, the hood less pointed and lower.

Taphonomy

Coleoid remains are often incomplete in Nusplingen and often show traces of predation and/or scavenging (Schweigert 1999; Schweigert & Dietl in press; Klug et al. in press). The spatial proximity of gladius and beak combined with the dark matter which presumably represents ink remains (hence the dark colour; mantle remains are often in a greyish colour due to phosphatisation) and the incompleteness of the specimen indicate that probably all these parts belonged to one specimen which was mutilated prior to embedding by a scavenger and/ or a predator. Based on its overall morphology which resembles modern pelagic squid, it appears likely that *Plesioteuthis* was a nektonic predator. Apparently, it was killed by another predator which destroyed the soft-parts partially. The absence of the arm crown cannot be interpreted with certainty because it might have been preserved but on the adjacent plate which was not found in the field.

Phylogeny

Coleoid phylogeny is still poorly understood which can partially explained by the fact that well-preserved coleoid fossils are largely restricted to some Fossillagerstätten which yield such specimens in exceptional preservation. Although these rather complete coleoids from the Fossillagerstätten mentioned in the introduction have been known since over 150 years (Quenstedt 1852), not much was known about the beak morphology until recently (Klug et al. 2005). The new information about beak morphology is also a valuable contribution to the discussion of the systematic assignment of these fossil coleoids. The low jaw angle, the large outer lamella, the long hood, and the ratio between outer lamella size and inner lamella size resembles the upper beaks of decabrachian cephalopods rather than that of octobrachian cephalopods. This somewhat contradicts the phylogeny proposed by Fuchs (2006: figs 3.6-13, 4.1-1) who placed *Plesioteuthis* in the Prototeuthina which are supposedly ancestral to the octobrachian Vampyropoda. An early stem-group position of *Plesioteuthis* is, however, corroborated by the short octobrachian-like rostrum, which is often longer in Recent Decabrachia. Nevertheless, the new information on the beak morphology of *Plesioteuthis* represents only one additional character in the still incomplete character matrix, and thus, further information is needed to test the existing phylogenetic models like the one presented by Fuchs (2006).

Conclusions

A new specimen of *Plesioteuthis prisca* is described from the Kimmeridgian of Nusplingen (SW Germany). In this specimen, the fairly well-preserved beak-remains are longitudinally aligned with the gladius and surrounded by a dark field which we interpret as ink remains. This arrangement suggests that the listed parts belong to one specimen, thus confirming the previously tentative assignment by Klug et al. (2005) of this type of upper beak to *Plesioteuthis prisca*. The fairly good and complete preservation of the upper beak allows a correction of the reconstruction (Fig. 2H) published by Klug et al. (2005: fig 12B). According to our new reconstruction, an early stem-group position within the Coeloidea appears likely but its resemblance to the beaks of Recent Decabrachia suggests a closer relation to this group. Additionally, the incompleteness of the specimen in combination with the arrangement of preserved hard parts and the ink suggest exposure to the activities of scavengers and/ or predators.

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Fossil Coleoids from the Late Cretaceous (Campanian & Maastrichtian) of the Western Interior

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Abstract

Coleoid cephalopods have been reported from the Campanian and Maastrichtian of the Western Interior for more than 150 years, yet they remain poorly described, scantily represented, and rarely published. Meek & Hayden (1856) described *Belemnitella bulbosa* from the Fox Hills group of the Upper Missouri Cretaceous series (upper Maastrichtian). Jeletzky (1966) figured a single specimen; Kennedy et al. (1998), Stoffer et al. (2001) & Stoffer (2003) examined several specimens and compared *B. bulbosa* to *B americana.* This paper revisits *Belemnitella bulbosa* and describes many previously undescribed features of the phragmocone.

Actinosepia canadensis was reported from the upper Campanian *Baculites compressus* through *B. reesidei* Zones to the upper Maastrichtian *Jeletzkytes nebrascensis* Zone sediments throughout the northern portions of the Western Interior (Whiteaves 1897; Landes 1940; Waage 1965). *A. canadensis* is found to be restricted to the upper Campanian (and possibly the lower Maastrichtian) of the Western Interior while *A. landmani* n. sp. and *A. mapesi* n. sp. are described from the upper Maastrichtian.

Tusoteuthis longa Logan, 1898 was described from the Santonian or lower Campanian Smoky Hill Chalk, Niobrara Formation of western Kansas. Miller & Walker (1968) described *Enchoteuthis melanae* from the lower Campanian, Smoky Hill Member of the Niobrara Formation. *T. longa* was also reported from the lower middle Campanian Pierre Shale (Nicholls & Isaak 1987). The types along with new specimens of *T. longa* and *E. melanae* are re-examined; *Tusoteuthis cobbani* n. sp. is described from the upper Campanian Pierre Shale; and new family, Enchoteuthidae, is erected for these unique coleoids.

Meek & Hayden (1860) described *Phylloteuthis subovatus* from the Fox Hills group. *P. subovatus* is examined and confirms what Green (1977) suspected, *Phylloteuthis* is nothing more than a leaf.

Introduction

Coleoids from the Western Interior are rare and scantily represented in literature and collections. Although they have been known and reported on for more than 150 years, predation, poor preservation, diagenesis and lack of recognition have resulted in few being found and described. Most coleoid species have been named from the Niobrara Formation or Niobrara Chalk of west-central Kansas (Coniacian through lower Campanian) yet they occur throughout the Pierre Shale, Bear Paw Shale and Fox Hills Formation. This paper looks at many collections of Campanian and Maastrichtian coleoids from the Western Interior and re-examines their taxonomy and morphology.

While the rostrum of *Belemnitella bulbosa* Meek & Hayden, 1856 has been described and illustrated (Meek 1876; Kennedy et al. 1998; Stoffer et al. 2001; Stoffer 2003), its phragmocone has not been completely described or adequately illustrated. A large number of *B. bulbosa* specimens from the Fox Hills Formation in north-central South Dakota are examined.

The holotype of *Actinosepia canadensis* Whiteaves, 1897 (from the mid-upper Campanian) has proven to be a problem. Waage (1965) revisited *A. canadensis* and came very close to naming a distinct species from the upper Maastrichtian of the Western Interior. This paper revisits Waage (1965), looks at many other specimens and discovers additional characters and describes *A. landmani* n. sp. and *A. mapesi* n. sp. from the upper Maastrichtian.

Tusoteuthis longa Logan, 1898 has long been at the center of discussion and comparison for the description of Western Interior coleoids (Miller 1957; Miller & Walker 1968; Green 1977; Nicholls & Isaak 1987). In preparation for this paper, the type of *T. longa* was examined, measured and photographed. This study revisits those publications and introduces new specimens from the lower middle Campanian of Wyoming. Larson et al. (1997) illustrated a specimen attributed to *Tusoteuthis longa* from the *Baculites compressus* Zone (upper Campanian) of Pierre Shale in South Dakota. This paper looks at that specimen and names a new species. The shape of the vanes, median field, lateral fields and conus were compared with dozens of other coleoid specimens including *Enchoteuthis melanae* Miller & Walker, 1968. Based on the anterior shoulder shape (Toll 1998), there are at least two genera of Enchoteuthidids in the Campanian of North America, *Tusoteuthis* and *Enchoteuthis*. This Western Interior coleoid group is further analyzed and Enchoteuthididae n. fam. is erected.

Previous studies

Belemnitella bulbosa has been poorly illustrated over the last 150 years. Meek & Hayden (1856) briefly described *B. bulbosa* from specimens found near the Moreau Trading Post in what is now north-central South Dakota from, what they called formation No. 5. Meek (1876: 504, Pl. 33, Figs 2a-e) would later illustrate three specimens of *B. bulbosa* and thoroughly describe the guard but only partially describe the phragmocone. Meek would also clarify formation No. 5 as being the Fox Hills Formation. Since Meek's description, B. *bulbosa* has remained incompletely described and poorly illustrated. Jeletzky (1966: 48, Fig 1a-e) illustrated a small fragment of a *B. bulbosa* phragmocone. Larson et al. (1997: 94) partially described and illustrated a specimen of *B. bulbosa* consisting of mostly a large phragmocone. Jeletzky (1966: 48, Pl.1, Fig. 1 A-E) illustrated a small section of *B. bulbosa* from the Trail City Member (UNAZ) that showed its proostracal growth lines on the guard (something not visible in any of the specimens from the BHI collection). Waage (1968: 139) reported finding belemnites throughout the formation. Kennedy et al. (1998: 760-764, Fig 9a-c) reported on *Belemnitella* cf. *B. bulbosa* from the *Baculites baculus* and *B. clinolobatus* Zones (lower Maastrichtian) and from the *J. nebrascensis* Zone in the Elk Butte Member, Pierre Shale in Nebraska and South Dakota. Stoffer et al. (2001: 16-19, Tab 1, Fig 11 & 12) and Stoffer (2003: 34, Fig 43) illustrated *B. bulbosa* rostrums and included strontium isotope geochronology from the Badlands of South Dakota comparing *B. bulbosa* to *B. americana*.

Actinosepia canadensis Whiteaves, 1897 was described from several specimens found from the Bearpaw Shale in southern Saskatchewan. Landes (1940) redescribed *A. canadensis* based on a nearly complete impression of *Actinosepia* along with of other specimens from the same zone but from different locations than the holotype. Waage (1965) examined both Landes and Whiteaves *Actinosepia* including a specimen collected by Jensen & Varnes (1964) and 18 specimens from the Fox Hills Formation (upper Maastrichtian) of South Dakota. Waage (1965) redescribed the species and came close to naming a new species for the upper Maastrichtian *Actinosepia* but did not. The affinity of *Actinosepia* caused much debate in the Paleontological world, some believed it to be part of an ancient cuttlefish (Doyle et al. 1994) others were not so sure and claimed it to be merely the gladius of a Trachyteuthidid (Hewitt & Jagt 1999).

Logan (1898) described *Tusoteuthis longa* from the Niobrara Chalk (or Niobrara Formation) of western Kansas and was the first 'large' squid discovered in the Western Interior. *Tusoteuthis* was further described, separated and sometimes consolidated by later authors (Miller 1957, 1968; Miller & Walker 1968; Green 1977; Stewart 1977; Nicholls & Isaak 1987). The holotype of *Tusoteuthis longa* was collected from the *Hesperornis* beds of the Niobrara Chalk. Carpenter (2003) placed these beds in the lower Campanian. *Tusoteuthis longa* was reported by Nicholls & Isaak (1987) as occurring in the Pembina Member of the Pierre Shale (lower, middle Campanian) in southern Manitoba.

Meek & Hayden (1860) and Meek (1876) published on what they believed was a second coleoid, *Phylloteuthis subovatus*, from the Fox Hills Formation along the Moreau River. Meek (1876: Pl. 33, Fig. 3) illustrated his 'gladius' of *Phylloteuthis*. Zittel (1885), Fischer (1887), Boyle (1893) and Naef (1922) accepted that diagnosis and continued to publish *P. subovatus* as a valid fossil squid. Miller (1957, 1968), Miller & Walker (1968) and Green (1974) did as well, citing the differences between *Phylloteuthis* and coleoids from the Niobrara Formation. Whiteaves (1897), Landes (1940) and Waage (1965) ignored it even though Meek's illustration looked like a portion of an *Actinosepia*. However, Green (1977, following personal communication with G. Jeletzky), wrote that Meek's 'squid" was nothing more than the impression or remains of a fossil leaf.

Methods

Fossils were prepared with pneumatic tools. Micro-jacks were used to remove the rock from the fossil when needed. The marks created by the tool were than removed with an air-abrasive system using ground powders of Aluminum oxide, calcium carbonate and sodium bicarbonate. Most specimens were coated with PVA (polyvinyl acetate) as a protective coating that can be removed with either acetone or air-abrasive methods. All fossils photographed by the author utilized a Nikon D-300 with different lenses.

Comparative measurements were made on figured *Actinosepia*. When the posterior end was incomplete or missing, a measurement was taken from between the first two ribs on either side of the median keel and a calculation was made to determine how much was missing from the apex, so that rib angle measurements could be made. A 360º protractor with an 8 cm radius was used for rib angles with the center of the protractor as close to the apex of the gladius as could be calculated. The terminology for the gladius of *Actinosepia* follows Naef (1922) and Fuchs, Engeser & Keupp (2007).

In Enchoteuthididae n. fam., all that is preserved is a calcareous spear-shaped gladius consisting of a long free rachis that extends into the dorsal midline of the mantle and broad vanes (Green 1977). Toll (1998) published on the morphology of modern teuthid gladii, explaining their terminology, parts, differences and measurements. The terminology for the gladius of enchoteuthoids attempted to follow Toll (1998) and Fuchs, Engeser & Keupp (2007) but the definitions were inadequate to fully describe this unique gladius. Vanes include the large, posterior portion of the gladius that contains the conus, lateral and median fields and the non-free rachis. There is no term for the combined terminology of vanes, non-free rachis, median field, lateral fields and conus, except the posterior expansion of the gladius (Nicholls & Isaak 1987: 729). Since these animals' gladii resemble a shovel, spear, spoon or spatula the descriptive term 'blade' is used in this paper to include all parts of the posterior expansion of the gladius behind the free rachis.

The enchoteuthoid gladius is a fascinating structure composed of many different characters. According to Toll (1998), the characters used to separate genera and families include size, complexity, length, and position of the rachis; the number and position of the vanes; the relative width, length and shape of the vanes and the rachis; and the presence and type of conus. Naef (1922), Bandel & Boletzky (1988: 233) and Fuchs, Engeser and Keupp (2007) illustrated several different types of gladii shapes known in most Mesozoic coleoids.

Shell architecture, muscular fin attachments and ontogenetic development in fossil coleoids were discussed by Bandel & Boletzky (1988) but these subjects are not presented in this manuscript. Complete classification of some of these coleoids remains unknown or speculative. The author tried to remain as open as possible and when necessary compared the fossil and extant record.

Geological setting

The Campanian and Maastrichtian Late Cretaceous marine sediments of the Western Interior are primarily deposited within the Pierre Shale of Colorado, Kansas, Nebraska, North Dakota, South Dakota, eastern Montana, eastern

Wyoming, and Manitoba; the Bearpaw Shale in central and northern Montana, southern Alberta, and southern Saskatchewan; and the Fox Hills Formation within south central North Dakota, north central South Dakota, eastern Montana and eastern Wyoming. The Coniacian - lower Campanian Niobrara Chalk (Formation) is deposited in eastern Colorado, western Kansas, and around the Black Hills in eastern Wyoming and western South Dakota.

Several authors have published on the biostratigraphic zones of the Smoky Hill Chalk (Niobrara Formation) and Pierre Shale, most notably Gill & Cobban (1966), Miller (1968), Gill et al. (1972), Hattin (1982), Stewart (1990), Carpenter (1996, 2003), Larson et al. (1997) and Cobban et al. (2006). Some have published on the zones from the Fox Hills again most notably Waage (1968), Landman & Waage (1993), Kennedy et al. (1998) and Cobban et al. (2006). There are currently 28 ammonite Range Zones (biozones) and 13 inoceramid Interval Zones named within the Campanian and Maastrichtian marine rocks of the Western Interior (Cobban et al. 2006).

The Pierre Shale is composed of 300m to 3000m of mostly dark grey to black, fine-grained, homogenous shales sometimes separated by silty, sandy, bentonitic or calcareous units (Larson et al. 1997). There are more than 20 described members depending on which state, region or types of sediments that are deposited and 26 defined ammonite zones, within this formation, that help unravel its history and stratigraphy. Most of the fossil squids (gladii) found within the Pierre Shale have been collected from the Sharon Springs and Mitten Members (lower, middle Campanian). The gladii are usually associated with abundant, vertebrate remains.

While the biostratigraphy of the Pierre Shale has been well published (see above), the invertebrate biostratigraphy of the Smoky Hill Chalk has not. Correlation of the two formations is difficult because different fauna is often preserved in each formation since one is a chalk (which preserves calcite) and the other is shale (which preserves aragonite). Both formations preserve bone as well as some calcareous material.

The Fox Hills Formation conformably overlies the Pierre Shale and is a shallow water, regressional deposition of the Pierre Seaway (Kennedy et al. 1998). There are two ammonite zones in this formation: the *Jeletzkytes nebrascensis* Zone in the upper, Timber Lake Member and the *Hoploscaphites nicolletii* Zone in the lower, Trail City Member. According to Waage (1968) this *Hoploscaphites nicolletii* Zone was divided into four subzones. These subzones from top to bottom are: POAZ - *Protocardia-Oxytoma* assemblage subzone (non ammonite): UNAZ - Upper *Hoploscaphites nicolletii* assemblage subzone; LGAZ - *Limopsis-Gerevilia* assemblage subzone (non ammonite) and the LNAZ - Lower *Hoploscaphites nicolletii* assemblage subzone. This paper identifies the biostratigraphic zones (and subzones) where each different coleoid species have been found (see Table 1).

General mode of preservation

From the Fox Hills and throughout most of the Pierre and Bear Paw Shale, fossil coleoids are generally preserved inside of calcareous concretions. Within these concretions, the gladius of coleoids is often associated with abundant ammonites, bivalves, and gastropods. Aragonite is well preserved within these concretions but calcite tends to fuse with the rock or is often dissolved in the creation of the concretion.

While the guard, or rostrum, is normally the only part preserved in *Belemnitella*, and in nearly all belemnites, within the Fox Hills Formation the guard (rostrum) is poorly preserved and is nearly impossible to separate or clean from the host rock. The host rock is usually composed of calcitic/sideritic concretion, making preparation difficult. The phragmocone is often well preserved with only traces or portions of cross sections of the guard visible.

Most specimens of *Actinosepia* are poorly preserved, and none of the Fox Hills specimens exhibit a complete gladius. Waage (1965: 7) stated that all showed signs of decomposition "riddled with holes and frayed about the edges". He imagined them to have exfoliated during decomposition similar to exfoliation as on horns of cattle. If this were the case, the gladius would have laid on the bottom of the sea for some time before a concretion would have formed around it. Concretions probably would have had a difficult time forming around naked gladii unless there was some fleshy, organic material still attached. Since

Tab. 1: The Western Interior Ammonite Range Zones and their known Coleoid Fauna (all data for Ammonite Range Zones and radiometric dates in this table were taken from Cobban et al. 2006).

Period	Biozone	Million years BP	Known coleoids
Upper Maastrichtian	*Jeletzkytes nebrascensis*	~ 67.00 ± 0.45	*Actinosepia landmani, A. mapesi, Belemnitella bulbosa*
	Hoploscaphites nicolletii		*Actinosepia landmani, Belemnitella bulbosa*
	Hoploscaphites birkelundae		
Lower Maastrichtian	*Baculites clinolobatus*	~ 69.59 ± 0.36	*Belemnitella* cf. *bulbosa*
	Baculites grandis	~ 70.00 ± 0.45	*Actinosepia* cf. *canadensis*
	Baculites baculus		*Belemnitella* cf. *bulbosa*
	Baculites eliasi (upper)		
Upper Campanian	*Baculites eliasi* (lower)		*Actinosepia canadensis?*
	Baculites jenseni		
	Baculites reesidei	~ 72.94 ± 0.45	*Actinosepia canadensis*
	Baculites cuneatus		*Actinosepia canadensis, Tusoteuthis cobbani* n. sp.
	Baculites compressus	~ 73.52 ± 0.39	*Actinosepia canadensis, Belemnitella*? sp.
	Didymoceras cheyennense	~ 74.67 ± 0.15	
	Exiteloceras jenneyi	~ 75.08 ± 0.11	
	Didymoceras stevensoni		
	Didymoceras nebrascense	~ 75.19 ± 0.28	
Middle Campanian	*Baculites scotti*	~ 75.56 ± 0.11	
	Baculites reduncus		
	Baculites gregoryensis		
	Baculites perplexus		
	Baculites sp. (smooth species)		
	Baculites asperiformis		
	Baculites mclearni		
	Baculites obtusus	~ 80.58 ± 0.55	*Enchoteuthis melanae*
Lower Campanian	*Baculites sp.* (weak flank ribs)		
	Baculites sp. (smooth)		
	Scaphites hippocrepis III		*Enchoteuthis melanae*
	Scaphites hippocrepis II		
	Scaphites hippocrepis I		*Tusoteuthis longa*?
	Scaphites leei		*Enchoteuthis melanae?*
Upper Santonian	*Desmoscaphites bassleri*	~ 84.30 ± 0.34	*Tusoteuthis longa*
	Desmoscaphites erdmanni		
	Clioscaphites choteauensis		*Niobrarateuthis bonneri*?

Fox Hills *Actinosepia* are not complete and many are missing large sections and often have holes, this is interpreted to be the result of predation similarly seen in ammonites from the same horizon (Larson 2003). Specimens often occur as 'splits' in the center of concretions, with the outer shell layer adhering to part and counterpart. Hence, only in very rare cases are ventral and dorsal surfaces exposed. Most concretions are also usually smaller than the gladius so the outer edges are often missing.

Within the Smoky Hill Chalk, calcite and corneous material is often preserved while aragonite is not (diagenesis). Coleoid preservation consists only of the gladius preserved as calcareous, corneous material, often flattened and sometimes folded. Specimens are generally incomplete, since they were all found weathering out of the sediments. Signs of predation are occasionally present on some specimens (Stewart & Carpenter 1990).

Preservation of enchoteuthidids from the lower Pierre Shale is similar yet different. Some specimens are preserved without concretion and covered in gypsum while other specimens are preserved in calcareous/sideritic concretion. When preserved within concretions, most of the organic remains of the gladius remain uncrushed and well preserved. As in the rest of the Pierre Shale, aragonite is preserved; calcite is usually not.

Repositories

BHI, Black Hills Institute of Geological Research, Black Hills Museum of Natural History, Hill City, South Dakota

FHSM, Fort Hays State University, Sternberg Museum of Natural History, Hays, Kansas

GSC, Geological Survey of Canada, Ottawa, Ontario

KU, University of Kansas, Natural History Museum, Lawrence, Kansas

MDM, Canadian Fossil Discovery Centre, Morden, Manitoba

MMMN, Manitoba Museum of Man and Nature, The Manitoba Museum, Winnipeg, Manitoba

TMP, Tyrrell Museum of Paleontology, Drumheller, Alberta

USNM, United States National Museum of Natural History, Smithsonian Institute, Washington, D.C.

YPM, Yale Peabody Museum, Yale University, New Haven, Connecticut

Systematic paleontology

Subclass COLEOIDEA Bather, 1888
Order BELEMNITIDA Zittel, 1895
Suborder BELEMNOPSEINA Jeletzky, 1966
Family BELEMNITELLIDAE Pavlow, 1914

Genus *Belemnitella* d'Orbigny, 1840

Type species: *Belemnites mucronatus* Schlotheim, 1813 (p. 111)

Diagnosis: According to Christensen (1975), *Belemnitella* is characterized by medium to large guards (rostrum); well-developed, longitudinal, dorso-lateral depressions; dorso-lateral double furrows and with the anterior of the guard forming a calcified ventral tongue-like extension.

Stratigraphical and geographical range: *Belemnitella* has been described from the lower Santonian through the upper Maastrichtian. The genus ranges from the Northern Eurasia to the northern-half of North America. Some representatives have also been reported from the Mediterranean Province.

Belemnitella bulbosa Meek & Hayden, 1856

Figs. 1A-L

* 1856 *Belemnitella? bulbosa* Meek & Hayden, p. 70.
v. 1876 *Belemnitella bulbosa* Meek & Hayden. - Meek, p. 504, pl. 33 Figs 2a-e.
1893 *Belemnitella bulbosa* Meek & Hayden. - Boyle, p. 64.
1960 *Belemnitella bulbosa* Meek & Hayden. - Jeletzky, p. 31.
1966 *Belemnitella bulbosa* Meek & Hayden. - Jeletzky, pp. 48, 115, pl. 1, Fig. 1.
1968 *Belemnitella bulbosa* Meek & Hayden. - Waage, pp. 139, 143.
1993 *Belemnitella bulbosa* Meek & Hayden. - Christensen, p. 444-445.
1997 *Belemnitella bulbosa* Meek & Hayden. - Riegraf, p. 126.
v. 1997 *Belemnitella bulbosa* Meek & Hayden. - Larson, et al., p. 94.
2001 *Belemnitella* sp. - Stoffer et al., p. 16-19, Fig. 11, 12.
2003 *Belemnitella bulbosa*. - Stoffer, p. 34, Fig. 43.

Holotype: The original description of *Belemnitella bulbosa* was by Meek & Hayden (1856). Holotype by designation is the original of Meek (1876: pl. 33, Figs. 2a-b), collected in present day South Dakota by Col. Vaughn. Meek returned the holotype to the Academy of Sciences in St. Louis and these collections were transferred to the St. Louis Science Center several decades ago. Several attempts were made to locate the holotype, but no record of the specimen or of Col. Vaughn's notes regarding it have been found. Either the holotype is lost or it has simply been misplaced among other specimens.

Paratypes: There were two paratypes collected from the Fox Hills Formation 'near the Moreau trading post [South] Dakota' and also illustrated by

Meek (1876: 504, pl. 33, Figs. 2c & 2d-e). Meek (1876) stated that 'the fine specimen with the guard' from the Academy of Sciences in St. Louis, that specimen was the holotype. Both paratypes (numbered as USNM PAL 412) are located in the USNM.

Type locality and horizon: The type locality and horizon for the holotype are unknown, the paratypes were found in present day Dewey County, South Dakota from the *Hoploscaphites nicolletii* Zone of the Fox Hills Formation.

Material studied: *Belemnitella* are not common in the Western Interior. Approximately 60 specimens from both zones and nearly every subzone of the Fox Hills Formation in north-central South Dakota, representing nearly 40 years of collecting reside in the BHI Collection. Meek (1876: 504) wrote regarding *Belemnitella* from the Fox Hills: "It is very rare, only a few specimens having been brought in among all the extensive collections from the Northwestern Territories." The second largest collection (about 35 specimens) of *B. bulbosa* is from the YPM and is currently stored at the Mineralogical-Geological Museum in Copenhagen, Denmark where they have been since Walter Christensen borrowed them before his death; none of those specimens were examined.

Stratigraphical and geographical range: Regionally confined to the Fox Hills Formation and upper Pierre Shale of the northern Plains of the Western Interior. Within the upper Maastrichtian Fox Hills Formation of South and North Dakota, *B. bulbosa* are rare, but occur throughout both members and all subzones (Tab. 1).

Diagnosis: According to Meek & Hayden (1856) and revised slightly by Meek (1876), the shell or guard (rostrum) is small, slender, subcylindrical, elongated, widening at the anterior end, contracting than enlarging slightly again before tapering to a point. The alveolus has a small groove down the dorsum and marks of transverse septa round the sides. The phragmocone tapers regularly at an angle of 20º and terminates in a minute bulb; its section is circular to slightly oval with septa thicknesses 1/6 their diameter, septa faintly sinuate on the dorsum. The phragmocone has a thin, distinctly pearly substance on the surface marked by scarcely visible, fine growth lines.

Descriptions: USNM PAL 412, Paratype (Fig. 1A), POAZ, illustrated by Meek (1876, Pl. 33, Fig. 2d & e) consists of a small phragmocone 2.2 cm long.

BHI-5911 (Fig. 1B) from the LNAZ is a partial phragmocone and partial guard. 51 septa are present over 3.66 cm of the protoconch. The phragmocone is ovate, and a slight saddle in the septa is well defined. The guard is 0.77 cm wide the maximum diameter of the phragmocone is 1.1 cm. The siphuncle is visible on the venter.

BHI-5909 (Fig. 1C), POAZ, is a phragmocone (with partial guard in cross-section) that shows unusual dorsal striations and a pair of white lines that parallel the angle of the cone on either side of the siphuncle. According to Fuchs (personal communication 2010) these are most likely the mid-line and the diverging lateral fields of the proostracum. These lines are quite interesting in that they show proostracal growth increments. The bulbous protoconch is preserved as clear calcite at the end of the phragmocone. Septa are about 1/5 per diameter. Measurements on the incomplete phragmocone are 3.65 x 1 cm; the rostrum is 0.76 cm across.

BHI-5893 (Fig. 1D), POAZ, is an incomplete phragmocone with much of the rostrum. The phragmocone is cylindrical (0.98 x 0.94 cm), shows

Fig. 1: *Belemnitella bulbosa*. A) Paratype, USNM PAL 412, illustrated by Meek (1876: Pl. 33, Fig. 2d & e), photo courtesy of Stephen Godfrey, John Pojeta & USNM; scale bar = 1 cm. B) BHI-5911, typical phragmocone with siphuncle, *H. nicolletii* Zone, upper Maastrichtian, South Dakota; scale bar = 1 cm. C) BHI-5909 exhibits unusual dorsal markings that show the diverging lateral fields of the proostracum; scale bar = 1 cm. D) BHI-5893 shows the minute bulbous protoconch; scale bar = 0.5 cm. E) BHI-5895 shows dorsal saddle on septa; scale bar = 1 cm. F) BHI-5895 shows siphuncle placement at the top of the septa from the Trail City Member, Fox Hills Formation; scale bar = 1 cm. G) BHI-5894 shows the typical preservation of the guard and phragmocone,, Trail City Member; scale bar = 1 cm. H) BHI-7021 shows a nearly complete guard and partial phragmocone note the minimal taper in the guard and the rapidly expanding phragmocone; scale bar = 1 cm. I) BHI-5878 shows a nearly complete phragmocone and partial guard, Timber Lake Member, Fox Hills Formation; scale bar = 1 cm. J) BHI-5883 shows preservation from Trail City Member, Fox Hills Formation; scale bar = 1 cm. K) BHI-5885, Trail City Member, Fox Hills Formation; scale bar = 1 cm. L) BHI-5885 shows siphuncle placement on the venter; scale bar = 1 cm. M) *Belemnitella?* sp., BHI 7009, *Baculites compressus* Zone, upper Campanian, South Dakota; scale bar = 1 cm.


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its bulbous-like calcitic protoconch, septa height is 1/5 of diameter. The rostrum is consistently 0.65 cm wide.

BHI-5895 (Fig. 1E, F), POAZ, is a very partial phragmocone (4 septa, 1.42 x 1.49 cm in diameter) with siphuncle preserved. The siphuncle is small, 1.52 mm in diameter. The septa on the dorsal side form a slight dorsal saddle.

BHI-5894 (Fig. 1G), POAZ, consists of an incomplete phragmocone and partial rostrum. Protoconch is well preserved with 41+ partial septa (minus the protoconch) in 2.6 cm. The maximum diameter of the phragmocone is 0.82 cm; the rostrum is 0.54 cm wide.

BHI-7021 (Fig. 1H), POAZ, is an incomplete phragmocone and a nearly complete guard. The rostrum measures 7.4 cm long and 0.6 cm in diameter except near the apex where it expands over the phragmocone. The surface is rough because the rostrum was prepared with aluminum oxide by an air abrasive machine, no other method could expose it.

BHI-5878 (Fig. 1I), *Jeletzkytes nebrascensis* Zone of the Timber Lake Member, is a nearly complete phragmocone (6.5 cm long x 2.22 cm x 2.22 cm in diameter). The rostrum is 0.77 cm wide and extends 2 cm down the phragmocone with some swelling near the end of the phragmocone.

BHI-5883 (Fig. 1J), *Jeletzkytes nebrascensis* Zone, is most of a phragmocone with most of the guard which extends most of the way down the phragmocone. The protoconch is preserved in clear calcite and is nearly 1 mm across. The phragmocone is 3.34 cm long, guard 5.86 cm long and consistently 0.7 cm wide, total specimen is 7.26 cm long.

BHI-5885 (Fig. 1K, L), *Jeletzkytes nebrascensis* Zone, is a nearly complete phragmocone separated between two of its septa. The phragmocone is cylindrical (1.54 x 1.54 cm at the break). The siphuncle is visible on the ventral side of the phragmocone and measures 1.8 mm at the break. The septa form a slight lobe on the venter and a slight saddle on the dorsum.

Remarks: *B. bulbosa* phragmocones differ slightly from the Trail City Member to the Timber Lake Member of the Fox Hills Formation. Phragmocones from the Timber Lake Member are all nearly cylindrical while those from the Trail City Member are nearly all ovate to sub-cylindrical. The angles of the phragmocones also differ with those from the Timber Lake Member between 18º and 19º and those from the older Trail City Member between 16º and 17º. This may or may not be a species distinction.

Kennedy et al. (1998: 762) described a single specimen of *Belemnitella* cf. *bulbosa* from the Elk Butte Member of the Pierre Shale (upper Maastrichtian) and analyzed 10 specimens of *Belemnitella* from the *Baculites baculus* and *B. clinolobatus* Zones (lower Maastrichtian), Pierre Shale of South Dakota; USGS Mesozoic Locality # D12229. The author has not looked at these specimens.

Belemnitella? sp.

Fig. 1M

Locality: Meade County, South Dakota, north side of the Belle Fourche River, near the Elm Springs road, Snook Ranch. Collector: Jim Michaud, Rapid City, South Dakota.

Material: A single specimen BHI-7009 (Fig. 1M) reposited in the BHI Collection is poorly preserved in concretion associated with *Baculites compressus* and *Hoploscaphites brevis*.

Stratigraphical and geographical range: Pierre Shale, *Baculites compressus* Zone, upper Campanian, Western Interior.

Description: BHI-7009 consists of an incomplete rostrum, apparently missing the apex and anterior section with the phragmocone. The rostrum is slender and 0.45 cm wide, and is 3.6 cm long (incomplete).

Remarks: This specimen is too incomplete to identify to genus. It is interesting, in that untold tons of material has been collected from this zone by the author yet this is the only belemnite specimen recognized within these rocks to date. It is probable that more specimens exist but their preservation makes them difficult to discern.

Order VAMPYROMORPHIDA Robson, 1929

Family ACTINOSEPIIDAE Doyle, Donovan & Nixon, 1994

Genus *Actinosepia* Whiteaves, 1897

Type species: *Actinosepia canadensis* Whiteaves, 1897

Diagnosis: According to Waage (1965) the gladius is broad, ovoid, arched, ventrally concave, deepening posteriorly. The main body expands forward like a fan from the apex and attains maximum width about one-third from the anterior end; it contains five dorsal, longitudinal ribs that originate from the apex; its median rib forms an acuminate tip and the remaining rib ends impart a slight scallop shape to the anterior margin; it is undivided by asymptotes with rounded conus vane on the rear third along a weak lateral asymptote. The gladius is corneous to corneous-calcareous in two laminated layers (ventral-smooth, dorsal-tuberculate). Surface of the gladius ornamented with fine to coarse tubercles except on the conus vanes. Late Campanian and Maastrichtian.

Emended diagnosis: Gladius is curved, dorsally convex, almost leaf shaped, scalloped anterior, characterized by at least 3 ribs radiating from the posterior. Contains a prominent, central rib or median keel, with lateral ribs on either side. Median rib is the longest and forms a point on the anterior of the gladius. The lateral ribs also have slight points or scallops but not as prominent as on the median keel. Lateral fields are separated from the median field by the hyperbolar zone on both sides of the posterior of the gladius, and form a rounded apex on the posterior end of the gladius. Ribs may or may not have tubercles, most noticeable only on the outer, surface and a corneous or knobby texture may be present on the rest of the surface. Growth lines are present under the outer surface and appear to be preserved as fibrous strands, except on the lateral fields where there are none. Differences between species seem to be: number of ribs in the median field; presence or absence of tubercles on ribs; tubercle size and spacing; and angle of the lateral ribs in relationship to the median keel.

Stratigraphical and geographical range: Late Campanian through Maastrichtian (Tab. 1), Upper Cretaceous from the Great Plains of southern Canada and northern United States (Whiteaves 1897; Landes 1940; Jensen & Varnes 1964; Waage 1965) and Pacific Northwest, Vancouver Island (Ludwigsen & Beard 1997; Tanabe et al. 2008).

Remarks: The placement of *Actinosepia* within Vampyromorphida has proved to be confusing. Whiteaves (1897) noted its many *Sepia*-like characters, and imagined it to be the ancestor of modern cuttlefish. Waage (1965) undertook extreme preparation and thin-sectioning several upper Maastrichtian specimens to compare them with modern cuttlefish and proved otherwise. As a result, Waage placed *Actinosepia* in the Family Trachyteuthididae (Naef, 1921) under the Order Teuthida (Naef, 1916). Doyle et al. (1994) created a new Family Actinosepiidae based on their unique characters but placed them, controversially under the Order Sepiida Zittel, 1895. Hewitt & Jagt (1999) ignored Doyle et al. (1994), and placed *Actinosepia* back into Trachyteuthididae, but in the Order Vampyromorpha (Pickford, 1936 [1938]). Because Actinosepiidae is similar with Trachyteuthididae and the vampyropod affinities of *Trachyteuthis* are well supported, the author regards Actinosepiidae as a vampyropod.

Species included: *Actinosepia canadensis* Whiteaves, 1897; *Actinosepia landmani* n. sp.; *Actinosepia mapesi* n. sp.

Actinosepia canadensis Whiteaves, 1897

Figs. 2-3

* 1897 *Actinosepia Canadensis* Whiteaves, p. 459-461, Pl. 2.
v. 1940 *Actinosepia canadensis* Whiteaves. - Landes, p. 180-181.
1964 *Actinosepia canadensis* Whiteaves. - Jensen & Varnes, p. F9.
v. 1965 *Actinosepia canadensis* Whiteaves. - Waage, p. 18-25; Fig. 1; Pl. 1, Fig. 1-3; Pl. 2, Fig. 1-3; Pl. 3, Fig. 1-2.
v non 1965 *Actinosepia canadensis* Whiteaves. - Waage, p. 19-20, 24-25; Pl. 1, Fig. 4-5; Pl. 4, Fig. 1-4.
1995 *Actinosepia canadensis* Whiteaves. - Riegraf, p. 150.
v. 1997 *Actinosepia canadensis* Whiteaves. - Larson, et al., p. 101.
1997 unnamed cuttlefish. - Ludwigsen & Beard, p. 138, fig. 102.
1998 *Actinosepia canadensis* Whiteaves. - Riegraf et al., p. 311.
1998 *Actinosepia canadensis* Whiteaves. - White, p. 2.
1999 *Actinosepia canadensis* Whiteaves. - Hewitt & Jagt, p. 322.

Holotype: Original of Whiteaves (1897), GSC 5379, by subsequent designation of Waage (1965, p. 25), reposited in the GSC collections, Ottawa, Ontario.

Paratypes: GSC 5379a, GSC 5379b, GSC 5379c; by subsequent designation of Waage (1965, p. 25)

Type locality: From opposite the mouth of the Swift Current Creek along the South Saskatchewan River, southwestern Saskatchewan (Canada).

Type horizon: *Baculites cuneatus* Zone, Bearpaw Shale.

Material: More than 30 specimens from the TMP along with dozens of specimens from the BHI and YPM collections were photographed and measured in the preparation of this manuscript.

Stratigraphical and geographical range: Late Campanian, Great Plains of southern Canada and northern United States. *Baculites cuneatus* through *B. reesidei* Zones of the upper Campanian, Upper Cretaceous, Western Interior and most likely into the *B. compressus* Zone (lower) and *B. jenseni* and *B. eliasi* Zones (above). The species is prevalent in the Bearpaw Shale of Alberta and Montana (Tab. 1).

Diagnosis: According to Whiteaves (1897) *Actinosepia canadensis* is elliptical to ovate in outline, slightly convex, marked with five narrow, acute, but not very prominent longitudinal ridges, with faint depressions or shallow grooves between them. It is bilaterally symmetrical and contains a median ridge and the two lateral ridges on either side that are slightly divergent. Has fine, numerous growth lines that point anteriorly into an acutely pointed lobe with its apex on the summit of the median ridge.

Emended diagnosis: Based on specimens discovered from the same biozone in the Bear Paw Shale as the type and utilizing specimens described by Waage (1965) the description of *A. canadensis* can be more fully defined. A dorsally convex pen or shield characterized by 5 distinct, tuberculate ribs radiating from the posterior end of the gladius. Ribs consist of a central median keel (rib) with two lateral ribs on either side at angles to the median rib. Of the 5 ribs, the median rib is the longest; the two outer lateral ribs are the shortest. The first lateral rib is angled at ~10º from the median keel, the second lateral rib angles at ~14º. The lateral fields are separated from the outer lateral ribs on the posterior of the gladius by the hyperbolar zone and are barren of rugose ornamentation. The lateral field is about 1/3 the length of the gladius, gladius length is about 2.5 times its width, maximum width about one-third the distance from the anterior end. Tubercles on the dorsal surface only are about 1 mm in diameter and are consistently spaced on all of the ribs (at 3 to 4 per cm) ribs are tuberculate for nearly their entire length. Rib tubercles are larger, uniformly spaced and better defined than the rough, small tuberculate, corneous structures (nodular ornamentation) that cover the rest of the median field. The angle of the apex is acute, between 55º-60º.

Descriptions: GSC 19888 (Fig. 2A, B) is a 'perfect' negative of *Actinosepia canadensis* described by Landes (1940: 180-182) and Waage (1965: 18-22, Fig. 1, Pl. 2). Waage used GSC 19888 to more completely describe the species. It has a corneous surface, five tuberculate ribs and well-defined hyperbolar zone separating its vanes from the median field. Complete description follows that of the emended diagnosis; see Landes (1940) and Waage (1965) for further description. Collected from the *Baculites cuneatus* Zone of the Bearpaw Shale near Manyberries, Alberta.

BHI-5845 (Fig. 3A) is the most complete specimen yet seen, other than GSC 19888 (which is a perfect negative) but is split and missing the outer, dorsal surface. The specimen is complete from the posterior end to the anterior end but is missing the ends of the anterior portion of the median rib. The lateral fields are well preserved and display the hyperbolar zones (see Fuchs, Engeser & Keupp 2007). The right lateral field is crushed a little inwards and is slightly distorted. The gladius is widest about 1/3 of the length from the anterior end, and if uncrushed would have measured ~10 cm wide. If complete, the gladius would have measured between 27 and 29 cm long. The length of the hyperbolar zone is between 7.5 and 8 cm. The first lateral rib, on either side of the median rib, is ~10º off the median rib and the second lateral rib is ~14º off the median rib. The specimen is inflated, very convex near the lateral fields and less convex and flattened on the anterior end. Tubercles are not visible on the ribs because the counterpart was not collected. It was collected from the *Baculites reesidei* Zone, Bearpaw Formation, along St. Mary's River near Lethbridge, Alberta.

BHI-5843 (Fig. 3B) is incomplete, dorsal view, still partially incased in concretion from the *Baculites reesidei* Zone, Bearpaw Formation, near the Korite Ammolite mines along St. Mary's River near Lethbridge, Alberta. The posterior end and shows much of the exterior of the early development of the median field, but lacks the lateral field, and the end of the anterior median field. It is very similar

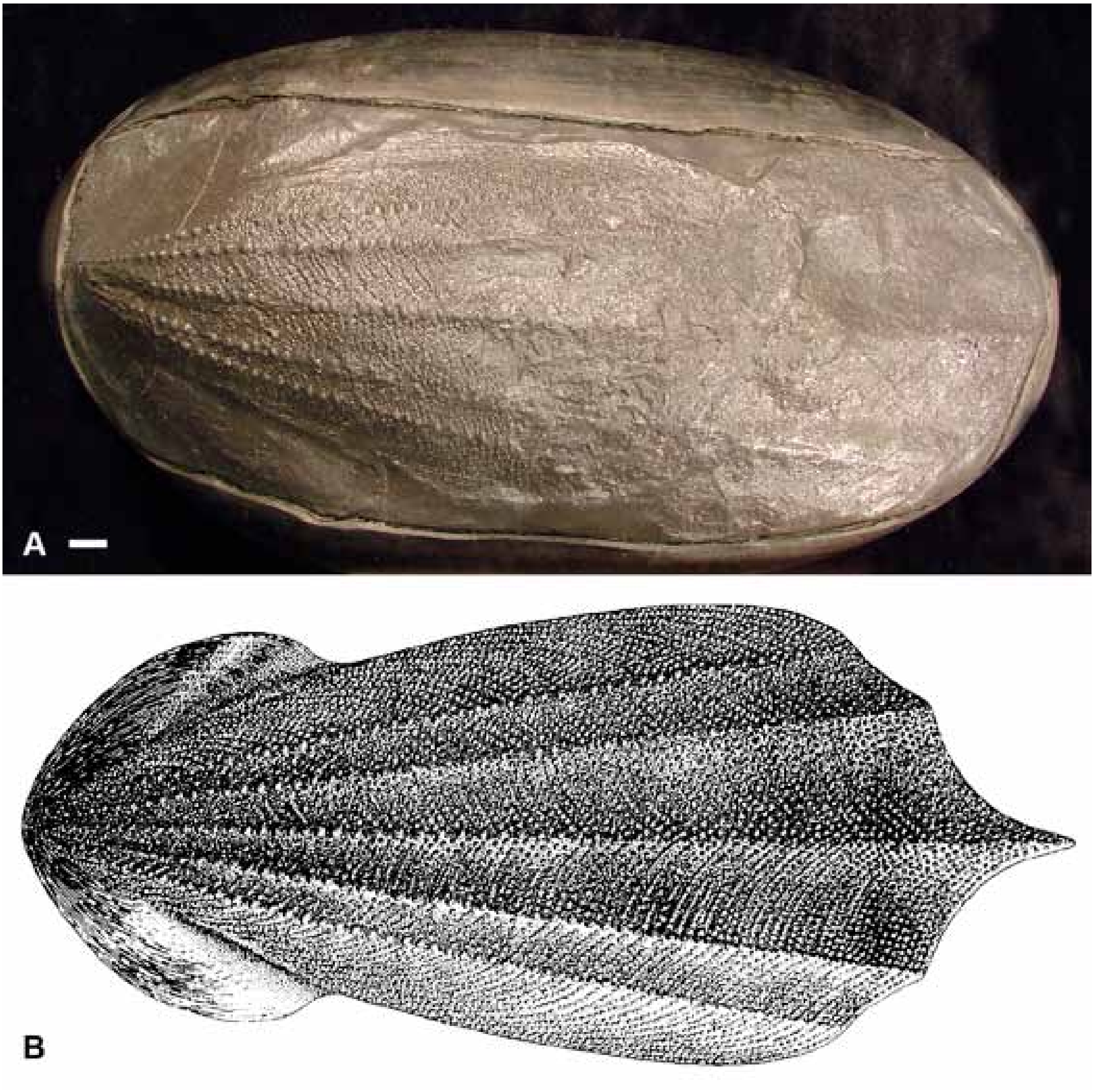

Fig. 2: *Actinosepia canadensis* Whiteaves, 1897. A) Cast of GSC 19888, *B. cuneatus* zone, Bearpaw Shale near Manyberries, Alberta, Yale specimen IP. 035101; scale bar = 1 cm. B) illustration of GSC 19888 taken from Waage (1965: Fig. 1).

in appearance to GSC 19888 (see Waage 1965: Fig. 1, Pl. 2). 1 mm tubercles spaced about every 3 mm run the length of the five ribs, much smaller tubercle like structures are present on the rest of the surface. The first lateral rib on either side of the median rib is ~9º-10º off the median rib; the second lateral rib is ~14º-15º off the median rib, which matches GSC 19888.

TMP 2004.666.05 (Fig. 3C) is from an unrecorded rock unit in Alberta, but the notes indicate that it probably came from the *Baculites reesidei* Zone, Bearpaw Formation, near the Korite Ammolite mines along St. Mary's River. The first lateral rib is angled at 11º from the median keel the second lateral rib angle is unknown. The specimen has large bullate tubercles the entire distance of the median and the first lateral rib. The vanes are slightly present near the apex but are still covered by rock.

Remarks: The holotype of *A. canadensis* (GSC 5379), Bearpaw Formation southern Saskatchewan, is

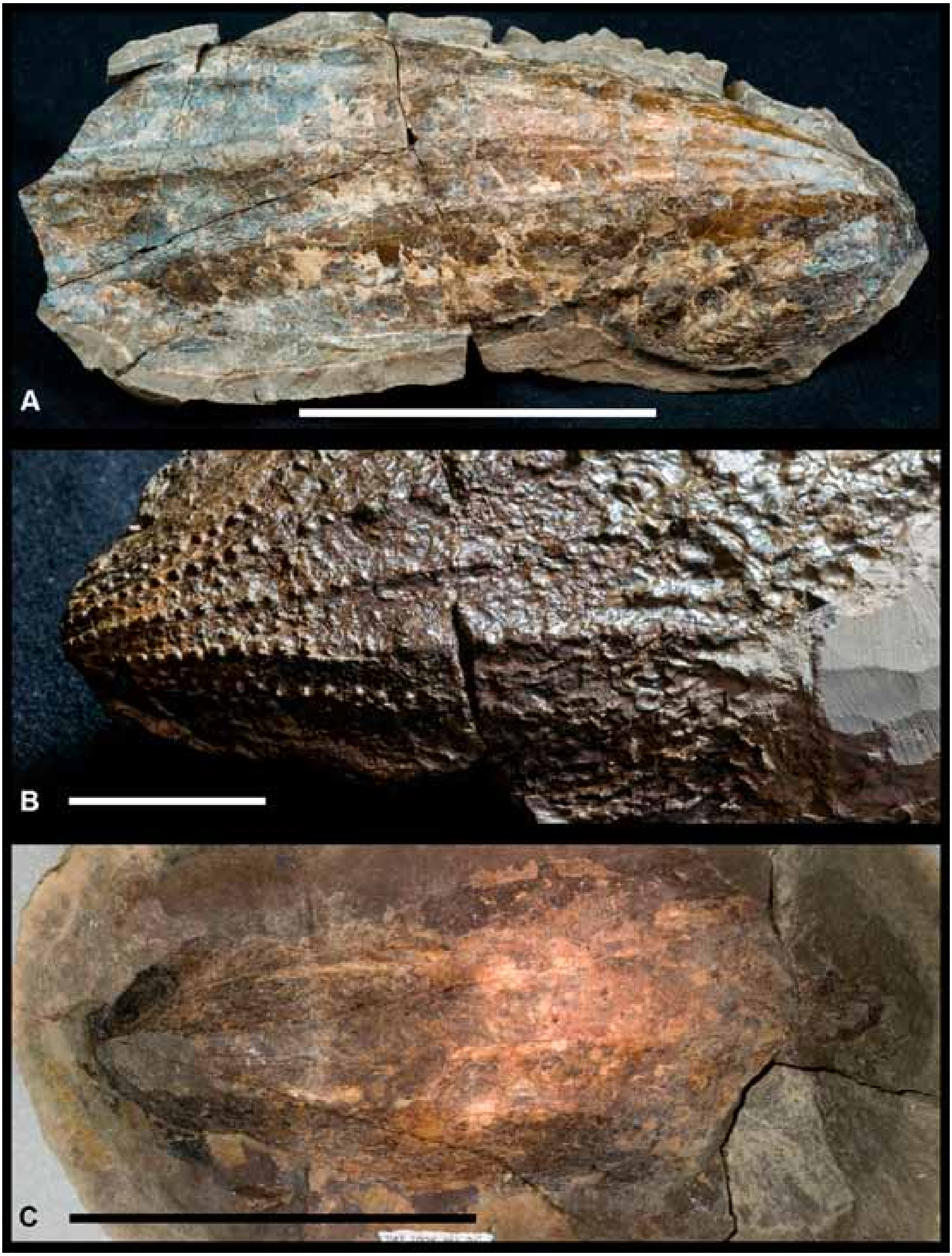

Fig. 3: *Actinosepia canadensis* Whiteaves, 1897. A) BHI-5845 is a nearly complete gladius with well defined lateral and median fields, *B. reesidei* Zone, Bearpaw Formation near Drumheller, Alberta; scale bar = 15 cm. B) BHI-5843 shows the prominent tubercles on the ribs from the *B. reesidei* Zone, Bearpaw Formation near Drumheller, Alberta; scale bar = 5 cm. C) TMP 2004.666.05 shows a nearly complete gladius; scale bar = 5 cm.

incomplete and lacking many of the characters needed for complete description and to adequately compare it with others with the exception of 5 tuberculate ribs and its growth lines. Its biozone is unknown, but based on the geology of the area, must have come either from the *Baculites compressus*, *B. cuneatus* or *B. reesidei* Zone. Likewise, the four paratypes from the same locality (Waage 1965) are even more incomplete. GSC 19888 is a very complete dorsal view of the gladius used by Waage (1965) for his comparisons and utilized in this paper for comparisons between the Fox Hills and Bearpaw actinosepiids. As noted in the above specimens, GSC 19888 is typical for upper Campanian *Actinosepia* specimens and is important because it shows so many characters.

Actinosepia landmani n. sp.
Figs. 4-7

v. 1965 *Actinosepia canadensis* Whiteaves. - Waage, p. 19-20, 24-25; Pl. 1, Fig. 4-5; Pl. 4, Fig. 1-4.
1998 *Actinosepia canadensis* Whiteaves. - White, p. 2.

Holotype: YPM 24809 collected and illustrated by Waage (1965: Pl. 1, Fig. 4-5); reposited in the YPM Invertebrate Collection, Yale University, New Haven, Connecticut.

Paratypes: BHI-5855, BHI-4097, and BHI-5867 reposited in the BHI Collection.

Type locality: Dewey County, South Dakota, Yale locality number: A673, collector Karl Waage.

Type horizon: Upper *nicolletii* Assemblage Zone (UNAZ), *Hoploscaphites nicolletii* Zone (Tab. 1), Trail City Member, Fox Hills Formation, Upper Maastrichtian, Upper Cretaceous

Derivation of name: After Dr. Neil H. Landman, curator of fossil invertebrates at the American Museum of Natural History in New York who has spent much of his career studying and publishing on the fossil cephalopods of the Pierre Shale and Fox Hills Formation.

Material: 39 specimens of *A. landmani* n. sp. currently reside the collections of Black Hills Institute of Geological Research and 34 in the collections of the Yale Peabody Museum. Even though there are many specimens, none are complete and only a few have enough characters preserved for comparison, measurement and identification.

Stratigraphical and geographical range: *Hoploscaphites nicolletii* through *Jeletzkytes nebrascensis* Zones of the upper Maastrichtian, Upper Cretaceous, Fox Hills Formation of the Western Interior in Wyoming, North and South Dakota (Tab. 1).

Diagnosis: *Actinosepia landmani* n. sp. consists of a long, broad, ovate, somewhat convex gladius, characterized by five, non-tuberculate to slightly tuberculate ribs radiating from the posterior apex or conus. The ribs form a scalloped edge on the anterior of the gladius. The ribs consist of a raised median keel (the longest and highest) with two lateral ribs on either side. The areas between the ribs are concave. The first lateral rib is angled between 11º and 13º from the median keel and the second lateral rib is very faint (the shortest of the ribs and quite often nearly non-distinct) at angles of 20º to 23º off the median keel. A narrow lateral field borders the outside of the ribs on the posterior end of the gladius and tubercles are present on the ribs only above the lateral fields.

Description: The selected holotype, YPM 24809 (Fig. 4A, B) of *A. landmani* n. sp. was previously figured by Waage (1965: Pl. 1, Fig. 4 & 5). The specimen is nearly complete (positive and negative) and measures 17.7 cm long by 8.7 cm wide. It has a nearly complete median field but is missing the lateral field, the apex, and the anterior ends of the ribs. The ribs number five and even though this is a 'split' all are non-tuberculate. The first lateral rib has an angle of 11º off the median and the second lateral rib has an angle of 20º off the median keel. There is a round hole present on the left side of the median rib close to the anterior edge resembling the cross-section of a tooth and indicating vertebrate predation.

BHI-5855 (Fig. 5), paratype, has a nearly complete median field but lacks the lateral fields and the apex. The anterior, scalloped edge is nearly complete. The first lateral rib has an angle of 11.5º off the median and the second lateral rib has an angle of 22º off the median keel. The gladius measures 10.8 x 27.2 cm long. There are two round "tooth" holes present on either side of the median rib close to the anterior edge (indicating vertebrate predation). The specimen consists of positive and negative in concretion, from the *Hoploscaphites nicolletii* Zone (UNAZ), Trail City Member, Fox Hills Formation, along the Grand River near Bullhead, South Dakota.

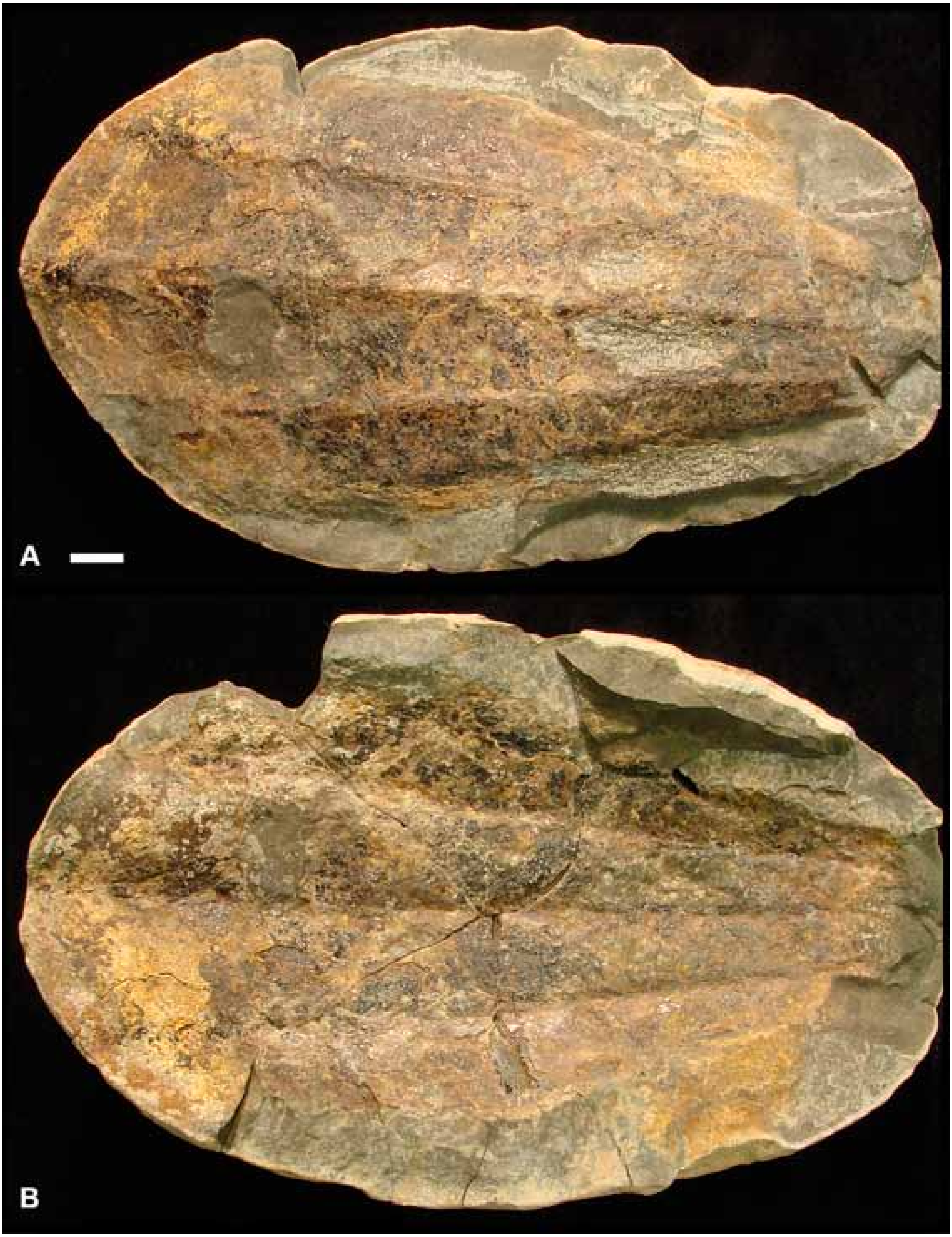

Fig. 4: *Actinosepia landmani* n. sp. A, B) YPM 24809, positive and negative of the ‚holotype, UNAZ, *H. nicolletii* Zone, Trail City Member, Fox Hills Formation, South Dakota; scale bar = 1 cm.

BHI-4097 (Fig. 6), paratype, is the only specimen of *A. landmani* that shows the posterior portion of the gladius, containing the apex and portions of the lateral fields. The specimen is very incomplete, missing the entire anterior sections of the median field from the lateral field forward. BHI-4097 measures 8cm long by 7 cm wide (mid-length). The lateral fields are fragmentary, but connect behind the apex. The apex is broadly rounded and the gladius is very convex. The outer surface is missing so there is little texture, the ribs are faint and rib angles were impossible to obtain. Specimen was collected from the, LNAZ, Trail City Member, Ziebach County, near Parade, South Dakota.

BHI-5867 (Fig. 7A-C), paratype, consists of positive and partial negative in concretion. This specimen has mostly exterior surface detail with very little being 'split'. The first lateral rib is angled at 11.5º off the median and the outer lateral rib is angled between 21º and 22º. Only on the posterior portion of the gladius is there any bullate or tubercle type of ornamentation on the ribs. Even though the outer surface of the gladius is visible, tubercles are almost indiscernible (Fig. 7B). In this region, the median rib has tiny, low, narrow, less than 1 mm bullae spaced about 3 mm apart for only 5 cm in length from the posterior end, after that the rib becomes non-tuberculate. The second lateral rib shows no sign of tubercles or bullae. The outer lateral rib has broad, ovate, tubercles about 3 mm across, and spaced one about every 5 mm for nearly the entire distance of the lateral fields and then they become non-tuberculate. Other then what has been mentioned, the remaining portions of the lateral ribs do not show any tubercle or bullate ornamentation but rather have a rough, corneous-like appearance. The rest of the surface is covered with a corneous texture, though not nearly as corneous as *A. canadensis* (Fig. 7C). The remnants of an ink sack are present on the right posterior section, near where the lateral fields would have attached. A section of *Jeletzkytes spedeni* microconch is preserved with the gladius, indicating that the specimen was collected from the *Hoploscaphites nicolletii* Zone, Trail City Member.

BHI-7017 (Fig. 8) (positive and negative) from the Timber Lake Member of the Fox Hills shows traces of tubercles on the ribs. As in BHI-5867 there are only low, bullate, small tubercles on the median rib till the end of the lateral fields and some larger, broad, ovate tubercles on the outer lateral rib near the apex. The lateral fields are partially preserved on the right side of the posterior portion of the gladius but the apex is missing. The gladius

Fig. 5: *Actinosepia landmani* n. sp. BHI-5855, paratype, POAZ, *H. nicolletii* Zone, Trail City Member, Fox Hills Formation, South Dakota; scale bar = 1 cm.

Fig. 6: *Actinosepia landmani* n. sp. BHI-4097, paratype, LNAZ, *H. nicolletii* Zone, Trail City Member, Fox Hills Formation, South Dakota; scale bar = 1 cm.

is inflated and convex. The specimen has been predated, yet much of the gladius remains intact. There are large sections and pieces of the gladius missing but the overall shape and size remain. The ornamentation on this specimen matches BHI-5867.

Differential diagnosis: *A. landmani* n. sp. differs from *A. canadensis* in the angle of the lateral ribs radiating from the apex and possessing only a few small bullate tubercles on the ribs in the posterior one-third of the gladius (above the lateral fields) while *A. canadensis* has large bullate tubercles present the entire length of the ribs. The middle rib on *A. landmani* is consistently the highest rib on the gladius while on *A. canadensis* the first two lateral ribs are higher than the median rib in the region above the lateral fields. The surface of *A. landmani* is not as corneous as *A. canadensis*.

Remarks: Waage (1965) revisited and studied *A. canadensis* carefully examining the holotype and

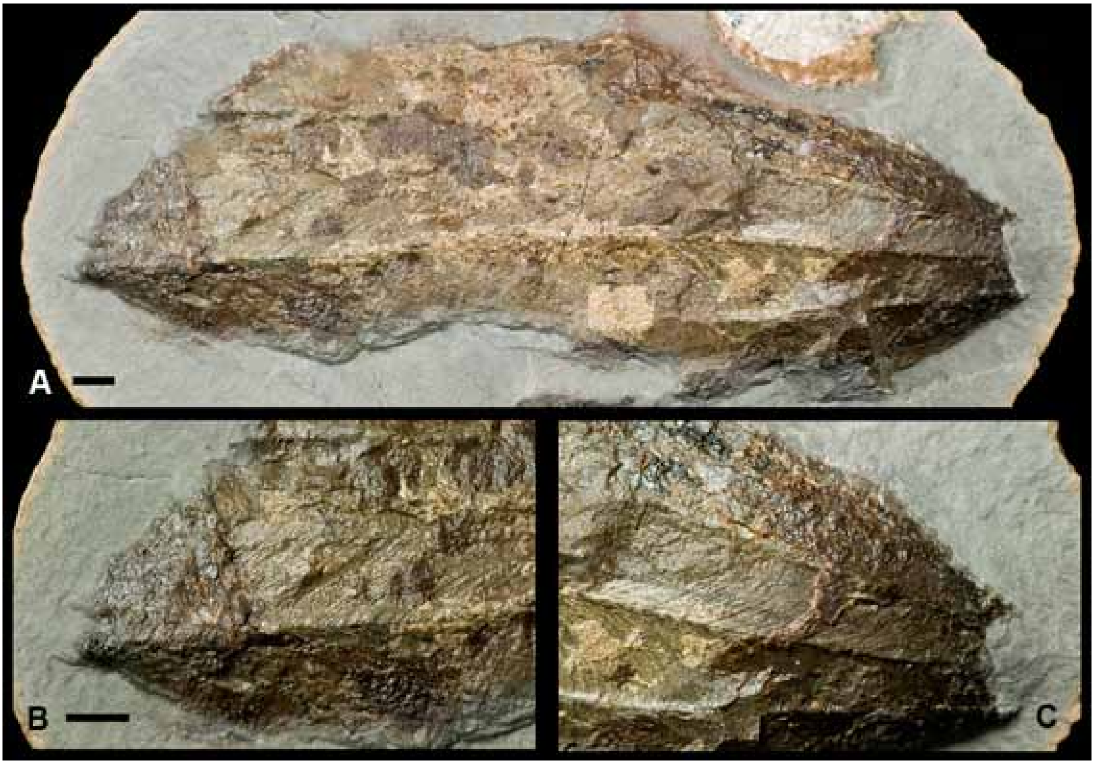

Fig. 7: *Actinosepia landmani* n. sp. A) BHI-5867, paratype, POAZ, *H. nicolletii* Zone, Trail City Member, Fox Hills Formation, South Dakota; scale bar = 1 cm. B) close-up of A) showing the posterior gladius with minor tubercles on outer rib; scale bar = 1 cm C) close-up of A) showing the anterior gladius.

Fig. 8: *Actinosepia landmani* n. sp. BHI-7017, *J. nebrascensis* Zone, Timber Lake Member, Fox Hills Formation, South Dakota; scale bar = 1 cm.

paratypes. He also obtained several other specimens from other locations in Canada (Landes 1940) and the U.S. (Jensen & Varnes 1964) to compare with specimens that he had collected from the Fox Hills Formation (upper Maastrichtian). Waage (1965) came just short of naming a new species from the Fox Hills Formation (p. 24-25) saying "Whiteaves' four specimens differ enough from most other specimens of *Actinosepia* to question whether they may be specifically distinct." There are only two specimens of *A. landmani* n. sp. within the BHI Collection which have tubercles present on the ribs (see BHI-5867 & BHI-5867). Cope MacClintock, curator of the Yale Peabody Museum, examined the YPM Collection for presence of tubercles on the Fox Hills *Actinosepia* and found none. Not to mention the time difference (5 million years), the angle of the ribs and the ornamentation is distinct enough between *A. canadensis* (upper Campanian) and *A. landmani* n. sp. (upper Maastrichtian) to designate a new species.

Actinosepia mapesi n. sp.

Fig. 9

Holotype: BHI-5863; reposited in the BHI collections.

Type locality: Bear Creek Gravel Pit, Dewey County, South Dakota, collected in 1983 by Neal L. Larson.

Type horizon: *Jeletzkytes nebrascensis* Zone, upper Maastrichtian, Upper Cretaceous, Fox Hills Formation, South Dakota.

Derivration of name: After Dr. Royal Mapes, professor emeritus at Ohio University, Athens, Ohio in recognition for his abundant contributions to the field of coleoid paleontology. He has been a major contributor with his discoveries and research on both extinct and extant cephalopods and has worked unselfishly with others.

Material: A single specimen from the BHI collection.

Stratigraphical and geographical range: Known only from the type locality and the type horizon (Tab. 1).

Diagnosis: Consists of a median, tuberculate rib with six or seven heavily tuberculate ribs on either side of the median rib. There are well-defined smooth valleys between the ribs. Like other species of *Actinosepia*, the posterior of the ribs appear to be divergent forming well defined angles (unmeasured) between the ribs.

Description: BHI-5863 (Fig. 9) is small with only the median fields preserved. The dorsal view of the gladius has been prepared. The specimen was slightly broken before preservation with one section of the median fields separated and at 60º to the plain of the median keel. The anterior section of the median field lies upside down to the rest of the specimen and lies directly on top of the specimen in the negative along with a *H. comprimus*. The specimen is incomplete on the right side, but has six (and maybe seven) lateral ribs on the left side, not including the median keel. Three ribs are all that are present on the left side of the median keel. There are abundant bullate-shaped tubercles on all ribs (2.5 - 3 per cm). The surface between the very pronounced ribs is deeply concave and smooth and the rough, corneous texture, common with *Actinosepia*, seems to be absent from the rest of the gladius.

Differential diagnosis: *A. mapesi* n. sp. differs from *A. canadensis* and *A. landmani* in having six (or seven) lateral ribs on either side of the median keel versus two lateral ribs on either side in *A. canadensis* and *A. landmani. A. mapesi* has more widely spaced tubercles (and larger) on the ribs than *A. canadensis*, *A. landmani* n. sp. has only a few on the posterior portion. *A. mapesi* lacks the very heavy texture on the rest of the gladius, *A. landmani* n. sp. has a rough, somewhat knobby texture near the anterior end of the median field and *A. canadensis* has the corneous texture over its entire surface.

Order TEUTHIDA? Naef, 1916
Family ENCHOTEUTHIDIDAE n. fam.

Type genus: *Enchoteuthis* Miller & Walker, 1968

Diagnosis: Gladius characterized by spoon, spatula, shovel (spade) or spear shape. Consists of corneous material with a long, free rachis extending into and terminating at the end of its two large vanes (lateral fields). The vanes form a patella or leaf-like structure also referred to as "wings" (Nicholls & Isaak 1987). The rachis forms a dorsal ridge above the vanes. The conus is preserved as a dome at the termination of the rachis in the middle portion of the blade. The blade, a combination of the vanes, non-free rachis and conus is also referred to as the posterior expansion of the gladius (Nicholls & Isaak 1987). It is generally two to three times longer than it is wide. Free rachis length is generally twice the length of the blade. In enchoteuthids, the rachis terminates with the lateral fields approximately one-half to two-thirds the distance into the vanes, a moderately convex posterior expansion of the conus and lateral fields complete the gladius.

Fig. 9: *Actinosepia mapesi* n. sp. Holotype, BHI-5863 *J. nebrascensis* Zone, Timber Lake Member, Fox Hills Formation, South Dakota; scale bar = 1 cm.

Stratigraphic and geographic range: From the Smoky Hill Chalk and Pierre Shale of the Western Interior, Coniacian to upper Campanian Stage, Late Cretaceous. Also perhaps extends downward into the Turonian, Carlile Shale (based on a single specimen of a rachis in the BHI collections). Known from western Kansas, eastern Colorado, eastern Wyoming, South Dakota, northeastern North Dakota and southern Manitoba.

Genera included: *Enchoteuthis* Miller & Walker, 1968; *Tusoteuthis* Logan, 1898; *Niobrarateuthis* Miller, 1957; and *Kansasteuthis* Miller & Walker, 1968.

Remarks: Gladius morphology is essential in the systematic description and study of extant teuthoids. The basic shape of the teuthoid gladius is so vital it is organized at the generic and family level (Toll 1998: 59). Even though the gladius might be of a similar shape, the difference in the anterior vane-shoulder shape, where the rachis inserts (and begins) and the length and width of the rachis determines genera (Toll 1998).

It is for this reason the shape of the vanes (or blade) of *Tusoteuthis* and *Enchoteuthis* are important for the genus. The rachis shape, complexity, length, width, the distance of the rachis into its vanes and their relationship to the overall size of the vanes is also important in the determination of the genus as is the percentage of the size and shape of its conus. Even though the gladius of enchoteuthidids look similar to some modern coleoid gladii, the conus is far different forming a dome-like structure near the middle of the blade.

Although the shape of the late Cretaceous Enchoteuthididae n. fam. resembles Muensterellidae Roger, 1952 (Doyle 1991; Naef 1922) the overall shape, size, conus, length of the free rachis (Fuchs 2009) and rachis complexity of Muensterellidae are unique enough to separate the two into distinct families.

Genus *Enchoteuthis* Miller & Walker, 1968

Type species: *Enchoteuthis melanae* Miller & Walker, 1968

Stratigraphical and geographical range: From the Santonian through mid-lower Campanian Stages of the Late Cretaceous. Presently only recognized from the Smoky Hill Chalk and Pierre Shale, Late Cretaceous, Western Interior from central Kansas to southern Manitoba (Table 1).

Diagnosis: According to Miller and Walker (1968), *Enchoteuthis* consists of a corneous, lanceolate gladius that is dorsally arched (spoon-like). The guard is rounded and confluent with a median keel that extends along two-thirds of the gladius (blade). They did not recognize any subdivisions of the gladius into conus, free rachis, asymptotes of hyperbolar zones or growth lines.

Emended diagnosis: The gladius is characterized by shovel or spade shaped blade. Gladius composed of a rachis that extends from one-half to two-thirds of the way into a broad blade. The blade is widest near the anterior portion and is two times longer than wide. The blade consists of two parts: 1) paired anterior vanes that are dorsally concave with lateral fields separated by the rachis and 2) a spoon shaped conus located at the end of the rachis between the lateral fields near the center of the blade. The free rachis is missing on the holotype but based on other specimens it is long with a complex cross-section made up of many rod-like and wavy structures, attached to and extending into broad vanes and median fields ending at the conus. The conus appear to be adjacent to (or a continuation of) the vanes. The posterior of the blade is moderately convex and rounded to bluntly acute.

Remarks: Differs from *Tusoteuthis* in its anterior shoulder shape, being spade or shovel-shape (versus spear shape), the ratios of the blade width to the blade length, rachis complexity, and the widest portion of the gladius. *Enchoteuthis* also attains a larger size, unseen in any *Tusoteuthis* specimens. The blade shape is distinct even on smaller specimens where there is some folding or distortion of the lateral fields.

Enchoteuthis melanae Miller & Walker, 1968

Figs. 10-14

*v.	1968	*Enchoteuthis melanae.* Miller & Walker, p. 176, 179-180.
	1987	*Tusoteuthis longus* Logan. - Nicholls & Isaak, p. 727-737, figs. 2-5.
	2006	*Tusoteuthis longus*. - Hoganson, p. 13.

Holotype: FHSM 13049, reposited within the collection of the Sternberg Museum of Natural History, Hays, Kansas.

Type locality: Near Russell Springs, Logan County, Kansas.

Type horizon: Smoky Hill Member of the Niobrara Formation, just above the *Uintacrinus socialis* Zone in either latest Santonian or earliest Campanian rocks.

Material: The holotype along with several specimens from KU and BHI are described.

Stratigraphical and geographical range: From the upper Santonian through lower, middle Campanian Stage of the Late Cretaceous (Tab. 1), Western Interior, Smoky Hill Chalk and Pierre

Shale. From Western Kansas, eastern Colorado, eastern Wyoming, South Dakota, northeastern North Dakota and southern Manitoba.

Diagnosis: According to Miller and Walker (1968), the rachis is made of two main rods often with two more auxiliary rods on either side. The blade consists of two, dorsally concave, lateral fields (vanes) separated by the rachis (which forms a ridge) and large posterior expansion of the lateral fields with a conus located at the end of the rachis in the lateral fields. The posterior of the lateral fields forms a more rounded or blunt end than *Tusoteuthis*.

Re-description: The holotype of *Enchoteuthis melanae* FHSM 13049 (Fig. 10) is spade shaped, wider near the anterior shoulders than mid-blade. The blade is 22 cm long and 10.5 cm wide with the widest portion is about one-third the distance into the blade. The view is dorsal and the free rachis is missing. Blade is twice as long as it is wide, and the free rachis is typically two to two and one-half times the length of the blade. The non-free rachis is 12 cm long and forms a high dorsal ridge above the vanes terminating a little more than half way into the blade at the conus. The posterior of the lateral fields comprises about 41% of the length of the blade. The specimen is very well inflated. As with the genus the vanes are dorsally concave and the conus is dorsally convex. The rachis is complex, with two main rods separated at mid-line. The gladius is much larger than *T. longus*, with some specimens from the lower, middle Campanian Pierre Shale attaining two to four meters in length.

Descriptions: BHI-5874 (Fig. 11A) is from the Smoky Hill Member of the Smoky Hill Chalk of Gove County, Kansas. It is an incomplete specimen, missing most of its rachis, some of its conus and most of its right vanes. It is preserved in dorsal view and is spade shaped. The non-free rachis is 25 cm long and forms a high dorsal ridge (~3 cm, which is folded over. The rachis is 3 cm wide and structurally complex, consisting of many rods and cones. The anterior shoulder of the vanes, extend nearly perpendicular from the rachis forming the shape of a spade. The width of the complete (yet slightly folded) left lateral field is 10.2 cm and is widest near the anterior of the blade. The conus is slightly convex, the posterior of the lateral fields is not preserved.

BHI-5873 (Fig. 11B) is also from the Smoky Hill Member of the Smoky Hill Chalk of Gove County, Kansas (upper Santonian or lower Campanian) and is prepared in ventral view. It has an incomplete rachis, but the spade-shaped blade

Fig. 10: *Enchoteuthis melanae* Miller & Walker, 1968. Holotype, FHSM 13049, Smoky Hill Member, Niobrara Formation, Logan County, Kansas, photo courtesy of Michael J. Everhart; scale bar = 1 cm.

Fig. 11: *Enchoteuthis melanae* Miller & Walker, 1968. A) BHI-5874, Smoky Hill Member, Niobrara Formation, Gove County, Kansas; scale bar = 10 cm. B) BHI-5873, Smoky Hill Member, Niobrara Formation, Gove County, Kansas; scale bar = 10 cm.

is complete. The vanes leave the rachis at nearly 90 degrees. The specimen is widest just above the anterior shoulders, as in the type. The vanes exhibit some structure, maybe for the hyperbolar zones. The width of both vanes is 30 cm and each side measuring from mid-rachis is 15 cm in width. The entire blade length is 49 cm. The non-free rachis is 26 cm long the posterior of the free rachis is 4 cm wide. The rachis is quite complex. The conus is complete, the lateral fields terminate in a blunt or rounded end. A rachis-like structure (or markings) extends all of the way to the posterior of the conus. The conus is ventrally concave.

KU 314494 (Fig. 12A-C) is from the *Baculites obtusus* Zone, Sharon Springs Member of the Pierre Shale, near Redbird, Wyoming. The ventral side of a complete gladius is cleaned and measures 140 cm long. The blade (Fig. 13B) measures 66 cm long by 38 cm wide (19 cm wide on the unfolded side). The anterior shoulders are spade like with only slight distortion of the vanes. The conus is cup shaped and adjacent to the vanes. The posterior of the lateral fields comprises 22% the entire gladius length. The entire blade is 47% the length of the entire gladius. The rachis measures 107 cm long (Fig. 12C). The free-rachis is consistently 5 cm wide and is 73 cm long.

KU 150409 (Fig. 12D), dorsal view, is from the *Baculites obtusus* Zone, Sharon Springs Member of the Pierre Shale, Niobrara County, Wyoming. The specimen has an incomplete, free rachis that measures 27.5 cm long, and is 3.5 cm wide and

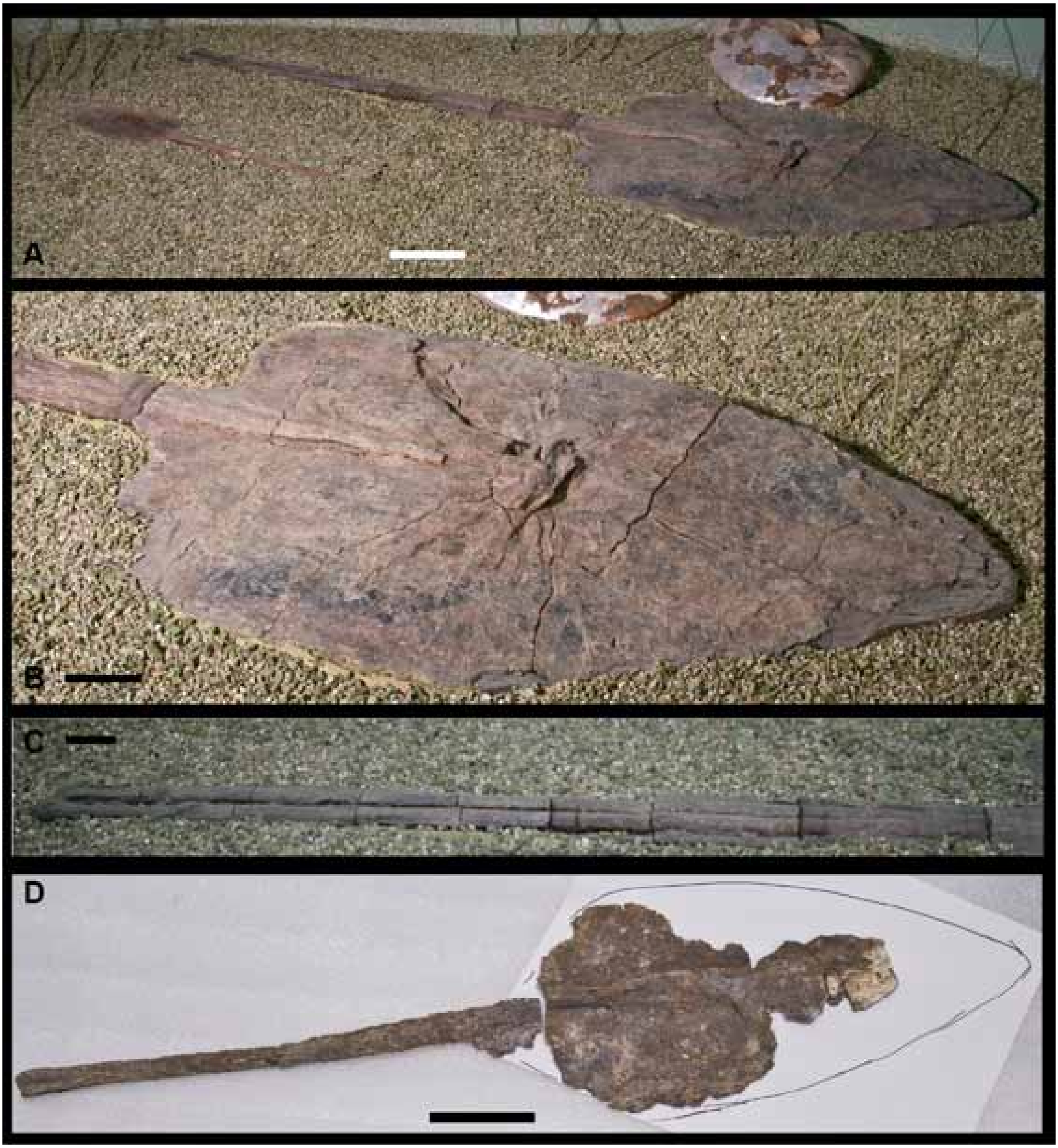

Fig. 12: *Enchoteuthis melanae* Miller & Walker, 1968. A) KU 314494, ventral view, *B. obtusus* Zone, Pierre Shale, Niobrara County, Wyoming, with a cast of the type of *Niobrarateuthis bonneri* (left side), photo courtesy of Corinne Meyers; scale bar = 15 cm. B) Close up of A); scale bar = 5 cm. C) Rachis of KU 314494 *e*, photo courtesy of Corinne Meyers; scale bar = 5 cm. D) KU 150409, dorsal view, *B. obtusus* Zone, Pierre Shale, Niobrara County, Wyoming; scale bar = 15 cm.

the non-free rachis is 16 cm long. The blade is 12.3 cm wide, the length is unknown, but believed to be somewhere near 25 cm in length the keel is 3.5 cm high. The blade is incomplete, but shows the shovel like shape, typical of *Enchoteuthis*. The conus is flattened, possibly in preservation. The specimen was prepared so both sides of the gladius can be examined.

KU 151925 (Fig. 13) is the cross section of a rachis from the Smoky Hill Member of the Smoky Hill Chalk (upper Santonian or lower Campanian). It measures 3.4 x 5.5 cm, and shows the typical complexity of rachii in the larger Campanian *Enchoteuthis*.

BHI-5812 (Fig. 14A-D) is also from the *Baculites obtusus* Zone, Sharon Springs Member of the Pierre Shale, near Redbird, Wyoming. The specimen is preserved as two sides of a split concretion, is uncrushed with most of the original material missing from in between. The specimen is missing the majority of its gladius due to weathering and preservation. It terminates at the end of the free rachis and is missing its conus and most of the vanes. The partial preserved negative of the lateral fields and non-free rachis is 46cm long by 27cm wide, yet if it were complete the blade would have measured (based on other specimens from the Pierre Shale) nearly a meter in length and a half-meter in width. Some of the free rachis is preserved within concretion. The width of its uncrushed rachis is 7.2 cm across (Fig. 14C), or 40% larger than MMMN I 794 and structurally very complex in cross-section (Fig. 14D). If BHI 5812 had been complete, through comparison with other specimens, the blade of BHI 5812 would have measured nearly a meter long by one/half meter wide (Fig. 14A) and its gladius would have been nearly 3 meters in length. BHI 5812 would have rivaled many of the large vertebrate reptiles in the Cretaceous oceans for dominance.

Fig. 13: *Enchoteuthis melanae* Miller & Walker, 1968. KU 151925, cross section of a rachis from the Smoky Hill Member of the Niobrara Chalk, Kansas; scale bar = 1 cm.

Remarks: *Enchoteuthis melanae* differs enough from *Tusoteuthis longa* in shape, size and rachis complexity to keep it separated as a different genus. The uniform gladius shapes, as seen in the figures, seem to confirm this diagnosis. Certainly more specimens need to be collected and studied. Three other Pierre Shale 'giants' of *Enchoteuthis* are known to exist but were not measured and photographed for this manuscript: one from the Pierre Shale in the collections of the Colorado School of Mines Geology Museum at Golden (Ken Carpenter, personal communication 2009); one from the lower, middle Campanian, Pierre Shale, eastern Wyoming in the collections of AMNH (Neil Landman, personal communication 2008) and one from the Pembina Member of the Pierre Shale from northeast North Dakota reposited at North Dakota Heritage Center in Bismarck, ND (Hoganson 2006).

Six specimens referred to as *Tusoteuthis longa* Logan, 1898 were reported by Nicholls & Isaak (1987) from the Pembina Member of the Pierre Shale (lower, middle Campanian) in southern Manitoba. MDM Q77.02.07 is spade shaped with a complex rachis (Nicholls & Isaak (1987) published the ventral view as dorsal and the dorsal view as ventral). The blade shape of the other specimens is unknown, however Nicholls & Isaac (1987) described the complex structure of the rachis and illustrated two of them (1987: Figs. 4 and 5-3). Based on those figures and measurements, it is believed that MDM Q77.02.07 and MMMN I 794 are *Enchoteuthis*. MDM Q84.03.18 (Nicholls & Isaak 1987: 732, Table 1) is a nearly complete (but unfigured) gladius from the Pembina Member. Based on measurements from that specimen that has the blade length of 42.3 cm and total length of the gladius at 121.3 cm, it is estimated that the gladius of MMMN I 794, which has an incomplete blade 68 cm long by 35 cm wide and a partial rachis 5 cm wide, would have been between 210 and 250 cm long.

Genus *Tusoteuthis* Logan, 1898

Type species: *Tusoteuthis longus* Logan, 1898

Species included: *Tusoteuthis longa* and *Tusoteuthis cobbani* n. sp.

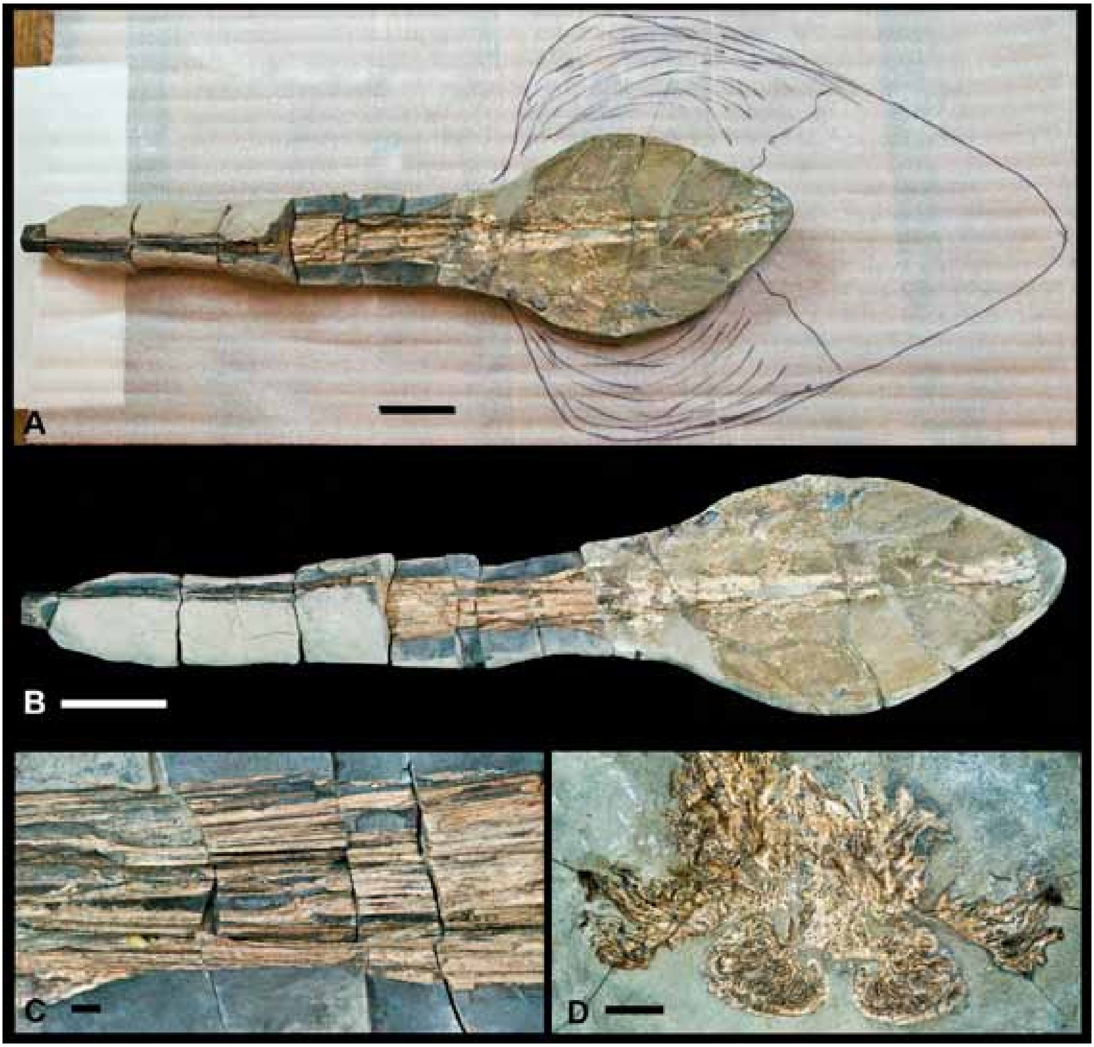

Fig. 14: *Enchoteuthis melanae* Miller & Walker, 1968. A) BHI 5812, ventral view, *B. obtusus* Zone, Pierre Shale, Niobrara County, Wyoming, showing in sketch how much of the blade is believed to be missing; scale bar = 15 cm. B) same specimen, dorsal 'split' view, with partial rachis (in concretion); scale bar = 15 cm. C) same specimen, close up of the rachis; scale bar = 1 cm. D) same specimen, close up of the rachis (cross section); scale bar = 1 cm.

Stratigraphic and geographic range: From the Santonian through mid-upper Campanian Stages of the Late Cretaceous (and possibly into the older Coniacian and Turonian). Presently only recognized from the Smoky Hill Chalk and Pierre Shale (Tab. 1), Late Cretaceous of the Western Interior from central Kansas and South Dakota. Possibly exists elsewhere in the Western Interior as well.

Diagnosis: According to Logan (1898), the gladius is moderately convex, lanceolate, and corneous in texture, greatest breadth centrally; anterior point is obtuse to rounded and covered with a calcareous layer. Striations on the surface of the gladius run parallel with the border. Rachis at base is broad and rounded, with a cylindrical shaft inclosing a hollow cone.

Emended diagnosis: Gladius characterized by spear-like shape. Rachis is long, attached to-and extending into vanes with large, primary conus behind the fin attachments. Gladius composed of corneous and chitinous material consisting of a long, free-rachis extending from one-half of the way into the blade. The rachis is cylindrical with small rods and cones may become more complex in larger specimens. The blade is two to three times longer than wide and consists of two parts: paired anterior vanes that are dorsally concave with the lateral fields separated by the rachis and the conus. The vanes terminate one-half way into the blade. The posterior of blade is made up of a large, primary conus that is a continuation to and adjacent to the vanes. The dorsal view of the conus is moderately convex and acutely blunted. The lateral fields (vanes) are widest in the central portion of the blade; the rachis points anteriorly, the conus points posteriorly.

Tusoteuthis longa Logan, 1898

Figs. 15-16

*v.	1898	*Tusoteuthis longus.* Logan, p. 497-98, Pl. 110, Fig. 1.
	1922	*Tusoteuthis* Logan. - Naef, p. 45.
	1928	*Tusoteuthis longus* Logan. - Whitney, p. 428.
	1957	*Tusoteuthis* Logan. - Miller, p. 809, 810.
v ?	1957	*Niobrarateuthis bonneri*. - Miller, p. 809-811, figs. 1, 2 (Holotype).
	1968	*Tusoteuthis longa* Logan. - Miller, p. 53-54, Pl. 8, Fig. 6.
?	1968	*Niobrarateuthis bonneri*. - Miller, p. 54-56, Pl. 8, Fig. 5.
	1968	*Tusoteuthis longa* Logan. - Miller & Walker, p. 176, 179-180.
	1974	*Tusoteuthis longa* Logan. - Green, p. 53, 55-57.
	1974	unidentified teuthid. - Green, p. 56, Fig. 5.
	1977	*Tusoteuthis longa* Logan. - Green, p. 992, 994.
	1976	*Tusoteuthis longa* Logan. - Stewart, p. 94.
non	1987	*Tusoteuthis longa* Logan. - Nicholls & Isaak, p. 727-737, figs. 2-5.
	1995	*Tusoteuthis longus* Logan. - Riegraf, p. 156.
non v	1997	*Tusoteuthis longa* Logan. - Larson, et al. p. 99-100.
	1998	*Tusoteuthis longus* Logan. - Riegraf et al., p. 186.
	2005	*Tusoteuthis longus* Logan. - Everhart, p. 37.

Holotype: Published as KU 4208; renumbered as KU 113463; reposited in the Invertebrate Collection, University of Kansas, Lawrence, Kansas.

Type locality: Exact locality is unknown, probably southern Logan County or western Gove County, Kansas (see Green 1977).

Type horizon: "from the Hesperornis beds of the Niobrara Cretaceous on the Smoky Hill river (sic) by Mr. Martin" (Logan 1898: 497), Smoky Hill Member, Niobrara Formation of western Kansas, upper Santonian.

Material: Several previously undescribed specimens exist at the KU, and one in the BHI Collection. KU 113463, the holotype, is incomplete but was carefully examined, measured and photographed for this paper.

Geographical and stratigraphical range: From the Smoky Hill Chalk and lower Pierre Shale, upper Santonian through the lower Campanian Stage of the Late Cretaceous, Western Interior (Tab. 1). Also perhaps extends downward into Coniacian, Smoky Hill Chalk. From western Kansas and eastern Colorado, possibly in the Niobrara Formation around the Black Hills.

Diagnosis: According to Logan (1898), the gladius is lanceolate (spear-shaped), corneous in texture, having its greatest width centrally; posterior point is obtuse and rounded; striations (growth lines) run parallel with the border (exterior shape). Midrib (rachis) at base is broad and rounded, cylindrical inclosing a hollow cone with parallel striations (separate rods).

Emended diagnosis: Although the holotype is incomplete, it is apparent that the gladius is spear –shaped, characterized by a free-rachis and blade. The blade is narrow, two to two & one-half times as long as it is wide, with slightly dorsally concave lateral fields, and dorsally convex conus. Based on other specimens, the rachis extends into the blade and forms a tall, thin ridge above the depressed lateral fields before it terminates near mid-blade. The gladius is widest at the central portion of the blade. The conus and an extension of the lateral fields forms the posterior of the gladius. Logan (1898) and Miller (1968) described the rachis as cylindrical with a hollow, central portion; the author has been unable to determine what that meant. The rachis is cylindrical and seems to contain small rods or cones. The rachis on the type consists as a single shaft and does not

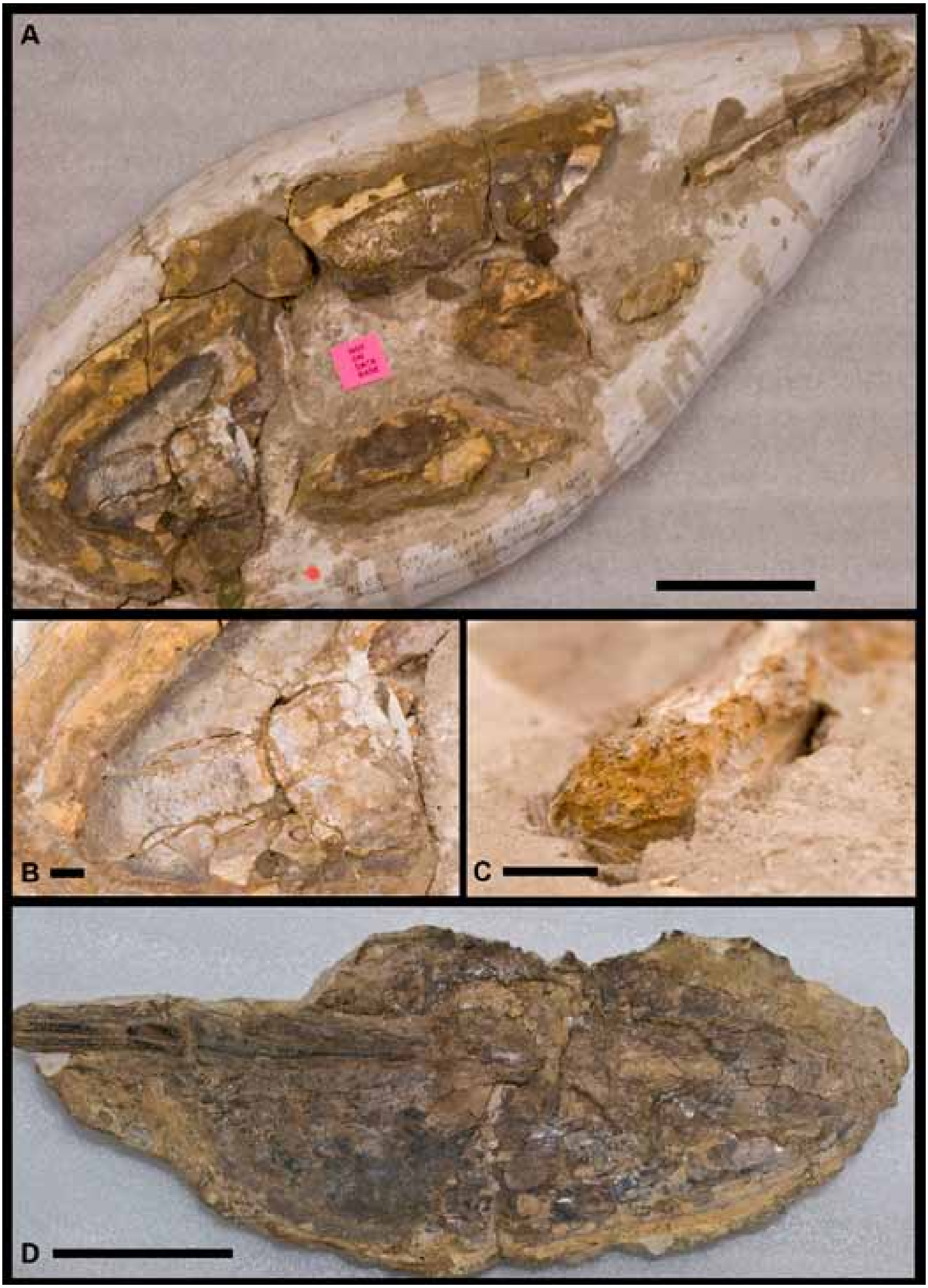

Fig. 15: *Tusoteuthis longa* Logan, 1898. A) KU 113463, holotype, Smoky Hill Member, Niobrara Formation of western Kansas; scale bar = 15 cm. B) close up of A) showing the conus; scale bar = 1 cm. C) close up of A) showing the rachis; scale bar = 1 cm. D) KU 113464, Smoky Hill Member, Niobrara Formation of western Kansas; scale bar = 10 cm.

Fig. 16: *Tusoteuthis longa* Logan, 1898. A) KU 150477, Smoky Hill Member, Niobrara Formation of western Kansas; scale bar = 1 cm. B) BHI 7010, Smoky Hill Member, Niobrara Formation of western Kansas; scale bar = 15 cm.

indicate the complexity as seen in *Enchoteuthis* from the Smoky Hill Chalk or Pierre Shale.

Descriptions: Logan (1898) designated an incomplete specimen as the holotype for *Tusoteuthis*. The holotype, KU 113463 (Fig. 15A), is missing most of the lateral fields and nearly its entire rachis. It has a spear-shaped blade. The anterior shoulder of the vane increases in width till the mid-point of the blade and then gently curves back to the apex. The holotype is a dorsal view based on the convex shape of the conus. The conus is nearly complete, dorsally convex (ventrally concave) the posterior is acutely blunt (Fig. 15B). The right lateral fields (vane) are missing along with the dorsal rachis ridge and most of the free rachis. The rachis is very partial, with only a small portion present at the anterior portion of the lateral fields. The rachis is cylindrical and does not indicate any complexity (Fig. 15C). There is enough preserved to determine the overall shape of the blade but other characters remain unknown unless compared with similar specimens. The blade is 35.5 cm long and 13.5 – 14 cm wide (based on evidence present on the type) or about twice as long as it is wide. It is unknown how far into the blade the rachis extended (unless compared with similar specimens).

KU 113464 (Fig. 15D), dorsal view, from the Smoky Hill Member of the Smoky Hill Chalk of western Kansas (either Gove or Logan County). As with the holotype, the blade is spear shaped. It consists of a partial free-rachis extending into the vanes terminating at the conus. There are growth lines that parallel the curvature of the apex. The vanes contain a well-defined hyperbolar zone. The non-free rachis extends about one-half way into the blade. The free rachis shows some minor complexity not seen in the holotype. The central portion of the blade is the widest. The non-free rachis is 19 cm long, the blade is 32.5 cm long, the one complete side of the blade (to mid-rachis) is 8.3 cm, meaning it would have been about 16.6 cm wide, or half as wide as long.

KU 150477 (Fig. 16A), ventral view, from the Smoky Hill Member of the Smoky Hill Chalk, Gove County Kansas. It is also incomplete, though it has one nearly complete side of the blade. The gladius is spear-shaped with the central portion of the blade being the widest, identical to the holotype. It is folded almost perfectly in half, is 32 cm long, missing its free-rachis, and its one complete side is 8 cm wide (at mid-rachis) meaning it would have been 16 cm wide or twice as long as wide. The rachis extends into the blade approximately one-half way into the blade.

BHI 7010 (Fig. 16B), dorsal view, from the Smoky Hill Member of the Smoky Hill Chalk of Gove County, Kansas (upper Santonian or lower Campanian). The specimen is nearly complete but poorly preserved. As with the type, the blade is spear shaped. The anterior end of the rachis is nearly complete the dorsal rachis ridge is well exposed and lying near the center of the blade. The vanes and conus are very poorly preserved. Total length is 49 cm long, rachis length is 36.5 cm long with free-rachis is 24.5 cm long and non-free rachis 12 cm long. The blade is 23.3 cm long and nearly 10 cm wide.

Remarks: The holotype of *Tusoteuthis longus* Logan, 1898 is fragmentary, consisting of a dorsal view of a very incomplete blade containing only one partial vane, the conus, and a nearly incomplete rachis. *Niobrarateuthis bonneri* Miller, 1957; *Enchoteuthis melanae* Miller & Walker, 1968; *Kansasteuthis lindneri* Miller & Walker; 1968; and *Niobrarateuthis walkeri* Green, 1977 have been described as differing from *Tusoteuthis* in regards to the shape of the blade (spoon, spear or shovel), the anterior shoulders, how far the rachis extends into the lateral fields, and the shape and cross-section of the rachis. After examining the type and dozens of other like specimens, I too am convinced that there are distinct differences in the shape of the anterior shoulder and rachis to separate these different genera.

Tusoteuthis cobbani n. sp.

Figs. 17-18

v. 1997 *Tusoteuthis longa* Logan. Larson et al. p. 99-100

Holotype: BHI-4138, reposited in the collection of Black Hills Museum of Natural History, Hill City, South Dakota.

Paratype: BHI-5847, acquired by James A. Honert in the early 1980's, from a cut bank near Wasta, SD. Reposited in the BHI Collection.

Type locality: Pierre Shale, along the Cheyenne River, Wasta, South Dakota.

Type horizon: *Baculites cuneatus* Zone, upper Campanian, Upper Cretaceous,

Etymology: After Dr. William "Bill" A. Cobban of the United States Geological Survey in Denver Colorado. Bill is an extraordinary biostratigrapher, paleontologist and geologist who fundamentally changed our understanding of the Upper Cretaceous Western Interior.

Material: Two specimens, one prepared from the dorsal side and the other split are all that are known to exist. The holotype (BHI-4138) was originally split in two, but was put back together and prepared down, exposing the dorsal side. The paratype (BHI-5847) was broken open at collection and remains a 'split' showing much of the interior structure of the gladius.

Stratigraphical and geographical range: *Baculites cuneatus* Zone (and possibly in the underlying *B. compressus* Zone), Pierre Shale, Meade and Pennington counties of South Dakota (Tab. 1).

Diagnosis: The gladius of *T. cobbani* n. sp. is spear to spoon shaped, composed of corneous, chitinous-like material. Gladius is characterized by a long, free-rachis and a blade. The rachis extends into the blade, and terminates near the middle of the blade at the conus. The blade is narrow, two and one-half times as long as it is wide, with dorsally concave lateral fields (vanes), and dorsally convex conus. On the dorsal side, the rachis forms a tall, thin ridge above the depressed lateral fields before it terminates near mid-blade. It is widest at the central portion of the vanes. The rachis consists as a single shaft. The posterior of the gladius is composed of an extension of the lateral fields (and perhaps the conus) terminating in an acute end (inferred by the specimen).

Descriptions: The holotype BHI-4138 (Figs. 18A, B) is an uncrushed, undistorted, three-dimensional gladius, consisting of a dorsal view with a nearly complete blade and a partial rachis. Its description follows the diagnosis for *T. cobbani*. The non-free rachis is 8.6 cm long, its dorsal ridge is 1.5 cm above the vanes, the width of the free rachis near the vanes is 1.1 cm; the partial free rachis is 6 cm long. The vanes are ventrally depressed, leaving a deep (1.5 cm) concave depression next to the rachis ridge. The vanes are each 3.2 cm wide; the width at its greatest point (mid-vanes) is 6.4 cm. The vanes exhibit some structure to suggest a hyperbolar zone and inner and outer asymptotes. The lateral field meets the conus with a sinuous line that extends from the end of the rachis. The dorsal shape of the conus is like that of an upside down spoon. There are well-preserved growth lines around the edge of the blade and some slight iridescence on the outer surface indicates perhaps the ostracum or hypostracum layers. The posterior termination appears to probably be acute.

Fig. 17: BHI-5847, ink sack exposed in concretion on ventral side of gladius; scale bar = 1 cm

The paratype, BHI-5847 (Figs. 18C-D), consists of an uncrushed split, showing much of the interior (ventral) structure of the gladius. It has a nearly complete conus but is missing its posterior end and most of its free rachis. The structure consists of growth lines, hyperbolar zone, inner and outer asymptotes, the median and lateral fields and conus. Some of these features are much better preserved than on the holotype. The large dorsal depression and the conus dome is well defined, the rachis ridge is encased in concretion. An exploded ink sack is preserved on the back of the ventral side (Fig. 17). The width of both vanes is 5.3 cm; the length from the anterior shoulder to the conus bulge (end of the rachis) is 6 cm; rachis diameter is 0.7 cm near the anterior shoulder.

Differential diagnosis: *T. cobbani* n. sp. differs from *T. longa* in that the blade is more spoon-shaped than in *T. longa* (the anterior shoulders are more rounded), the vanes are much more dorsally concave than could be imagined on *T. longa,* it has a large conus bulge (larger than seen on *Enchoteuthis*) and the rachis is moderately complex. *T. cobbani* n. sp. differs from *Enchoteuthis* in its more spear-like shape, rachis complexity, and how far the rachis extends into the blade.

Other

During research for this paper, I examined the illustration of Meek (1876) and was convinced that *Phylloteuthis* must be portion of an *Actinosepia*, something overlooked for over 110 years. I contacted John Pojeta of the Smithsonian Institute, he and Stephen Godfrey went to the collections, located the specimen and photographed it for this paper (Fig. 19). They also photographed a note from R. Brown dated Jan. 23 1940 which stated that he (Brown) thought *Phylloteuthis* was a cycad frond - *Nilssonia gibbsi* Newberry, 1898. Upon examination of the photos and comparison with *Actinosepia* from the Fox Hills, I too was convinced it was a leaf. Photos were sent to paleobotanist Kirk Johnson, Denver Museum of Nature and Science, for confirmation and identification and he replied, "Brown was right. It is a cycad and I would use the name *Nilssoniocladus yukonensis*." Hopefully *Phylloteuthis subovata* will no longer be published as a coleoid.

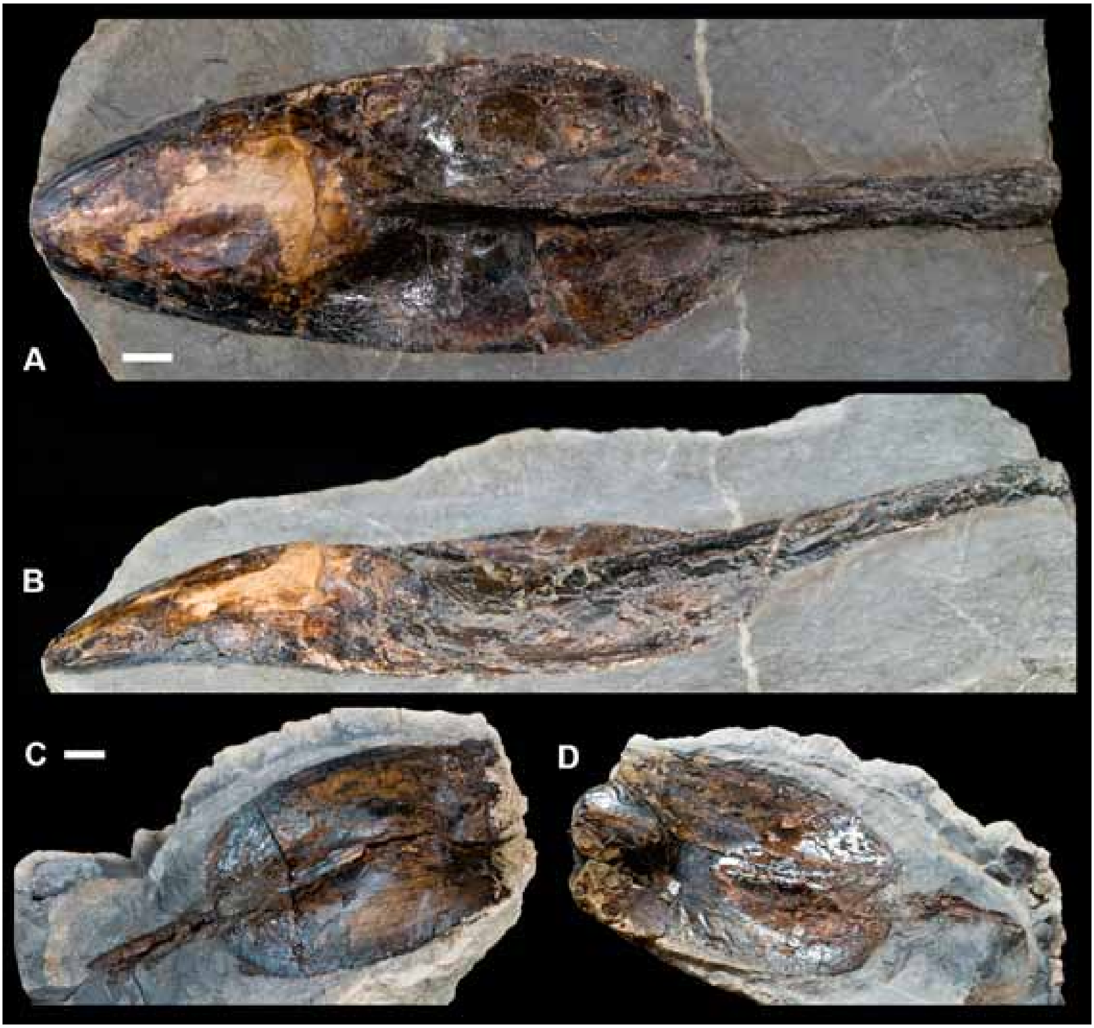

Fig. 18: *Tusoteuthis cobbani* n. sp. A) BHI-4138, holotype, dorsal view, *B. cuneatus* Zone, Pierre Shale, South Dakota; scale bar = 1 cm. B) side view of holotype; scale bar = 1 cm. C) BHI-5847, paratype, *B. cuneatus* Zone, Pierre Shale, South Dakota showing 'split' ventral view; scale bar = 1 cm. D) paratype, 'split' dorsal view; scale bar = 1 cm.

Comments

35 years ago (1974) while hunting for fossils in the Fox Hills Formation we collected a strange fossil that we could not identify, it resembled a duck's foot. We showed it to Karl Waage and he identified it as an *Actinosepia* gladius. Karl sent us a copy of Waage (1965) with a note on it "the duck foot treatise". For the next 15 years we would save every scrap of fossil coleoid for his research. Karl always said that someday he would get around to describing the fossil coleoids of the Pierre Shale and the Fox Hills Formation. That day never came but that collection became the nucleus for this paper. When Karl Waage retired from his position at Yale University he returned many of the fossil squids that we loaned him. It was at this time, I realized that someone needed to describe the Western Interior coleoids that Karl had started. As a result, this paper has been twenty years in the 'to-do-someday' stage.

While this paper brings together most of the known species of coleoids from the Campanian and Maastrichtian of the Western Interior, there is still more work to do. There are some new specimens of gladii and jaws of coleoids discovered that need descriptions. And more collecting should be done with an emphasis to locate new and better specimens from zones where the coleoids have been found as well as discovering them in zones where none have yet been recognized.

Acknowledgements

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This paper could not have been done without the assistance, cooperation and help of the following people from these different institutions: Corinne Meyers, Larry Martin, David Burnham, Talia Karim and Amanda Falk from the University of Kansas, Lawrence, Kansas gave the author access to specimens, found notes and publications, made copies and even held lights for photographs, going completely out of their way to assist the author. Dan Spivak and Brandon Strilisky of the Tyrrell Museum of Paleontology, Drumheller, Alberta allowed the author access to those collections for photographs and measurements. Mike Everhart from Oceans of Kansas Paleontology provided photographs and measurements of the FHSM Type specimens. René Trudel and Pierre Paré from Korite International and Canada Fossils, Lethbridge and Calgary, Alberta allowed me access to their land for collecting along St. Mary's River where most of the *Actinosepia* specimens at the Tyrrell Museum originated, enabling me to determine which ammonite zones *Actinosepia canadensis* came from and thus better understanding the Bearpaw Shale of southern Alberta. Melinda Frillman from the Saint Louis Science Center, St. Louis, Missouri tried to locate the type of *Belemnitella bulbosa*, but to no avail. John Pojeta from the U.S. National Museum and Stephen Godfrey of the Calvert Marine Museum, Solomons, Maryland went out of their way to photograph the type specimens of *Phylloteuthis subovata* and *Belemnitella bulbosa* at the Smithsonian. Kirk Johnson, from the Denver Museum of Nature and Science, provided taxa identification on *Phylloteuthis* and confirmed what some suspected. Cope MacClintock from the Yale Peabody Museum, New Haven also took much needed photographs, measurements and data from some *Actinosepia* in that collection for this manuscript.

Fig. 19: Type of *Phylloteuthis subovata* Meek & Hayden 1860 (actually a plant *Nilssoniocladus yukonensis*). Photo courtesy of Stephen Godfrey, John Pojeta & USNM (leaf 4 cm long x 2 cm wide).

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The oldest known (Lower Carboniferous-Namurian) protoconch of a rostrum-bearing coleoid (Cephalopoda) from Arkansas, USA: phylogenetic and paleobiologic implications

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Abstract

The early ontogeny of *Hematites barbarae*, which is the earliest known coleoid, has been unknown because the protoconch and initial chambers were truncated. Now a single specimen has been recovered that is not truncated, and therefore, the protoconch and first chambers of the phragmocone are preserved within a thick rostrum. The spherical protoconch in *Hematites* is relatively large (0.9 mm long and 1.0 mm wide), which is nearly comparable to the maximum size seen in bactritoid protoconchs (0.3 mm long and 1.0 mm wide). The *Hematites* protoconch is also larger than the protoconchs in belemnitids. The first septum is thin and appears to be calcified. It has a sub-marginal siphuncular foramen and short indistinct retrochoanitic septal neck. The latter is shown by the slight apicad curvature of the septum around the foramen. The ceacum was not observed, apparently not preserved. The protoconch shows no evidence of an enclosing membrane, which is a characteristic of the Order Belemnitida. There is no evidence of a primordial rostrum. The initial portion of the phragmocone in the Order Belemnitida shows no hatching constriction (= primary constriction), although such a feature is present in the phragmocones of the Order Bactritida. The lack of a hatching constriction may be a significant difference between early coleoids and bactritoids. Based on the protoconch morphology, namely: 1) the position of the first septum, 2) the lack of an enclosing membrane, 3) the septal foramen on the first septum, and 4) the slightly retrochoanitic shape of the septal neck on the first septum. There is strong support for the conclusion by Doguzhaeva, Mapes & Mutvei (2002) that *Hematites* can not be assigned to the Order Belemnitida or to the Order Aulacocerida, and for that reason they erected the new order Hematitida to accommodate *Hematites*, *Paleoconus*, and *Bactritimimus*. Therefore, the presence of the rostrum in the coleoid orders Hematitida, Aulacocerida, and Belemnitida can be considered a product of parallel evolutionary development. With this being the case, the Order Hematitida must stand as a separate short-lived evolutionary experiment.

Introduction

In the last 15 years, there have been great advances in revealing the wide systematic diversity of coleoid cephalopods in the Late Paleozoic. During this time, two new orders have been established, and seven new genera and species have been described. Indeed, the numerous reports of new discoveries have been phenomenal given that a mere 50 years ago, the community of fossil cephalopod workers was impressed by the report of Flower & Gordon (1959) of three uncontested coleoid taxa from the Lower Carboniferous. The specimens they described were recovered from the Lower Carboniferous (Upper Mississippian - Fayetteville Formation in the Arkansas and the Chainman Formation in Utah). Reports of Devonian and other Early Carboniferous coleoids by De Koninck (1843), Flower (1945), Termier & Termier (1950), and Bandel, Reitner & Stümer (1983) have been rejected for various reasons [see Mapes et al. (in press) for a more extended discussion and references]. However, despite these advances, the study of the initial chamber or protoconch on any of these Lower Carboniferous coleoids remained out of reach until the mid 1980's when a rostrum of *Hematites barbarae* Flower & Gordon, 1959 was recovered from the Fayetteville Shale in northern Arkansas. The rostrum was mechanically broken in the laboratory, and an intact protoconch and phragmocone was discovered inside the rostrum. Unfortunately, after this discovery the labeled specimen was misplaced and was considered lost (Doguzhaeva, Mapes & Mutvei 2002). Later, it was rediscovered in a cabinet drawer with non-related specimens. It is that specimen that is the focus of this report and its significance.

Materials & Locality

More than 100 coleoid specimens have been recovered from the lower part of the Fayetteville Shale in Arkansas during the past 40 years by R.H.M. Based on his experience, a typical collecting trip for an experienced collector in the Fayetteville Formation at selected known fossiliferous sites yields between one and four coleoid specimens per collecting trip; whereas, by comparison, hundreds and sometimes more than a thousand ammonoids (mostly crushed) and a modest number of orthoconic and coiled nautiloids can be observed and/or recovered at the same time (see Gordon (1964) for the cephalopod diversity). All of the coleoids recovered from the Fayetteville Formation are fossilized as discrete, isolated specimens in the shale or as nuclei in concretions composed mostly of phosphate and/or calcium carbonate with pyrite.

In the lower part of the Fayetteville Formation in Arkansas, phosphate concretions are locally somewhat common. These phosphate concretions are formed around a nucleus, which in most cases is a coprolite with distinctive textures, and sometimes they contain vertebrate and/or invertebrate pieces (see Mapes (1987) for a more extended discussion and examples of cephalopod mandibles recovered). Thus, as far as can be determined, many (all?) of the coleoids recovered from the phosphate concretions are parts of coprolites, and therefore, the rostrum had to have passed through the digestive tract of a predator. Detritus feeders were probably uncommon or not present since the lower Fayetteville sea bottom was probably extremely dysaerobic and/or anoxic. This interpretation is supported by the fact that phosphate/pyrite deposition requires a dysoxic/anoxic reducing sedimentary environment. This explains why there are essentially no benthic organisms such as crinoids, corals, and trilobites found in the lower part of this formation.

The phosphate concretions formed quickly and relatively early in the taphonomy of the coleoid specimens. The preservation of these and other concretion-encased specimens is excellent unless modern weathering has interacted with the pyrite. This high quality of preservation is supported by the recovery of numerous cephalopod mandibles and in some cases ammonoids with mandibles and radulae *in situ* in the body chamber of the cephalopod [see Mapes (1987), Tanabe & Mapes (1995) and Doguzhaeva, Mapes & Mutvei (1998) for examples]. The specimens recovered directly from the shale and that were not encased in concretions were typically not well preserved, with the delicate internal structures of the phragmocone and the rostrum being partly crushed or distorted and the exterior of the rostrum being "etched" by taphonomic processes. This "etching" was probably the result of biodegradation of the organic portions of the rostrum [see the ultrastructure study of Doguzhaeva, Mapes &

Mutvei (2002) for details of the organic material in the rostrum of *Hematites*]. As is pointed out in the systematic section of this report, this "etching" feature figures prominently in the systematic decisions of Flower & Gordon (1959) and Gordon (1964). In so far as can be determined, most, if not all of the material described by Flower & Gordon (1959) and refigured by Gordon (1964) was probably obtained directly from the shale and not from concretions. The specimens preserved in the phosphate concretions are ultrastructurally much better preserved (see Doguzhaeva, Mapes & Mutvei 2002). Typically, in the concretion-encased specimens, there has been little "etching" of the external surface of the rostrum and the phragmocone is usually undistorted. This is true of even those specimens that are in coprolites. Of the more than 75 specimens that have been recovered, at least 50 are preserved in phosphate concretions.

The *Hematites* specimen with the protoconch is reposited in the Ohio University Zoological Collections with the number OUZC 5700. The phosphate concretion with the protoconch was recovered from an approximately 1 km long exposure of the lower part of the Fayetteville Shale at the community of Durham, Arkansas in N ¼, W ¼, sec. 29, T14N, R28W (Durham 7 ½ minute Quadrangle). At this locality, the lower 5 m of the lower Fayetteville shale is exposed in the sides and bottom of the Middle Fork of the White River. Fossils are typically distributed throughout the shale, though the best collecting is usually in the shale near the water line where weathering and stream erosion is maximized. Typical ammonoid genera recovered are *Cravenoceras*, *Paracravenoceras*, and *Tumulites*; most are crushed. Additionally, a variety of bactritoids (see Mapes 1979), and both coiled and orthoconic nautiloids have been recovered. Gordon (1964) provided a list of most of the cephalopods from this formation in northern Arkansas. Fossil plants [see Dunn (2003) for a comprehensive summary] and sharks and other fish (Lund & Mapes 1984; Zangerl et al. 1969; J. Maisey, personal communication, 2007) have also been recovered from this exposure.

The initial discovery of the protoconch in a specimen of *Hematites barbarae* Flower & Gordon 1959 (OUZC 5700) occurred when a phosphate concretion was cracked open using a hammer on a steel anvil. The initial fracture surface exposed part of the protoconch and the phragmocone inside the rostrum (Fig. 1). The plane of the break was approximately along the lateral side of the phragmocone exposing the interior of some of the chambers. In addition to retaining the protoconch and the early part of the phragmocone, OUZC 5700 retains approximately 20 undistorted septa. In places in the phragmocone, the cameral chambers were without mineral infilling and some septa were destroyed in these parts of the phragmocone when the concretion was broken. At the orad end of the phragmocone septal telescoping and crushing occurred, and mineral infilling has obscured details of the septa in this part of the specimen. The orad terminal end of the rostrum and the associated phragmocone beyond the edge of the phosphate concretion is not preserved.

The specimen (OUZC 5700) is identified as *Hematites barbarae* on the basis of the internal features of the phragmocone and the external morphology and appearance of the rostrum. The following morphological features require special explanation.

Description of the protoconch-bearing specimen

1. Protoconch and First Septum

The protoconch is relatively large (0.9 mm long and 1.0 mm wide) (Fig. 2). Thus, the protoconch is nearly spherical and has a bactritoid-like constriction at its orad end at the position of the first septum. The external surface of the protoconch is smooth with no trace of ornament. The interior of the *Hematites* protoconch shows no evidence of an enclosing membrane. The ceacum is not preserved. The first septum is thin, and because organic membranes are not preserved in the phragmocone, the septum is probably calcified. The first septal neck is short and is distinguished by the slight apicad curvature of the septum around the septal foramen. The septal foramen in the first septum is like that in bactritoids. The foramen has a sub-marginal position, and there is no evidence of a primordial rostrum.

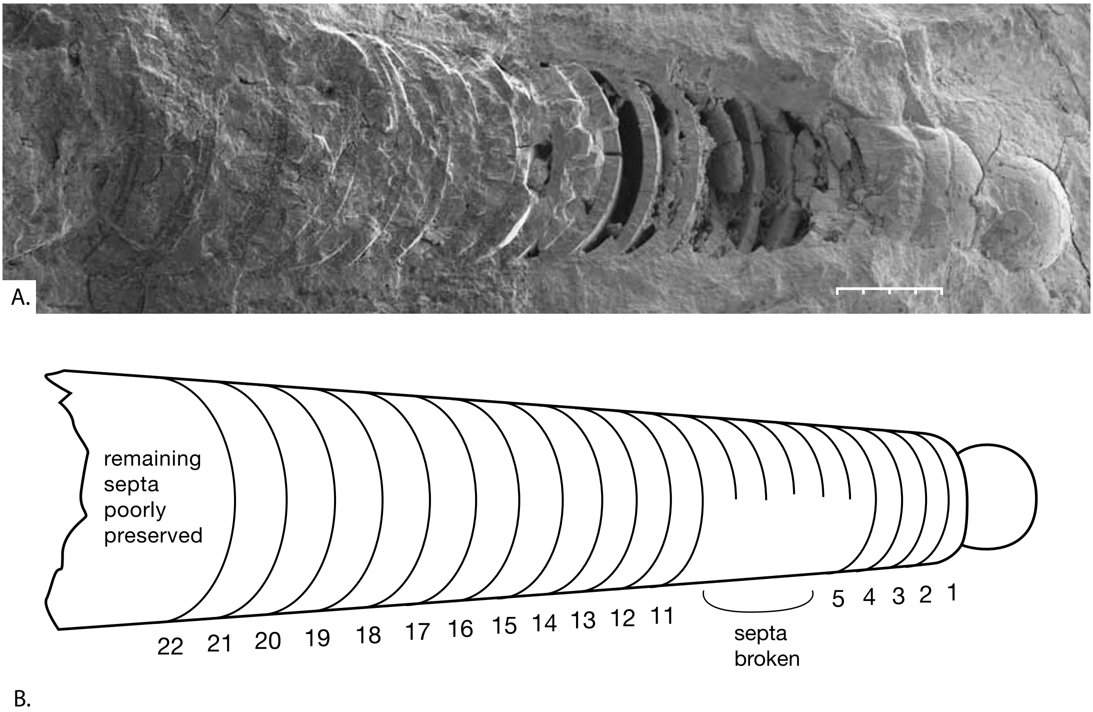

Fig. 1: A) Scanning electron photograph of *Hematites barbarae* Flower & Gordon, 1959 (OUZC 5700) from the Fayetteville Formation showing the protoconch and the phragmocone. Scale bar = 5.0 mm. B) Generalized diagram showing the positions of the protoconch and septa.

2. Phragmocone Orad of the First Septum

There are approximately 22 intact septa within the uncrushed part of the phragmocone (Fig. 1B). These septa appear to be in place, but some contemporaneous breakage during preparation has occurred. The protoconch and septa 1 - 4 and part of septum 5 orad from the protoconch are intact; whereas, septa 6 -10 have been broken and lost at the time the concretion was split in the preparation room. Septa 11 - 22 are present without significant distortion. Orad of septum 22, there are eight partial septa in place; these septa may have been originally complete prior to breakage in the laboratory. All of the septa except the first septum have episeptal and hyposeptal deposits above the cyrtochoanitic septal necks. In the apical end of the specimen some of the chambers (between septa 6 - 10) were hollow (i.e., without mineral infilling in the chambers) at the time the concretion was broken, and this caused some of the septa and deposits attached to those septa to be broken and dislodged; those pieces were lost. Orad of septum 10 the deposits and septa are well pyritized, and the deposits are sufficiently large that each episeptal deposit is in contact with the hyposeptal deposit. This configuration of the deposits, together with mineral infilling, seems to have provided septum stability, which reduced septal displacement during diagenesis and contemporary breakage.

The part of the conch orad of septum 1 to the position of about septa 3 or 4 shows a very rapid expansion from the protoconch/septum 1 position to septum 2 and then the rate of expansion of the phragmocone decreases to a uniform angle of 12 degrees. This angle of expansion continues without change into the post-embryonic shell for the length of the preserved phragmocone/rostrum. At the orad end of the specimen there are eight broken septa, and these are not considered in this discussion because they conform to and add nothing more to the descriptions provided by Flower & Gordon (1959). The septal spacing from the septum 1 to septum 3 increases slightly with the increased diameter of the phragmocone. The distance between septa 3 and 4 shows a slight decrease in distance, and as best as can be determined, the distance between septa again increases as the phragmocone diameter increases from septum 4 to the orad end of the well-preserved part of the phragmocone (septum 22).

3. Rostrum

The rostrum is similar in external appearance and ultrastructure to those illustrated by Flower & Gordon (1959) and Doguzhaeva, Mapes & Mutvei (2002). No new information was revealed during the preparation of specimen OUZC 5700.

Discussion

Bactritoid and Coleoid Comparisons

1. Hatching

Arnold, Landman & Mutvei (1987) provided an extensive discussion on the background and shell characteristics of *Nautilus* at the time of hatching. Among the features they list as characteristics are the nepionic constriction and the septal approximation; either of these features can serve as hatching signals that can be applied to bactritoids and coleoids.

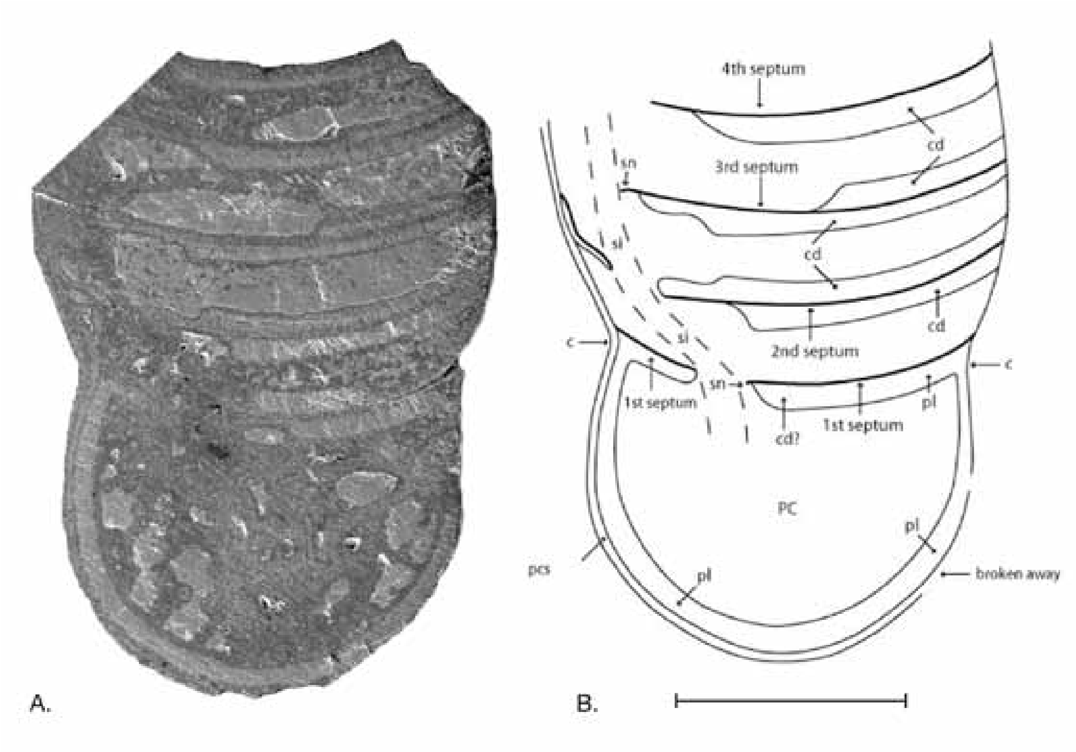

Fig. 2: A) Scanning electron photograph and B) diagram to scale with the photograph in A), respectively, of the protoconch and initial chambers of *Hematites barbarae* (OUZC 5700) showing the positions of the septa, the constriction at the end of the protoconch, and the first four septa at the beginning of the phragmocone shaft. The following features are indicated: PC = protoconch, si = siphuncle, cd = cameral deposit, c = constriction, pl = pyritized lining, sn = septal neck, pcs = protoconch shell. Scale bar = 0.5 mm.

The time of hatching in the bactritoids (*sensu Bactrites*) is marked by a gradually developed constriction, which is seen as a diameter decrease of the body chamber at the time the third septum is secreted (see discussion in Mapes & Nützel 2009; Doguzhaeva 2002: Pl. 1, Fig. 1; Pl. 2, Figs. 1-5; Pl. 4, Figs. 5, 6, 10; Pl. Pl. 8, Figs. 1, 3, 4). While this decrease is gradual in bactritoids, it is much more pronounced in ammonoids where it forms a conspicuous constriction at about one whorl (see Landman, Tanabe & Shigeta 1996 for an extensive discussion). This difference is probably due to the way that the embryonic shell in bactritoids and ammonoids is secreted. In *Nautilus* the hatching constriction is relatively faint (Stenzel 1964), and it may not be present at all or expressed as a slight constriction in some orthoconic nautiloids (R.H.M., unpublished observation). In *Hematites*, there is no constriction, and this may prove true for all Paleozoic coleoids. If so, this may eventually prove to be a characteristic that can be used to separate the embryonic phragmocones of bactritoids from those of coleoids. However, we are reluctant to make this determination on the basis of a single specimen.

The other characteristic that can be used is the decrease in septal spacing followed by an increase that coincides with the timing of the constriction in the earliest part of the shell. In *Nautilus* this change takes place between septa 7 and 8 (Stenzel 1964), and in bactritoids (*sensu Bactrites*) it takes place between septa 3 and 4 (Mapes & Nützel 2009). Additionally, in bactritoids sometimes the fourth septung is inclined while the first three septa are transverse; this inclination is probably due to the decreased diameter of the phragmocone shaft (i.e. the constriction) at the time of hatching.

Using septal approximation as a hatching guide, the time of hatching in *Hematites* is indicated by the slight decrease in septal spacing between septa 3 and 4. There is no indication of a phragmocone constriction and there is no inclination of the septa. With the reservation that this observation is based on a single specimen, it is possible that the lack of inclined septa in the early stage of phragmocone development may be an additional indicator of how bactritoids can be separated from early coleoids.

2. Protoconch

The early embryonic shell of the bactritoids (*sensu Bactrites*) has a nearly round to slightly egg-shaped protoconch, which is sharply separated by a constriction that separates the protoconch from the shaft of the phragmocone. The protoconch of *Hematites* is relatively large (0.9 mm long and 1.0 mm wide) with a conspicuous constriction separating the protoconch from the phragmocone shaft (Kröger & Mapes 2007).

In comparison with co-occurring bactritoid protoconchs from the Fayetteville Formation, only the protoconch for *Bactrites fayettevillensis* Mapes 1979 is known. The bactritella (= embryonic bactritoid shell) has a protoconch that is 0.6 mm long and wide and has a strong constriction separating the protoconch from the phragmocone shaft. By comparison, the protoconch on the *Hematites* is approximately twice as wide. However, other bactritoid protoconchs from the Lower Carboniferous (see Mapes 1979) range in size both above and below that of the Lower Carboniferous coleoid from the Fayetteville Formation, and all have a conspicuous constriction between the protoconch and the phragmocone shaft.

A comparison of Upper Carboniferous coleoid protoconchs is also not especially informative. The only coleoid protoconch known from this time interval is that of *Mutveiconites* from Russia and a single specimen of this genus from Texas [Doguzhaeva, (2002) and Doguzhaeva, Mapes & Dunca (2006), respectively]. The coleoid protoconch of *Mutveiconites* from Texas, which is sectioned almost on the plane of symmetry, is 0.5 mm long and 0.3 mm wide, and therefore, it is also smaller than that of the *Hematites* coleoid specimen. However, in the latter case the protoconch measurements of the Texas specimen are less than they would be if the section plane was through the siphuncle. Even with this observed measurement problem and the overall limited sample, this feature is not sufficiently different in size or shape to allow separation of Carboniferous coleoids from bactritoids.

3. Phragmocone Angle

There is a significant difference in the angle of expansion of the *Hematite*s phragmocone in the embryonic through the post-embryonic growth stages (12 degrees) when compared with some Carboniferous bactritoids. Some bactritoids have relatively small phragmocone angles (< 5 degrees) and others have relatively large phragmocone angles (> 30 degrees) with many taxa between these two expansion rates (see Erben (1964), Shimansky (1954), Mapes (1979) for bactritoid examples). However, even though *Hematites* has a somewhat large expansion rate, this feature alone cannot be used to reliably separate bactritoids from coleoids.

Post-Hatching Development

1. Phragmocone and Rostrum Relationship

Despite examining and sectioning the coleoid specimens in their collection, Flower & Gordon (1959) were not able to recover a specimen with a protoconch. Because of this, they were not able to determine any of the characteristics of the morphology of the embryonic shell or the early post hatching growth of the phragmocone and rostrum. Thus, in the sectioned specimens they prepared, examined, and illustrated, all were lacking the protoconch and early chambers, even though the rostrum was rather robust and, diagenesis permitting, their specimens should have preserved this critical early stage of shell development. Flower & Gordon (1959: 839) were puzzled by this, and they tentatively speculated that the missing early parts of the phragmocone could be due to reabsorption, which has been suggested to occur in some Mesozoic belemnite specimens that lacked the protoconch and early chambers [for example see Krymgol'ts (1958)].

We reject the possibility of reabsorption in these early Carboniferous coleoids because 1) there is no evidence there was any mantle tissue inside the rostrum after the rostrum was secreted that could have reabsorbed the phragmocone and 2) there is a large number (*n* = 100+) of coleoid specimens (most are *Hematites barbarae,* several appear to be *Paleoconus sp.*) from two geographically separated regions (Arkansas and Utah are separated by a distance of approximately 1,725 km) and basins/stratigraphic units (i.e., the Fayetteville Shale and the Chainman Shale – these faunas from these *Hematites* -bearing shale intervals are thought to be isochronous) that are lacking the early ontogenetic portion of the phragmocone. Indeed, the specimen described herein is the only specimen that has a preserved protoconch and early chambers of the phragmocone.

Having rejected the reabsorption hypothesis, we suggest as an alternative hypothesis that rostrum development was dependent on either involuntary loss of the apical end of the phragmocone prior to the secretion of the rostrum, or if sufficient maturity was attained without the loss of the apical end, the animal would begin depositing the rostrum material as part of the maturation process. To determine the loss of the apical end of the phragmocone as a case of involuntary decollation, some of the best-preserved specimens from the Fayetteville Shale were selected and various features of the phragmocone and rostrum were measured to see if any trend was apparent or if it is a case of random removal (Table 1). These features include the total length of the specimen, the diameter of the terminal apical septum, the overall diameter of the specimen at the terminal septum, and the length of the rostrum from the end of the terminal septum to the apical tip.

Comparisons of these features (Fig. 3A-E) indicate that the loss of the early phragmocone is random, and that it was not a genetically programmed separation that occurred at a specific stage of the ontogeny. Thus, there is support for our contention that the reabsorption hypothesis cannot be applied to *Hematites* as an explanation for the loss of the early developmental stages of the phragmocone.

These conclusions imply that the phragmocone was either not covered by mantle tissue early in ontogeny or that the mantle tissue covering the phragmocone was very thin. Also, it implies that the phragmocone and eventually the phragmocone/rostrum combination were located at the apical end of the animal and that the phragmocone and perhaps the phragmocone/rostrum formed the apical tip of the animal.

2. Ontogenetic reconstruction

The loss of the apical end of the phragmocone in orthoconic cephalopod shells (orthoconic nautiloids, bactritoids, and even ammonoids like *Baculites*) during life was probably more common than is presently realized because today the shells of these animals are most commonly recovered

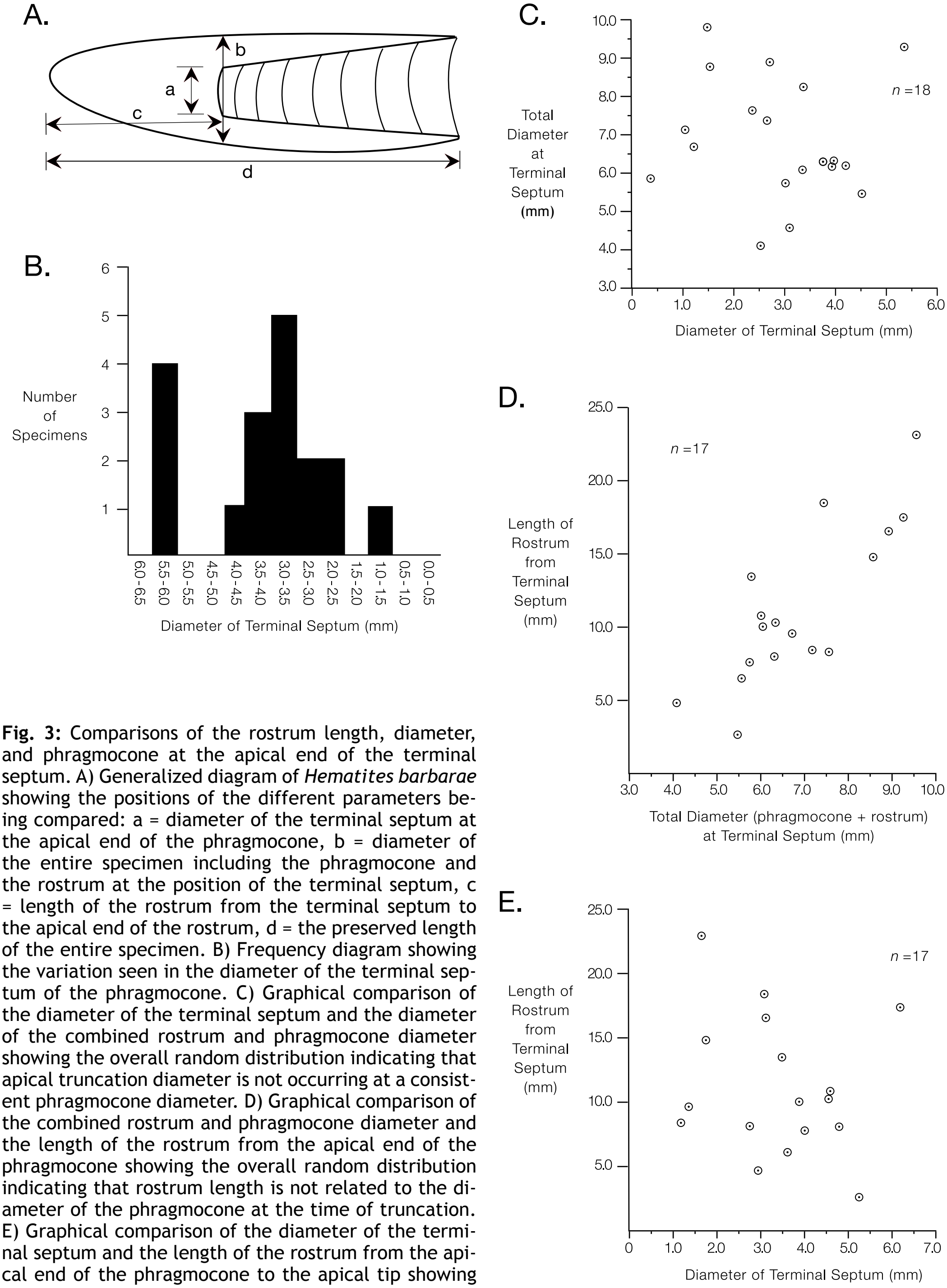

Fig. 3: Comparisons of the rostrum length, diameter, and phragmocone at the apical end of the terminal septum. A) Generalized diagram of *Hematites barbarae* showing the positions of the different parameters being compared: a = diameter of the terminal septum at the apical end of the phragmocone, b = diameter of the entire specimen including the phragmocone and the rostrum at the position of the terminal septum, c = length of the rostrum from the terminal septum to the apical end of the rostrum, d = the preserved length of the entire specimen. B) Frequency diagram showing the variation seen in the diameter of the terminal septum of the phragmocone. C) Graphical comparison of the diameter of the terminal septum and the diameter of the combined rostrum and phragmocone diameter showing the overall random distribution indicating that apical truncation diameter is not occurring at a consistent phragmocone diameter. D) Graphical comparison of the combined rostrum and phragmocone diameter and the length of the rostrum from the apical end of the phragmocone showing the overall random distribution indicating that rostrum length is not related to the diameter of the phragmocone at the time of truncation. E) Graphical comparison of the diameter of the terminal septum and the length of the rostrum from the apical end of the phragmocone to the apical tip showing there is no relationship in terms of rostrum growth and the diameter of the terminal septum.

Tab. 1: Measurements of specimens of *Hematites barbarae* selected because of preservational state. Features that are measured include A) diameter of the terminal septum at the apical end of the phragmocone, B) Overall diameter (rostrum and phragmocone) at the terminal septum at the apical end of the phragmocone, C) length of the rostrum from the terminal septum at the apical end of the phragmocone to the apical tip of the specimen, and D) total length of the specimen. This latter measurement proved to be unreliable because of the lack of complete preservation and earlier preparation in the laboratory removed parts of specimens; these specimens are noted with an asterisk.

Specimen No	**A**	**B**	**C**	**D**
OUZC 5701	3.4	5.7	7.6	19.1
OUZC 5702	4.5	5.5	2.8	17.0
OUZC 5703	4.2	6.3	8.0	16.3*
OUZC 5704	3.9	6.3	10.2	22.7
OUZC 5705	3.0	5.7	13.5	22.8
OUZC 5707	2.7	8.9	16.7*	33.2*
OUZC 5708	1.2	6.7	9.7	24.1
OUZC 5709	1.4	9.7	23.0	36.2*
OUZC 5710	2.3	7.6	8.2	21.7
OUZC 5711	1.5	8.7	14.9	34.7
OUZC 5712	3.3	6.1	10.0*	34.4*
OUZC 5713	1.0	7.2	8.4	31.1
OUZC 5714	3.3	8.3	----	----
OUZC 5715	2.6	7.4	18.4*	22.6*
OUZC 5718	3.9	6.0	10.9	27.0*
OUZC 5719	5.3	9.3	17.4	22.8
OUZC 5720	2.5	4.1	4.9	18.9
OUZC 5721	3.1	5.6	6.5	13.8*
OUZC 5722	3.1	4.9	5.7	22.5

as pieces and segments when they are collected from outcrops. Thus, it is usually impossible to determine if the shells were broken in life, as there has generally been reworking by various agents after death but prior to burial, diagenetic crushing and dissolution, and even as the specimens were weathered or broken out of the outcrop when they were collected. Most (perhaps all) of these considerations do not apply to the phragmocones that are encased within the rostrum in coleoids.

Within the Coleoidea, well-developed rostra are present in the coleoid orders Belemnitida, Aulacocerida, and Hematitida. In the reconstruction presented below, we are not considering the orders Belemnitida and Aulacocerida; although, it seems likely that some parts of the scenario presented herein may be applicable to these two orders as well.

There is a wide variation in shell diameter seen on the apical ends of the phragmocone within uncrushed rostra (Fig. 3). Based on this supporting information, the conclusion that Doguzhaeva et al. (2002) suggested is correct, in that when the initial chambers of the phragmocone of *Hematites* was lost during growth, this loss triggered the development of the rostrum. However, the specimen of *Hematites* (OUZC 5700) with its protoconch and early chambers intact and covered by a well-developed rostrum adds a new dimension to the growth scenario proposed by Doguzhaeva et al. (2002). We suggest here that if the protoconch and early chambers were not lost in *Hematites*, at a certain stage of growth the animal would automatically begin to secrete a rostrum. This would explain the presence of the protoconch and initial chambers in OUZC 5700. If this scenario is correct, then the rostrum development in *Hematites* was probably inevitable even if the early growth stages of the phragmocone were not lost by involuntary decollation.

Conclusion

Separation of Upper Paleozoic bactritoid and coleoid protoconchs and the early developmental stages of shell growth of the phragmocone of these two cephalopod groups has been a recurring problem for researchers. Unfortunately, the protoconch of the *Hematites* protoconch and initial chambers described herein do not provide any resolution to this problem, although the presence or absence of a hatching constriction may eventually prove to be an important feature in separating the two groups. At the present time, the only way of reliably determining if a specimen is a coleoid is to have a definitive feature such as an enclosing membrane at the first septum, ink, arm hooks, ultrastructure, or rostrum development. Thus, specimens that lack these distinctive coleoid features must be relegated artificially to the Bactritoidea at this time.

The protoconch and initial chambers of OUZC 5700 provide important new information about the early phylogeny and ontogenetic development of the oldest known coleoids. Based on the protoconch morphology, namely: 1) the position of the first septum, 2) the lack of an enclosing membrane, 3) the septal foramen on the first septum, and 4) the slightly retrochoanitic shape of the septal neck on the first septum, there is strong support for the conclusion by Doguzhaeva, Mapes & Mutvei (2002) that *Hematites* can be assigned neither to the Order Belemnitida nor to the Order Aulacocerida. Thus, they were correct in erecting a new order Hematitida to accommodate *Hematites*, *Paleoconus*, and *Bactritimimus*. Therefore, we consider the presence of the rostrum in the coleoid orders Hematitida, Aulacocerida, and Belemnitida to be a product of parallel evolutionary development. With this being the case, and the relatively short time span [Lower Namurian = Upper Mississippian (Middle Chesterian)] that is presently known to produce coleoids that can be assigned to the Hematitida, this order must stand as a distinct, short-lived, evolutionary experiment in coleoid evolution.

In addition to the conclusions that the recovery and analysis of the protoconch and initial chambers of OUZC 5700 provide, there is also now a new understanding of the unique nature of the ontogenetic development that took place in *Hematites* and its evolutionary implications. Based on the available specimens, the conclusion of Doguzhaeva, Mapes & Mutvei (2002) is correct in that when the initial chambers of the phragmocone of *Hematites* were lost during growth, this loss probably triggered the development of the rostrum. For this involuntary loss of the apical end of the phragmocone to occur, the phragmocone must have been at the apical end of the body of the animal and it may or may not have been covered by a thin mantle when the involuntary decollation occurred. After the apical end breakage event, the remaining end of the phragmocone was mantle covered and rostrum secretion was initiated. The specimen with the protoconch adds an additional dimension to this growth scenario, because OUZC 5700 retains the protoconch and initial chambers, and there is a substantial rostrum in place. Thus, this single specimen forces one to conclude that if the protoconch and early chambers were not lost in *Hematites*, then at a certain stage of growth the animal would automatically begin to secrete a rostrum. This would explain the completeness of the phragmocone in OUZC 5700. Based on this, one must conclude that rostrum development in *Hematites* was inevitable even if the early growth stages of the phragmocone were not lost by involuntary decollation.

Given these conclusions, we consider the primary function of the rostrum in *Hematites* to be one of controlling buoyancy and equilibrium. The addition of the rostrum was a necessary part of the damage control to maintain buoyancy and equilibrium control despite massive injury to the phragmocone, which was the primary buoyancy and equilibrium control mechanism.

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A new late Carboniferous coleoid preserved with an ink sac from Nebraska, USA

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Keywords: Coleoidea, Upper Carboniferous, Floweritidae n. fam., *Flowerites bellevuensis* n. gen., n. sp., taphonomy

Abstract

The presence of an ink-filled sac within the crushed shell of an orthoconic cephalopod from the Stark Shale (Upper Carboniferous - Kasimovian), southeastern Nebraska, USA, leads to the conclusion that the specimen should be assigned to the Subclass Coleoidea. Because of incomplete preservation, interpretation of some of the shell morphology of this coleoid remains unresolved. The fossil shows the imprint and impression of a crushed, bluntly pointed shell with a short final chamber that contains a relatively large undistorted ink sac bearing the mural parts of four septa on its top. Septal fragments and/or shell debris are missing in the shale surrounding the fossil. The posterior of the shell appears to be missing. Three taphonomic scenarios are considered to explain the preservation of the specimen. The coleoid specimen is described as *Flowerites bellevuensis* n. gen. et sp. and referred to the family Floweritidae n. fam. with an as yet uncertain ordinal affiliation.

Introduction

During the First Palaeontological Congress in Sydney, Australia in 2002 the discovery of the oldest ink sacs of the Late Carboniferous coleoids was reported (Doguzhaeva et al. 2002). Before that report, the oldest known coleoid identified as *Phragmoteuthis*? *ticinensis* with preserved ink sac was from the Middle Triassic from Switzerland (Rieber 1970). In 2004, Doguzhaeva, Mapes & Mutvei (2004) described numerous cases of fossil ink in coleoids in the Upper Carboniferous and Mesozoic including specimens from the Stark Shale in Nebraska. Later, Mapes & Weller (2003) and Mapes, Weller & Doguzhaeva (2007, in press) reported that ink was preserved in a Lower Carboniferous coleoid from Montana, USA.

In the last decade several new fossil coleoids from the Late Paleozoic have been described (see Table 1). Based on the new discoveries, it seems to be a reasonable assumption that the general lack of fossilized coleoid specimens is the result of their low preservational potential, and that there was, in fact, a comparatively wide systematic diversity of coleoids living in marine environments during the Late Paleozoic. Another essential part of the coleoid rarity problem is the difficulty of separation of some orthoconic phragmocones of orthoconic nautiloids and bactritoids from those of phragmocone-bearing coleoids in the Paleozoic. This recognition difficulty lies in the fact that some orthoconic cephalopods (especially the Bactritoidea) have a phragmocone that can be identical to that of coleoids. In general bactritoid phragmocones have a ventral margin siphuncle with cyrtochoanitic or orthochoanitic septal necks, a simple shell ultrastructure, and a well-developed body chamber capable of containing the entire animal. Many Paleozoic coleoids can have most of the above bactritoid features, but they can be separated from the bactritoids by the presence of a short final chamber, ink sac, arm hooks, and a rostrum or similar structure deposited on the outside of the phragmocone. Using the above criteria, the evaluation of a set of fossils from the Stark Shale (Late Carboniferous - Pennsylvanian, Late Missourian = Kasimovian) in Nebraska has yielded a new coleoid cephalopod. The coleoid assignment is based on the presence of a well-preserved flask-like ink sac. Although the shell of the specimen is incompletely preserved, the overall shape of the shell of the single specimen can be determined, and some details of the phragmocone are present. The specimen is described as a new genus and species and referred to a new family of yet uncertain order affiliation.

Background Information & Material

1. Location and stratigraphy

The specimen was collected from a limestone quarry in the Upper Carboniferous (Pennsylvanian - Missourian) near the community of Bellevue, Nebraska (Fig. 1). In that region, the Winterset Limestone overlies the Stark Shale. Both the underlying Canville and Winterset limestones are mined and the thin (approximately 1 meter thick) Stark Shale, which is considered waste rock, is removed as irregular blocks and piled in abandoned parts of the quarry. After weathering, these shale blocks can be split along bedding planes to expose fossils.

2. Preservation

The Stark Shale in this region is rich in phosphate, which occurs as discrete ovoid concretions and as thin plates between the bedding planes. The shale is currently interpreted as having been deposited in an off shore, outer shelf environment in relatively deep water with the bottom of the water column being anoxic and conodont-rich (Heckel 1986, 1994). Other fossils in the shale include iniopterygians (Zangerl & Case 1973), articulated sharks and other fish tentatively assigned to the platysomoids and paleoniscoids, rare, small-sized, tightly umbilicate crushed ammonoids tentatively identified as *Neodimorphoceras*, *Schistoceras*, and *Gonioloboceras*, and arthropods including concavidcarids, tyrannophonitids, eocarids, and eurypterids (Schram 1984). This is the first coleoid cephalopod to be described from this unit. The fossil coleoid is split into a part and counter part (Holotype UNSM 16567A and B); both parts are reposited at The University of Nebraska State Museum (UNSM), Lincoln, Nebraska.

Taphonomic analysis

Because of the burial conditions and the way the specimen was exposed when collected, some unusual morphologic features are apparent on the crushed specimen (Fig. 2). These features include 1) the imprint of an incomplete body chamber that is too small to accommodate the organs of the coleoid, 2) relatively large undistorted ink sac filled with solidified ink, and the impression of the mural parts of 4 septa with precise septal spacing on top of the undistorted ink sac, 3) the overall lack of septal fragments in the specimen while the mural parts of the septa are well preserved, and 4) the lack of shell debris near the fossil impression.

The posterior tip of the shell is not well preserved and appears to be missing; however, there is a well-defined outline and impression of the shell

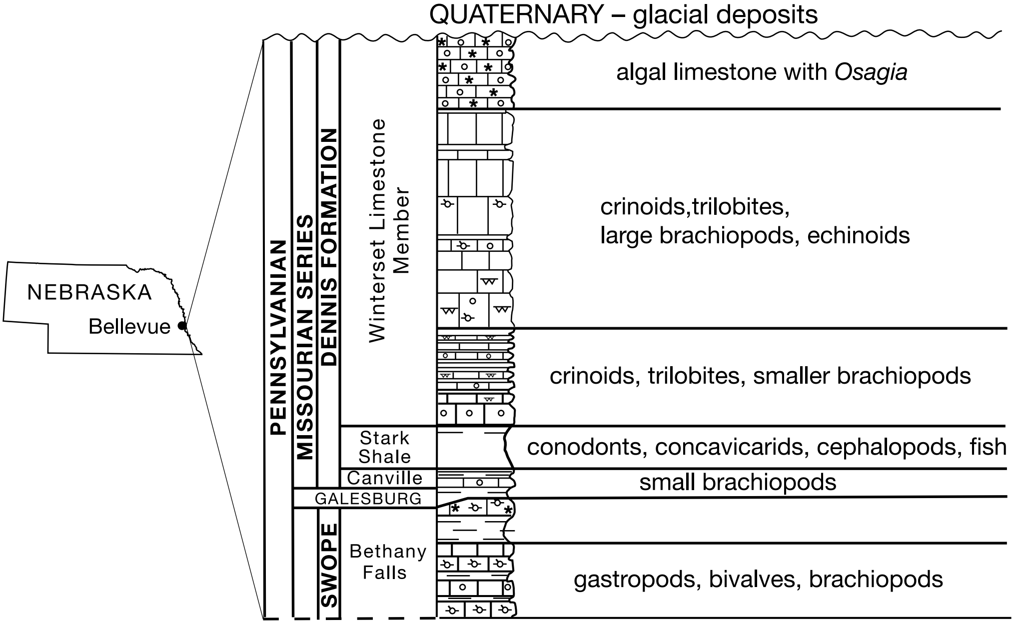

Fig. 1: Map and stratigraphic section showing the stratigraphic position of the Stark Shale. Note that this unit is considered the deepest part of the transgressive/regressive sequence, and that this shale unit is considered to have been deposited under anoxic conditions.

shape including the body chamber with some shell material preserved within the outline. We can visualize three potentially possible taphonomic scenarios that could be used to explain this unusual combination of morphologic features. The possibility that the shell damage and unusual position of the ink sac observed in the specimen could be the product of a predatory attack is a fourth possible scenario. However, we have concluded that there is no evidence to support this possibility including the fact that the body chamber, which must have contained some soft tissues, appears to be undamaged. Thus, this fourth scenario has been eliminated from additional consideration.

1. Scenario One (Fig. 3)

The phragmocone and soft body of the specimen were internal with a mantle coating. The final chamber can be viewed as relatively short, and given the volume of the ink sac, it could not have contained all of the visceral mass of the animal. The phragmocone was in a dorsal position above the soft body (panel A). When the animal died, the carcass settled to the bottom where the ink in the ink sac solidified (panel B). The animal was eventually buried, and during compaction, the septa collapsed directly onto the ink sac, which was filled with solidified ink. Diagenesis removed parts of the shell and septal fragments leaving traces of the mural parts of the septa on the ink sac (panel C). A few traces of the septa are present on the orad end of the ink sac, and this, plus the distribution of the shell fragments, supports the contention that the phragmocone was positioned over some of the viscera including the ink sac during life.

The nautiloid Order Ascocerida had a body plan at maturity that placed the phragmocone over the visceral mass of the body [see Furnish & Glenister (1964) for a more complete discussion of the Ascocerida]. Some modern coleoids such as the cuttlefish also have a similar (mineralized phragmocone over the internal organs including

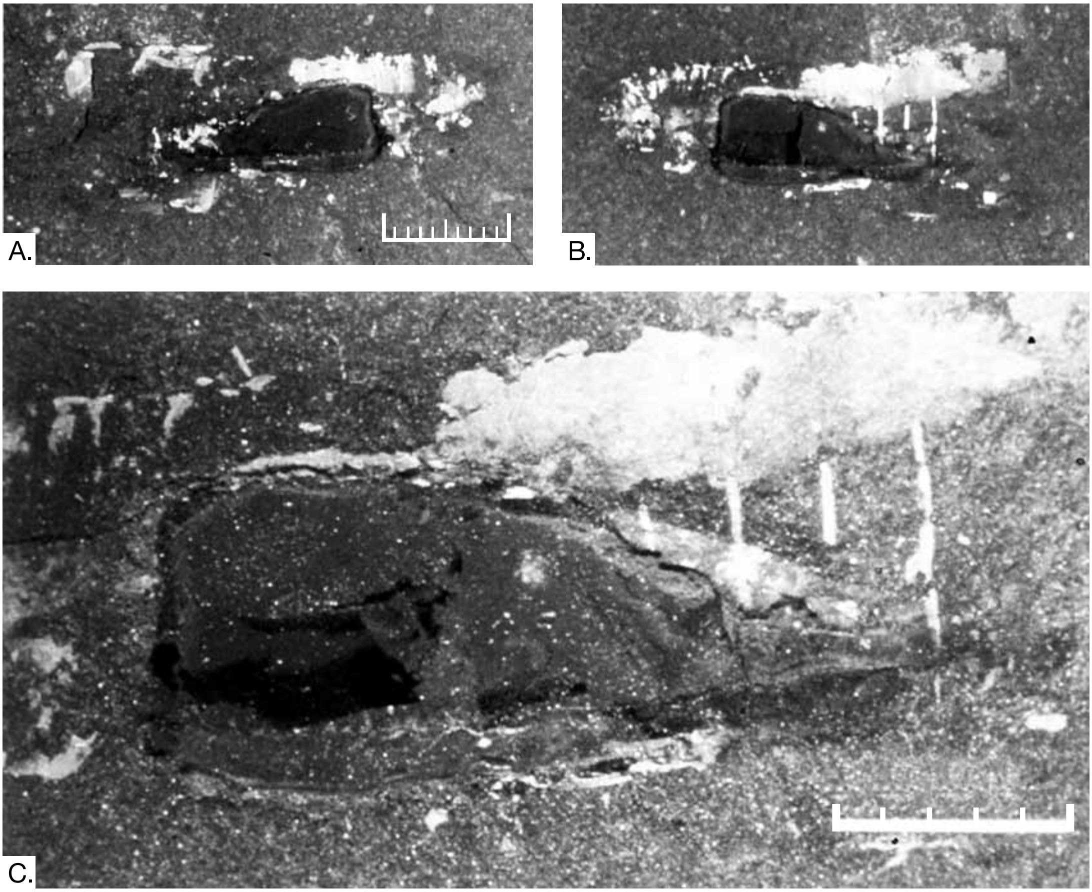

Fig. 2: A, B) Part and counter part (Holotype USNM 16567A and B, respectively) of *Flowerites bellevuensis* n. gen. et sp. C) Enlargement of USNM 16567 B showing the septa impressed onto the ink sac; scale bar = 1 mm.

the ink sac) body plan. Thus, such a body plan is not an unknown condition in the Coleoidea. However, coleoids are not known to have this kind of body plan in the Paleozoic or early Mesozoic.

2. Scenario Two (Fig. 4)

The phragmocone and soft body of the specimen were internal with a mantle coating as previously indicated in scenario 1. The phragmocone and body chamber were in a normal orthoconic nautiloid/bactritoid orientation. The shell wall and the septa were thin (panel A). After the animal died and the carcass settled on the bottom, the ink in the ink sac solidified (panel B). After burial and when lithostatic and hydrostatic pressures were crushing the shell, the septa collapsed, the solidified ink in the ink sac was pushed into the phragmocone. As the phragmocone collapse was completed, the mural parts of at least four septa were impressed on the ink sac (panel C).

The mechanism by which the ink sac was moved laterally into the phragmocone is uncertain. We can suggest that the ink sac was moved inside the phragmocone through the broken septa as the shell collapsed. If the shell simply collapsed, the fragments of the septa and the septal necks should be present. If the shell imploded and blew out the apical end of the phragmocone after burial, then the shell fragments of the phragmocone and the missing pieces of the septa and septal necks should be in the shale around or near the apex of the shell; there are no shell fragments at the apex of the specimen.

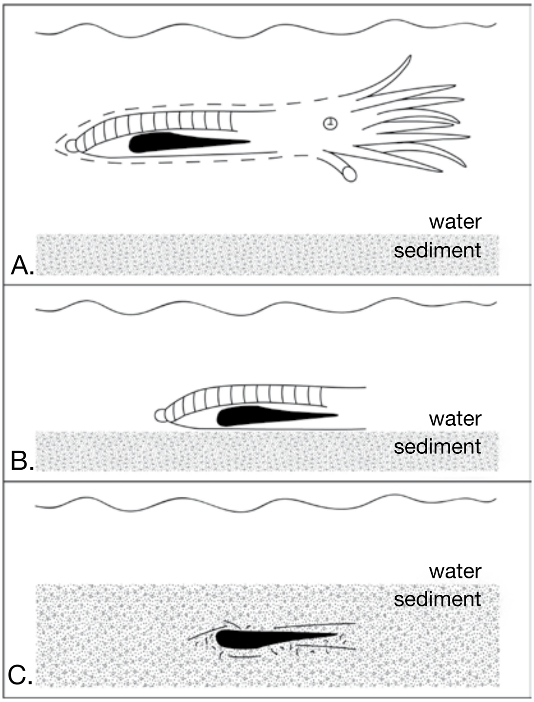

Fig. 3: Taphonomic scenario 1. Panel A is a representation of the animal living in the water column. The dashed line around the animal represents the mantle tissue. Panel B represents the animal dead on the seafloor prior to burial with the ink sac intact. Panel C represents the crushed shell after burial.

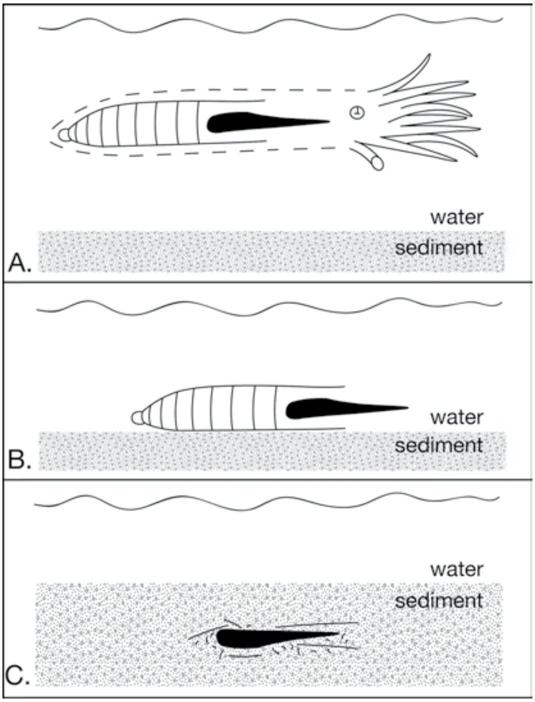

Fig. 4: Taphonomic scenario 2. Panel A is a representation of the animal living in the water column. The dashed line around the animal represents the mantle tissue. Panel B represents the animal dead on the seafloor prior to burial with the ink sac intact. Panel C represents the crushed shell after burial.

3. Scenario Three (Fig. 5)

The phragmocone was positioned in a normal nautiloid/bactritoid phragmocone/body orientation with the short body being in front of the relatively short final chamber at the orad end of the phragmocone; the entire shell was probably covered by the mantle (panel A). At the time of death, the ink solidified quickly and then the gas-filled chambers in the phragmocone imploded as the specimen drifted slowly to the bottom (panel B). The implosion destroyed the apical end of the shell, removed most of the thin septal fragments, and allowed the ink sac to simultaneously move into the damaged phragmocone. When the animal settled to the bottom, all of the phragmocone septa were breached, and the ink sac had moved into the phragmocone. After burial, the phragmocone collapsed leaving the impressions of the mural parts of the septa on the ink sac (panel C).

Of the three scenarios presented above, the third seems to us to best explain all of the morphological features that are present on the specimen. During an open water implosion, the expulsion of the cameral gases as the animal settled to the bottom would have blown out the apex of the phragmocone. This would remove many of the septal fragments, which would fall away from the main body of the animal. Also, by following scenario 3, an as yet undiscovered anatomical relationship of the phragmocone –ink sac position (see scenario 1 above) does not have to be justified. However, we recognize that scenario 1 cannot be completely eliminated, and that with new discoveries in the future, scenario 1 may become a viable option to explain the relationships seen

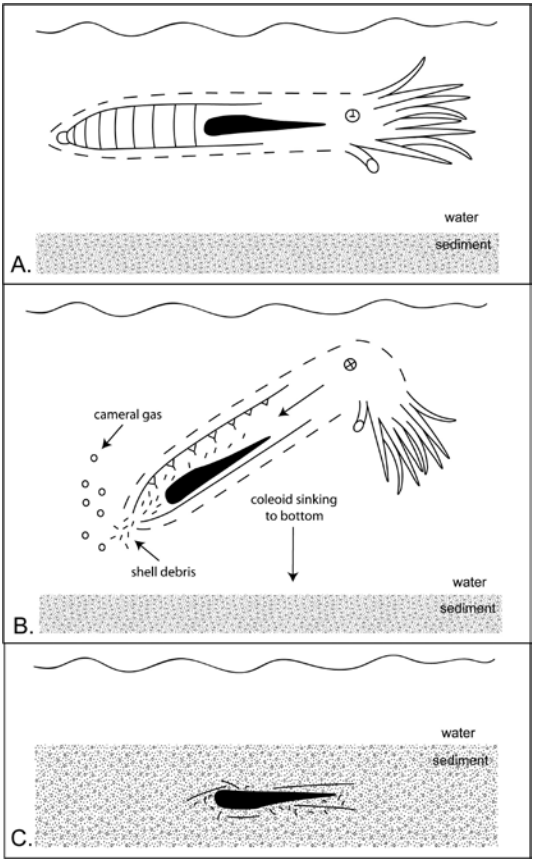

Fig. 5: Taphonomic scenario 3. Panel A is a representation of the animal living in the water column. The dashed line around the animal represents the mantle tissue. Panel B represents the animal imploding in the water column as it sinks to the seafloor. The fragmentation of the septa and the explosive release of cameral gases from the apical and allowed the solidifying ink in the ink sac to move apicad prior to burial. Panel C represents the crushed shell after burial with an uncrushed ink-filled ink sac.

in this coleoid specimen from the Stark Shale. If this proves to be the case the ordinal assignment of the specimen must be reconsidered. One item that is not explained adequately is how fast does the coleoid ink in the ink sac solidify? Would this happen fast enough so that the ink solidified in the slowly descending coleoid before the phragmocone of the animal imploded? These questions remain unresolved at this time.

Systematic paleontology

Class CEPHALOPODA Cuvier, 1797
Subclass COLEOIDEA Bather, 1888

Discussion: The ordinal assignment of *Flowerites* is uncertain at present. *Flowerites* does not show a rostrum; however, the possibility that the apical part of the phragmocone was covered with a small-sized, loosely mineralized rostrum in a similar way as that in *Saundersites* (Doguzhaeva, Mapes & Mutvei 2007b: Fig. 6.1A, B) cannot be eliminated.

In terms of morphological similarities of described Carboniferous taxa at the generic level belonging to the superorder Belemnoida, Flowerites is more closely related to Donovaniconus in that both 1) lack a well-developed rostrum, (the apical portion of the shell has not yet been observed in either genus, and the possibility that a small rostrum is present cannot be eliminated at this time), 2) have a breviconic phragmocone with closely spaced septa, 3) have a relatively long body chamber as compared to the length of the phragmocone, and 4) have a large mural attachment of the septum to the shell. Donovaniconus differs from Flowerites in the fact that the final chamber in Donovaniconus is sufficiently long to accommodate the internal organs (gills, crop, stomach intestinal tract, ink sac, mantle cavity) of the animal, and this is not the case for Flowerites. In fact, it is possible that the final chamber of Flowerites is mostly obsolete as a structure to protect the internal organs and that it mainly functioned as a support and muscle attachment structure as does the pen in modern squids.

The order Donovaniconida as established by Doguzhaeva, Mapes & Mutvei (2007b) presently contains genera with a weakly developed rostrum. Emendation of this order or the ordinal reassignment of the genera that have a poorly mineralized rostrum (Saundersites, Gordoniconus, Rhiphaeteuthis) from the genus (Donovaniconus) that does not show this critical feature (because the apical end of this genus has not yet been recovered) is desirable. Because of the confusion in the ordinal assignments of the rostrum bearing genera presently assigned to the Donovaniconida, we will leave the ordinal assignment of Flowerites as undetermined at this time.

Geological range: The geologic range of this order is currently restricted to the Carboniferous.

Table1: Classification of Paleozoic coleoids (modified from Nishiguchi & Mapes 2008; Doyle, Donovan & Nixon 1994; Doguzhaeva, Mapes & Mutvei 1999, 2002a, b, 2003, 2007b; Haas 2003).

Subclass COLEOIDEA Bather, 1888

- Superorder BELEMNOIDA Gray, 1849 (Early Carboniferous – Late Cretaceous)
  - Order HEMATITIDA Doguzhaeva, Mapes & Mutvei, 2002b (Carboniferous)
    - Family HEMATITIDAE Gustomesov, 1976
      - *Hematites* Flower and Gordon, 1959 - Late Mississippian, Lower *Eumorphoceras* Zone (= Serpukhovan), Utah, Arkansas, USA.
      - *Bactritimimus* Flower & Gordon, 1959 - Late Mississippian, Early Eumorphoceras Zone (= Serpukhovan), Arkansas, USA.
      - *Paleoconus* Flower & Gordon, 1959 - Late Mississippian, Early Eumorphoceras Zone (= Serpukhovan), Arkansas, USA.
  - Order PHRAGMOTEUTHIDA Jeletzky in Sweet, 1964 (Late Permian – Early Jurassic)
    - Family PHRAGMOTEUTHIDIDAE Mojsisovics, 1882
      - *Permoteuthis groelandica* Rosenkrantz, 1946 – Late Permian, Foldvik Creek Formation, Clavering Island, East Greenland
  - Order DONOVANICONIDA Doguzhaeva, Mapes, & Mutvei 2007b (Carboniferous)
    - Family DONOVANICONIDAE Doguzhaeva, Mapes & Mutvei, 2002a
      - *Donovaniconus* Doguzhaeva, Mapes & Mutvei, 2002a – Late Carboniferous, Desmoinesian, Oklahoma, USA.
      - *Saundersities* Doguzhaeva, Mapes & Mutvei, 2007b, Late Carboniferous, Desmoinesian, Illinois, USA
    - Family uncertain
      - *Gordoniconus* Lower Carboniferous – Bear Gulch Limestone, Montana, USA (Mapes, Doguzhaeva &Weller, in press)
  - Order AULACOCERATIDA Stolley, 1919 (Late Carboniferous - Jurassic)
    - Family MUTVEICONITIDAE Doguzhaeva, 2002b
      - *Mutveiconites* Doguzhaeva, 2002 - Late Carboniferous, Orenburgian, Southern Urals, Kazakhstan Republic (former USSR) and USA.
  - Order UNCERTAIN
    - Family RHIPHAEOTEUTHIDAE Doguzhaeva, 2002a
      - *Rhiphaeteuthis* Doguzhaeva, 2002a - Late Carboniferous, Orenburgian, Southern Urals, Kazakhstan Republic (former USSR).
    - Family FLOWERITIDAE new family
      - *Flowerites* new genus, Late Pennsylvanian, Virgilian = Stephanian); Nebraska, USA.
- Superorder DECEMBRACHIATA Winckworth, 1932 (Carboniferous - Holocene)
  - Order SPIRULIDA Pompeckj, 1912 (Carboniferous - Holocene)
    - Family SHIMANSKYIDAE Doguzhaeva, Mapes & Mutvei 1999
      - *Shimanskya* Doguzhaeva, Mapes & Mutvei 1999 - Late Pennsylvanian, Virgilian (= Stephanian); Texas, USA.
- Superorder VAMPYROPODA Boletzky, 1992 (Late Carboniferous – Holocene)
  - Order OCTOPODA Leach, 1817 (Late Carboniferous – Holocene)
    - Family PALAEOCTOPODIDAE Dollo 1912
      - *Pohlsepia mazonensis* Kluessendorf & Doyle 2000, Middle Carboniferous, Desmoinesian, Francis Creek Formation, Illinois, USA

Family FLOWERITIDAE n. fam.

Diagnosis: Thin-walled, small-sized (total length 33.7 mm), bluntly-pointed phragmocone (27.4 mm long and 12.2 mm wide at the midpoint of the length) and terminal chamber (10.3 mm long and 13.8 mm wide at the aperture); camerae comparatively short (about 5.5 to the phragmocone diameter long); long mural parts of septa (about 1/4 of a camera length); sutures straight; siphuncle small, marginal; septal necks probably cyrtochoanitic; ink sac flask-like, large (19.6 mm long and 6.8 mm wide at the base) compared to overall shell size; terminal chamber short (10.3 mm); rostrum unknown, but if it is present, it must relatively small and confined to the tip of the apical end of the phragmocone, which is missing on the specimen.

Derivation of name: Named for Rousseau Flower for his early pioneering work on Paleozoic Coleoidea.

Discussion: See ordinal discussion.

Genus *Flowerites* n. gen.
Fig. 2A-C

Type species: *Flowerites bellevuensis* n. gen. n. sp.

Diagnosis: As for the family.

Etymology: As for the family.

Occurrence: The single specimen was recovered from the Stark Shale from either the now abandoned Public Works Administration (PWA) Quarry, which is called the LaPlate Quarry, or from the 36th Street Quarry. Both of these quarries are near Bellevue, Nebraska. The specimen was collected in the 1970's by Mr. W. D. "Ted" White.

Discussion: As for the family.

***Flowerites bellevuensis* n. sp.**

Holotype: UNSM 16567A+B

Type locality: Abandoned quarry near Bellevue, Nebraska.

Type horizon: Stark Shale Member of the Dennis Formation (Kasimovian, Upper Carboniferous).

Diagnosis: As for the family.

Description: As for the family.

Etymology: The species is named for the community of Bellevue in Nebraska near where the specimen was found.

Discussion: As for the family.

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Annex

PROBLEMATIC PALEOZOIC SPECIMENS

1) *Boletzkya longa* Bandel, Reitner & Stürmer 1983 - Devonian (Emsian), Hunsrückschiefer, Kaisergrube, Hunsrück, Germany

2) *Naefiteuthis breviphragmoconus* Bandel, Reitner & Stürmer 1983 - Devonian (Emsian), Hunsrückschiefer, Kaisergrube, Hunsrück, Germany

3) *Protoaulacoceras longirostris* Bandel, Reitner & Stürmer 1983 - Devonian, Hunsrückschiefer, Kaisergrube, Hunsrück, Germany

4) *Eoteuthis* sp. Termier & Termier, 1971 - Devonian of Morocco, North Africa

5) *Aulacoceras*? sp. De Koninck, 1843 - ?Devonian/Carboniferous, Belgium

6) *Eobelemnites caneyensis* Flower, 1945 - Unknown locality and age

7) Jeletzkya douglassae Johnson & Richardson 1968 - Late Carboniferous, Desmoinesian, Francis Creek Formation, Illinois, USA

8) Unnamed coleoid from Czech Republic (Kostak et al. 2002) - Early Carboniferous, Moravica Formation, Northern Moravia, Czech Republic

9) Unnamed coleoid by Allison (1987) - Late Carboniferous, Desmoinesian, Francis Creek Formation, Illinois, USA.

10) "*Bactrites" woodi* Mapes, 1979, plate 18, figs. 8,12) - Late Carboniferous, Missourian, Kansas, USA

11) Undescribed Stark coleoids (see Doguzhaeva et al. 2002) - Late Pennsylvanian, Missourian (= Kasimovian), Nebraska, USA

12) *Palaeobelemnopsis sinensis* Chen & Sun, 1982 - Upper Permian from China

13) *Glochinomorpha stifeli* Gordon, 1971 - Lower Permian, Utah, USA

14) "*Diectyoconites*" cf. "*D.*" *groenlandicus* Fischer, 1947 in Gordon, 1966 - Lower Permain, Montana, USA

15) *Stenoconites idahoensis* Gordon, 1966

New data on the paleobiogeographic and biostratigraphic distribution of *Holcobelus* Stolley, 1927 and its allies (Belemnitida) in the Middle Jurassic

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Abstract

Previous reports of belemnite faunas composed by *Holcobelus* Stolley, 1927 and associated Pachybelemnopseina in the European Middle Jurassic generally lack precise biostratigraphic information. Recent field research in Luxembourg, Italy (Calabria) and France (Normandy and Haute-Provence) has shown that *Holcobelus* appeared and flourished in the Aalenian (*Murchisonae* and *Concavum* Zones), and disappeared in the early Bajocian (*Discites* Zone, *Propinquans* Zone for Normandy) with the sudden rise of *Pachybelemnopsis*. Our data suggest a peculiar and formerly unrecognized distribution pattern for *Holcobelus* in the north-western European area and the peri-mediterranean northern margin of the Tethys Ocean. The faunal associations at the Aalenian-Bajocian boundary (*Murchisonae-Propinquans* Zones) show strong Submediterranean affinities in southern and south-eastern Europe (SE France, Italy, Bulgaria, Romania), while a clear Subboreal compositional pattern is observed in NW Europe (Luxembourg, NE France, Germany, N Switzerland). The Submediterranean fauna is dominated by Pachybelemnopseina (*Holcobelus* ssp., *Pachybelemnopsis* and *Hibolithes*), meanwhile the Subboreal fauna is characterised by Belemnitina (*Brevibelus*, *Megateuthis*, *Eocylindroteuthis*, *Homaloteuthis*) and by the absence of *Holcobelus*, with the exception of a single migrant species, *Holcobelus blainvillii*. Both faunas mingled in the western Anglo-Paris and the Aquitaine Basins (SW England, NW France) where Belemnitina and Pachybelemnopseina are equally well represented.

Introduction

This paper stems from our recent studies (Mariotti, Santantonio & Weis 2007; Weis & Mariotti 2007) on the systematics, biostratigraphy and paleobiogeography of Middle Jurassic belemnites, particularly Holcobelidae Gustomesov, 1977 and related taxa. The use of belemnites in paleobiogeography is a research field that is especially promising owing to the nektonic-pelagic mode of life of these cephalopods, and to their widespread distribution in the Jurassic and the Cretaceous. Belemnitida originated in the Early Jurassic (Hettangian) (Weis & Delsate 2006, *cum bibl.*) of the European Archipelago, and reached a world-wide distribution in the later Early Jurassic, with widely cosmopolitan faunas except for Canada and northern Siberia, where a distinct Boreal-Arctic fauna developed in the Toarcian. Development of a peculiar Tethyan faunal association began in the early Middle Jurassic with the rise of the suborder Pachybelemnopseina Riegraf, 1998, announced by the genus *Holcobelus* Stolley, 1927 that spread rapidly on the European borders of the Tethys Ocean in the late Aalenian.

Holcobelus is now considered the oldest representative of Pachybelemnopseina (*sensu* Riegraf, Janssen & Schmitt-Riegraf 1998), which are characterised by a ventral groove extending over more than the half of the rostrum. *Holcobelus*, descending most probably from early Aalenian *Acrocoelites* Lissajous, 1915, flourished during the middle and late Aalenian and is considered the ancestor of *Pachybelemnopsis* Riegraf, 1980. *Pachybelemnopsis* appeared during the late Aalenian-early Bajocian on the European borders of the Tethys Ocean, and soon became the dominant belemnite genus in the Middle Jurassic. Both genera were mingling in southern and south-eastern Europe, from where *Holcobelus-Pachybelemnopsis* associations have been described (Stoyanova-Vergilova 1985; Combémorel et al. 1994; Mariotti, Santantonio & Weis 2007). The same association was reported by Krimholz (1931) from the Caucasus.

Further *Holcobelus* assemblages, recovered from other localities mainly in Europe, were repeatedly described (for an overview, see Weis & Mariotti 2007). To date, these faunas have not been put in a broader context, so that little is known about the actual paleobiogeographic and biostratigraphic distribution of *Holcobelus*. In this paper we compare literature and recent field data from the Calabria-Peloritani Arc (Mariotti, Santontonio & Weis 2007) and the eastern Paris Basin (Weis & Mariotti 2007) with unpublished material from the western Anglo-Paris Basin (Normandy) and the Dauphinois Basin (Castellane, SE France). In the ligth of this new data, the biostratigraphic and the paleobiogeographic distribution of *Holcobelus* in the European Jurassic is illustrated. Finally, the causes for this distribution are tentatively discussed. The systematics here used is that proposed by Riegraf, Janssen & Schmitt-Riegraf (1998).

Belemnite paleobiogeography in the Early and early Middle Jurassic

Very little is known about the geographic distribution of Early Jurassic (Hettangian-Sinemurian) belemnites. Only few findings are cited, the oldest (205 Myrs) records of Belemnitina being those from the Lower Jurassic (Hettangian, *Planorbis* and *Liasicus* Zones) of the European archipelago (Schwegler 1939; Weis & Delsate 2005, 2006). Sinemurian belemnites have a similarly restricted geographic distribution. Through the early Pliensbachian belemnites become more and more abundant. The Pliensbachian faunas from Turkey (Doyle & Mariotti 1991), Bulgaria (Stoyanova-Vergilova 1982), England (Lang 1928) and, with relatively rare specimens, Italy (Mariotti 1994) display a rather homogeneous composition.

Within the Boreal Realm, a clear provincialism was not established until the late Early Jurassic (Toarcian), when distinct Boreal-Atlantic and Boreal-Arctic faunas developed (Doyle 1987). At that time, Belemnitina were already distributed in the southern Hemisphere, as reported by Stevens (1965) and by Doyle & Pirrie (1999, cum bibl.). It is generally admitted that the development of distinct Boreal and Tethyan Realms only started in the early Middle Jurassic (Bajocian), with the rise of Pachybelemnopseina and the decline of Megateuthididae. However, Doyle (1987) mentioned the possibility of a characteristic Tethyan fauna already in the Toarcian (including *Dactyloteuthis*, *Rhabdobelus* and *Salpingoteuthis*), a subject that needs further investigation. At the end of the Aalenian, the earliest Pachybelemnopseina (*Holcobelus*) expanded on the European borders of the Tethyan Ocean. *Holcobelus* then rapidly declined during the early Bajocian (*Discites-Propinquans* Zones), when it co-existed with early *Pachybelemnopsis* and *Hibolithes*.

Observations

New data on the distribution of *Holcobelus* and its allies

Holcobelus has been reported by several authors (see Weis & Mariotti 2007), but no comprehensive overviews exist, with the exception of Stolley (1927). The stratigraphic distribution of *Holcobelus* was only broadly defined as ranging around the Aalenian-Bajocian boundary. New data, resulting from sampling in a number of key European localities (Fig. 1), demonstrate that certain species range into the early Bajocian (*Propinquans* Zone), whereas others disappear in the late Aalenian.

Western Anglo-Paris Basin (Normandy)

In the upper Aalenian (*Murchisonae* and *Concavum* Zones), *Holcobelus* is the dominant genus. Eudes-Deslongchamps (1878) cited in the "Couches à *Ammonites murchisonae*" in Normandy the following species: *Holcobelus blainvillii* (Voltz, 1830), *H. munieri* (Eudes-Deslongchamps, 1878) (including *Belemnites subblainvillei* Eudes-Deslongchamps, 1878), *H. brevicanalis* (Eudes-Deslongchamps, 1878), *H. tetramerus* (Eudes-Deslongchamps, 1878) and *H. harleyi* (Mayer, 1866) (= *Belemnites canaliculatus sensu* Eudes-Deslongchamps 1878). The same author (1878) mentioned rare records of *Holcobelus blainvillii* already in the "Couches à *Ammonites opalinum*" of Évrecy. Stolley (1927) erected the species *Holcobelus trauthi* based on specimens sampled in Normandy, probably from the lower Bajocian "Oolithe ferrugineuse"; that is from younger levels than those investigated by Eudes-Deslongchamps (1878).

The revision of the historic collections stored in Paris (Muséum national d'Histoire naturelle) and Lyon (collection École des Mines, Claude Bernard University), coupled with recent field data collected by the authors at Feuguerolles-sur-Orne, results in a better understanding of the stratigraphic range of the different species (Mariotti and Weis, unpublished data). With the exception of *Holcobelus trauthi* (Lower Bajocian according to Stolley 1927) and *Holcobelus munieri*, reaching the *Propinquans* and possibly *Humphriesianum* Zones (unpubl. data), all the other species do not cross the Aalenian-Bajocian boundary. Besides *Holcobelus*, *Brevibelus breviformis* (Voltz, 1830) occurs in large numbers in the upper Aalenian, and continues into the Bajocian where it mingles with *Brevibelus gingensis* (Oppel, 1857), *Megateuthis elliptica* (Miller, 1826), rare *Holcobelus munieri* and the earliest *Pachybelemnopsis* (*Propinquans* Zone). Concerning this latter group, it is noteworthy that Eudes-Deslongchamps (1878) reported *Belemnites apiciconus* Blainville, 1827 as a rare element of the "Couches à *Ammonites murchisonae*" from Les Moutiers. Stolley (1927) mentioned that the specimens figured as *Belemnites apiciconus* by Eudes-Deslongchamps (1878) might instead be a new species, representing the earliest occurrence of *Pachybelemnopsis* in the European Middle Jurassic. It is interesting to note that Stoyanova-Vergilova (1985) recorded *Pachybelemnopsis apiciconus* and *P. belediensis* (Stoyanova-Vergilova, 1985) from the upper Aalenian of Bulgaria, while Krimholz (1931) reported *Pachybelemnopsis* sp. from the upper Aalenian of Daghestan (Caucasus). In some areas of the European archipelago, however, as in SW Germany and Luxembourg, the first belemnopseids - *Pachybelemnopsis baculiformis* Riegraf, 1980 and *P. roettingensis* (Schlegelmilch, 1998) - are recorded not earlier than the lower Bajocian *Humphriesianum* Zone (Schlegelmilch 1998; Weis 2006; Weis & Mariotti 2007).

Fig. 1: Geographic location of the four recently investigated outcrops.

Calabria-Peloritani Arc (Calabria, Italy)

Although Calabria is the geographic continuation of the Apennines in Southern Italy, the first-order tectonic unit to which it belongs, known as the Calabria-Peloritani Arc, is considered to be a former fragment of the Alpine chain, later (Miocene) accreted to the Apennines following opening of the Tyrrhenian Basin (Amodio-Morelli et al. 1979). As such, it does not represent the deformed margin of the Adria Plate, like the Apennines do, but rather the continuation of the Sardinia-Corsica European margin (Santantonio & Carminati 2009).

In the hemipelagic red marls of the Sant'Onofrio Formation near Caloveto, Combémorel et al. (1994) and Mariotti, Santantonio & Weis (2007) described a belemnite assemblage composed by Pachybelemnopseina (*Holcobelus munieri, H. trauthi, H. tetramerus, H. tschegemensis* (Krimholz, 1931), *Pachybelemnopsis baculiformis* Riegraf, 1980, *Hibolithes wuerttembergicus* (Oppel, 1856), *H.* sp.) and a few isolated Belemnitina (*Brevibelus breviformis*, *Megateuthis* sp.). The dominant form, however, is an undescribed taxon (Belemnitida *incertae sedis*, Mariotti, Santantonio & Weis 2007), which shares certain distinctive features of both *Holcobelus* and *Pachybelemnopsis* and therefore may be a transitional form between these two. This new taxon, associated with *Holcobelus* sp., was recently found in coeval strata in the Castellane area in SE France (Mariotti and Weis, unpublished data). Associated ammonites indicate an Aalenian-Bajocian age for the Caloveto outcrop (*Opalinum - Discites* Zones).

Eastern Paris Basin (Luxembourg and Lorraine)

From the eastern Paris Basin, Weis & Mariotti (2007) described a Belemnitina-dominated fauna associated with rare *Holcobelus blainvillii* from the upper Aalenian of Rumelange in the Grand-duchy of Luxembourg. The *Murchisonae* and notably *Concavum* Zones yielded several specimens ascribed to *Brevibelus breviformis* and *Homaloteuthis murielae* Weis & Mariotti, 2007, associated with less frequent and mostly juvenile *Holcobelus blainvillii*. In the lower Bajocian of the same area, *Eocylindroteuthis corneliaschmittae* Riegraf, 1980, *E. trautscholdi* (Oppel, 1862), *Brevibelus breviformis, B. gingensis, Megateuthis suevica* (Klein, 1773) and *M. elliptica* occur starting in the *Discites* Zone, while *Pachybelemnopsis baculiformis*, *P. roettingensis* and *Hibolithes wuerttembergicus* appear in the *Humphriesianum* Zone. In adjacent NE France (Lorraine), the picture is similar to the situation in Luxembourg.

Swabian Basin (SW Germany and NW Switzerland)

In SW Germany and NW Switzerland, data from Riegraf (1980, 1981), Weis & Mariotti (2007) and collection data from the Museums of Basel and Zürich (unpubl. data) indicate an analogous situation as in the eastern Paris Basin, although with a slight difference: *Homaloteuthis murielae* is absent in the *Concavum* Zone, whereas *Homaloteuthis spinata* (Quenstedt, 1848) characterises the *Murchisonae* Zone of the Swabian Alb (Weis & Mariotti 2007: 164). As in the eastern Paris Basin, *Holcobelus blainvillii* is the only holcobelid species reported, with a stratigraphic range extending from the *Murchisonae* Zone (Aalenian) to the *Discites* Zone (lower Bajocian) (Riegraf 1980).

Dauphinois Basin (Castellane area, SE France)

Unpublished material collected near Castellane (Riegraf collection) reveals an *Holcobelus*-dominated fauna with close affinities to the fauna of Southern Italy (Mariotti, Santantonio & Weis 2007). The section consists of a hemipelagic succession of marl and limestone ("Calcaires à Zoophycos"). The belemnites come from the base of the formation, overlying a condensed hardground with Pliensbachian ammonites (Riegraf, pers. comm. 2005) found all along the southern margin of the Dauphinois Basin. While the exact age of the belemnite-bearing strata has yet to be established, a late Aalenian age is most likely. The following taxa have been identified (Mariotti and Weis, unpublished data): *Holcobelus munieri, H. tetramerus, H. tschegemensis*, Belemnitida *incertae sedis* (*sensu* Mariotti, Santantonio & Weis 2007).

Biostratigraphic importance of *Holcobelus*

Certain species can be valid stratigraphic markers for a belemnite biostratigraphic scale: Combémorel (1997) proposed a belemnite subzone based on *Holcobelus brevicanalis*, equivalent to the *Murchisonae* Zone. Stoyanova-Vergilova (1990) indicated *Holcobelus* ssp. as a reliable marker for the *Concavum* Zone in Bulgaria. As available data suggest, *Holcobelus munieri* and *H. blainvillii* both have a wide stratigraphic distribution (*Murchisonae–Propinquans* or possibly *Humphriesianum* Zones), so they are of relatively little stratigraphic value. However, an assemblage consisting of several *Holcobelus* species may be a valuable indicator for a late Aalenian age (*Murchisonae-Concavum* Zones) in the Submediterranean area. In order to confirm this hypothesis and validate holcobelid species as biostratigraphic markers, further work is needed on material coming from North Africa, Iberian Peninsula, and the United Kingdom.

The paleogeographic distribution of *Holcobelus*: patterns and peculiarities

Holcobelus holds a special paleobiogeographic importance, being the earliest marker among Pachybelemnopseina of the Tethyan northern margin (Fig. 2). *Holcobelus* assemblages stretch from Morocco and Portugal to SW England, France, Italy, Bulgaria, Romania to the Caucasus and beyond (unpubl. Data; these assemblages predate the early Bajocian rise of *Pachybelemnopsis* at the margins of the Tethyan Ocean. Literature data and new records, collected by the authors, evidence a short period of strong radiation during the Aalenian (*Murchisonae-Concavum* Zones), characterised by numerous species and by an important intrageneric variation of the rostrum morphology. Some of these species probably disappeared rapidly. Just a few species, like *H. munieri* and *H. blainvillii*, survived into the early Bajocian, but then declined rapidly.

H. munieri and *H. blainvillii* are perhaps the best known species. *H. munieri*, the most widespread form, is reported from most areas mentioned above, with the exception of the eastern Paris and the Swabian Basins (Weis & Mariotti 2007). Adult specimens developed an epirostrum, a feature which proved misleading taxonomy-wise, a case in point being Eudes-Deslongchamps (1878), who erected the species *Belemnites subblainvillei* based on that single feature. *B. subblainvillei* is now considered to be a synonymous of *H. munieri* (see Combémorel, Fischer & Rioult 1994, and own observations).

Based on work by Eudes-Deslongchamps (1878), who reported rare specimens from the *Opalinum* Zone of Normandy, and by Riegraf (1980), *Holcobelus blainvillii* is believed to be the oldest representative of holcobelids. It is morphologically close to *Acrocoelites*, whose ventral groove does not extend to the tip of the rostrum, as is typical of holcobelids. The paleogeographic distribution of *H. blainvillii* is peculiar: it is common across the central European epicontinental platforms (Luxembourg, SW Germany, Northern Switzerland, NE France), where it is the only holcobelid species, whereas it is rarer in Normandy and southern Europe.

A particularly slender rostrum was originally described by Krimholz (1931) from Daghestan as *Belemnopsis tschegemensis*. Since then, this species was reported from Bulgaria (Stoyanova-Vergilova 1982, 1990, 1993) and Calabria (Mariotti, Santantonio & Weis 2007). It was found also in the Dauphinois Basin (Castellane area; pers. obs. Weis & Mariotti) and in the western Swiss Alps (Ooster 1857: pl.2, figs. 7-8, reported as *Belemnites blainvillei*). It is most remarkable that this species is not known from Normandy: it probably evolved in southern or south-eastern European Basins and did not migrate into the Anglo-Paris Basin.

Summarizing, three broad assemblages characterize the Aalenian-Bajocian boundary (Fig. 3). While the geographic boundaries separating these assemblages may indeed not be exact duplicates of those set for ammonites, we elected to maintain ammonite province names (Callomon 2003) here, so that the reader can make reference to a well-established paleobiogeographic framework:

- a submediterranean association, composed essentially by Pachybelemnopseina, with the most characteristic *Holcobelus munieri* (Fig. 4I-J) and *H. tetramerus* (Fig. 4G-H) associated with *H. brevicanalis* (Fig. 4C-D), *H. trauthi* (Fig. 4K-L), *H. blainvillii* (Fig. 4A-B), *H. tschegemensis* (Fig. 4M-N), and *H. harleyi* (Fig. 4E-F). These assemblages are recorded mainly from the Dauphinois Basin

Fig. 2: Paleobiogeographic distribution of *Holcobelus* Stolley, 1927 (in red) in the studied area: DO=Dorset, NM=Normandy, AQ=Aquitaine, LU=Luxembourg, SW=Swabia, WSA=Western Swiss Alps, CAS=Castellane, CPA=Calabro-Peloritani Arc, RO=Romania, BG=Bulgaria. Map modified after Thierry & Barrier 2000.

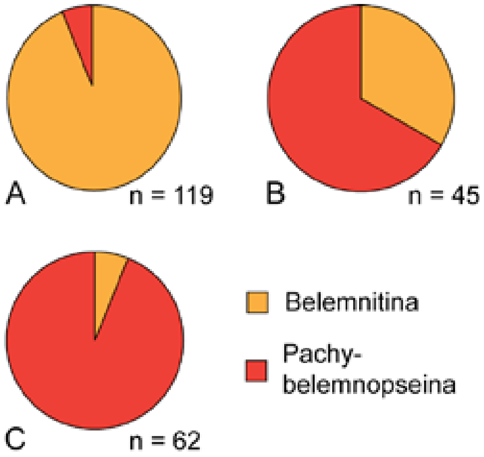


Fig. 3: Relative abundance (in per cent) of belemnitid groups in three representative outcrops. A, Rumelange, Luxembourg. B, Feuguerolles, Normandy. C, Caloveto, Calabria.

(Ooster 1857; Mariotti and Weis, unpublished data), the Calabria-Peloritani Arc (Combémorel et al. 1994; Mariotti, Santantonio & Weis 2007), Bulgaria (Stoyanova-Vergilova 1982, 1985, 1990, 1993), Romania (Preda 1975) and Caucasus (Krimholz 1931, 1947, 1953);

- a subboreal association dominated by Belemnitina (*Brevibelus, Homaloteuthis, Eocylindroteuthis, Megateuthis*). A similar fauna was recorded from the eastern Paris Basin (Weis & Mariotti 2007), the Swabian Basin (Riegraf 1980; Weis & Mariotti 2007), and the Hannover Basin (Kumm 1952). The single migrant *Holcobelus blainvillii* spread into the eastern Paris and the Swabian Basins, but there is no evidence for its presence in the Hannover basin;

- a mixed association, composed mainly by Belemnitina (*Brevibelus* and *Megateuthis*) and Pachybelemnopseina (*Holcobelus* and *Pachybelemnopsis*), was recorded from the western Anglo-Paris Basin (Eudes-Deslongchamps 1878; Phillips 1869) and the Aquitaine Basin (Mariotti and Weis, unpublished data).

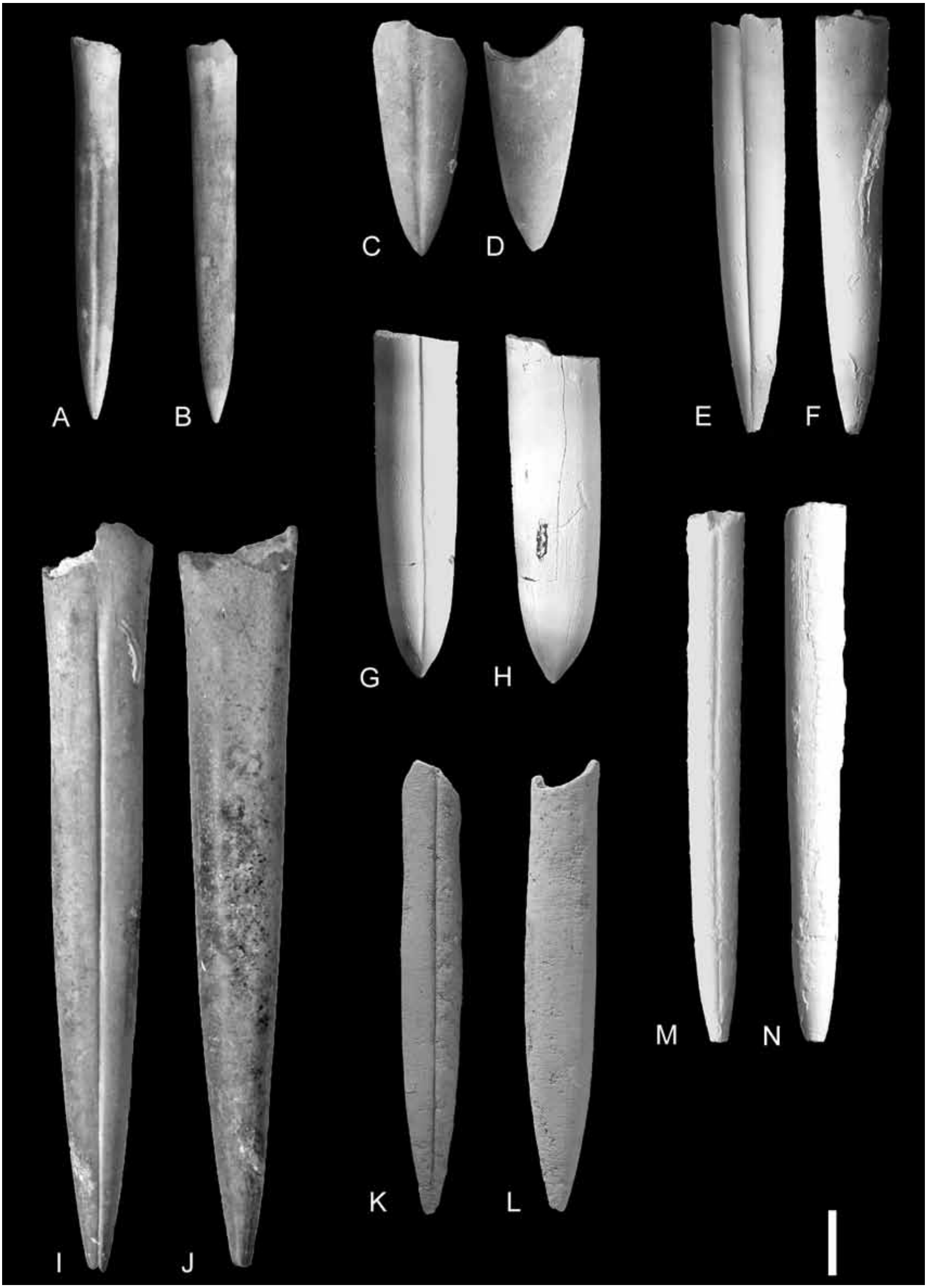
These assemblages cover the Aalenian (*Murchisonae-Concavum* Zones) and early Bajocian (*Discites-Propinquans* Zones). In the subsequent *Humphriesianum* Zone, *Holcobelus* disappeared, while *Pachybelemnopsis* and *Hibolithes* expanded deeply into the Subboreal Province, radiating from southern areas.

Discussion

The rise and fall of *Holcobelus* took place during a short time span, so certain species are potentially valuable biostratigraphic markers.

The peculiar distribution of the genus *Holcobelus* poses some questions regarding the causes that may have affected its inability to spread across the Tethys towards the south. In turn, this opens doors to a discussion regarding the possible relationships between the tectonic evolution of the western branch of the Tethys in the Early and early Middle Jurassic, and the paleogeographic and paleoceanographic changes that geodynamic processes must have produced at various scales. A first order conclusion, based on our review of geographic occurrences, reveals that *Holcobelus* thrived in areas surrounding continental masses, either along rifted margins (Calabria, SE France) or in epicontinental seas in tectonically more stable areas (S Germany, part of the Paris Basin).

Fig. 4: A-B) *Holcobelus blainvillii* (Voltz, 1830), Upper Aalenian, Normandy. Coll. Dubrulles at Maison du fossile, Lion-sur-Mer. C-D) *Holcobelus brevicanalis* (Eudes-Deslongchamps, 1878), Upper Aalenian, Normandy. Coll. Dubrulles at Maison du fossile, Lion-sur-Mer. E-F) *Holcobelus harleyi* (Mayer, 1866), Aalenian, Normandy. Coll. Ecole des Mines at Université Claude Bernard, Lyon (n° EM 18001).G-H) *Holcobelus tetramerus* (Eudes-Deslongchamps, 1878), Aalenian, Fontaine-Etapefour, Normandy. Coll. Ecole des Mines at Université Claude Bernard, Lyon (No. EM 18002). I-J) *Holcobelus munieri* (Eudes-Deslongchamps, 1878), Aalenian-Bajocian boundary, Croisilles, Normandy. Coll. Muséum national d'histoire naturelle de Paris (No.R07132). K-L) *Holcobelus trauthi* Stolley, 1927, Lower Bajocian, Normandy. Coll. Naturhistorisches Museum Wien (No.1868/0011/0016). Type figured by Stolley 1927. M-N) *Holcobelus tschegemensis* (Krimholz, 1931), Aalenian-Bajocian boundary, Blattenheid, Stockhornkette (Swiss Alps). Coll. Naturhistorisches Museum Bern (No.5015826, collected by Ooster and determined as *Belemnites blainvillei*); scale bar = 1cm.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On the other hand, a further spread to the north and north-west, toward northern England and the Boreal provinces was hampered, and so was the colonization of pelagic oceanic environments of the Adria margins. Facies-wise, this translates into a picture where *Holcobelus* is dominant in sediments wherever terrigenous input is significant, such as hemipelagic marls or siliciclastic-rich limestone, while it is generally missing in pure carbonate deposits.

Whereas the distribution of this genus demonstrates that it was unable to reach beyond the spreading axis of the Tethyan Ocean (the so-called Liguria-Piedmont branch), which by Aalenian/Bajocian had established, separating the Adria Plate from Europe (including Sardinia-Corsica and Calabria; Santantonio & Carminati 2009, and references therein; Schettino et al. 2009), the paleobiogeography of a nektonic organism cannot be directly related to a topographic feature of the sea bottom. Water depth, temperature, salinity, and nutrient content had to be the main parameters controlling the distribution of belemnite species. As concerns these parameters, we note the following:

1. water depth: whereas belemnites are conspicuously missing on shallow-water carbonate platforms and shelves, their occurrence is ubiquitous on relatively shallow continental shelves and in basins of any depth, the key here being connection with the open marine environment, rather than depth;
2. water temperature: this had to be relatively uniform, and generally high, across the Tethyan and the European shelves at the time, also due to the fact that no ice caps existed so the latitudinal temperature gradient was low (Hallam 1978): coral reefs and ooid shoals, typical features of tropical environments, existed as far north as Southern England in the Aalenian and Bajocian;
3. salinity: river discharge has practically no impact, at a large scale, on salinity in oceanic waters bordering a continental mass; this impact is greater wherever fresh water enters a shallow epeiric sea locally locked by landmasses, so this parameter must have had a greater impact in areas peripheral with respect to Jurassic oceanic circulation (e.g. the northern European/Boreal province);
4. nutrient content: this parameter is the result of the interplay of numerous variables, including (a) proximity to river deltas, resulting in the input of inorganic nutrients and organic matter, (b) orientation of coastline with respect to oceanic currents, resulting in local upwelling, (c) secular productivity changes in the oceanic waters, resulting in variation in the composition and abundance of plankton, (d) latitude (see also Muttoni et al. 2005).

It must be noted that parameters 2 to 4 are in turn linked with climate change, while all four exhibit various degrees of dependence from the plate tectonics evolution of a region.

Under this light, the distribution of *Holcobelus* is suggestive of a group of species requiring relatively high nutrient levels, unlike those of an open ocean, and perhaps sensitive (with the possible exception of *H. blainvillii*) to salinity changes. The Jurassic rifting-to-spreading (starting in the Toarcian) evolution of the African, European and Adria plate margins would lead to the progressive areal reduction, separation and foundering of land masses, resulting in changed sea-water circulation and affecting the migration paths of marine organisms.

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Ultrastructural characterisation of the adhesive area of *Sepia tuberculata* (Lamarck, 1798)

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Abstract

Four species of *Sepia* (*S. typica, S. papillata, S. pulchra* and *S. tuberculata*) use a mechanical mechanism to adhere to substratum. In all species dermal structures on the ventral mantle side and the fourth arm pair (*S. tuberculata, S. papillata*) act as a set of suckers and effect the bonding. Preliminary histochemical and morphological analyses for *Sepia tuberculata* indicate an additional chemical adhesion mechanism.

Ultrastructural investigations support this hypothesis. The adhesive epithelium is considerably higher (about 125 μm) than the non-adhesive mantle epithelium (about 50 μm). In the single-layered adhesive epithelium four gland cell types (type 1, type 2, type 3 and goblet cells) and the non-secretory interstitial cells can be distinguished by their structure and secretory composition.

Comparison with the adhesive system of *Euprymna, Idiosepius* and *Nautilus* indicate congruence concerning the number of cells and the secretory content (two cells with granules). For these species chemical adhesion is verified. The known mechanical adhesion of *Sepia tuberculata* seems to be supported by chemical substances of cell types 2 and 3.

Introduction

Adhesive systems are frequently used to capture prey, to camouflage or to hold on to substratum. In cephalopods mechanical adhesion is primarily achieved by reduced-pressure systems in suckers on the arms and tentacles (Kier & Smith 1990, 2002; Smith 1991, 1996). Apart from this, temporary chemical adhesion is known for three cephalopod genera (*Idiosepius, Euprymna* and *Nautilus)*. However, the function and occurrence of these adhesive systems vary between the genera: In *Idiosepius* the adhesive organ is located on the dorsal mantle side and partly on the fins (Sasaki 1921) and consists of five different cell types (columnar, granular, goblet, interstitial and basal cells) (Cyran, von Byern and Klepal 2005).

Euprymna scolopes covers itself with sand for camouflage via adhesive glands mainly located on its dorsal surface. In this species two cells form a duo-gland system: goblet cells responsible for adhesion and ovate cells for deadhesion (Singley 1982).

Nautilus has adhesive gland cells on the digital tentacles in order to hold prey, to attach to the substratum or to cling to other individuals for mating (Kier 1987; Muntz & Wentworth 1995). The adhesive system consists of two types of epithelial glandular cells: columnar cells with granular material and mucus cells without granules (Kier 1987; Muntz & Wentworth 1995).

Three species of Sepia (*S. typica, S. papillata and S. tuberculata*) use a mechanical mechanism to adhere temporarily to hard substratum (Adam & Rees 1966; von Boletzky & Roeleveld 2000). In *S. typica* the system consists of numerous pores on the ventral mantle side near the fin base (Adam & Rees 1966; von Boletzky & Roeleveld 2000), while for *S. papillata* and *S. tuberculata* wrinkled patches and the fourth arm pair act as a set of suckers and effect the bonding (Adam & Rees 1966, von Boletzky & Roeleveld 2000).

Observations of the adhesive behaviour of *Sepia tuberculata* reveal a participation of the entire body: first the animals attach with their ventral arms and then with the mantle shield (Adam & Rees 1966, von Boletzky & Roeleveld 2000). These results support the known mechanical adhesion. Further, histochemical analysis shows that glandular ovate and elongate cells are present in the adhesive system. The elongate cells, filled with granules (neutral hexose sugars) are more prominent than the ovate cells (containing acidic mucoproteins) (Scott pers. comm. 2000). The results suggest that the secretory products from both glandular cells might take part in the attachment of *S. tuberculata* (von Byern & Klepal 2006).

In order to verify the involvement of chemical substances in adhesion, ultrastructural investigations were made to characterize in detail the surface of and the different cell types within the adhesive area.

Materials & Methods

The adhesive epithelium of ten specimens (*S. tuberculata*) was examined by means of electron microscopy. The animals (mantle length 80-90 mm) were collected in September 2006 by the third autor during low tide in rock pools in the intertidal region in Wooley´s Pool and Miller´s Point along the coast of Cape Town, South Africa.

The animals were anaesthetised with 3% ethanol and decapitated accoring to the method of von Byern et al. (2008). The adhesive area was cut in 1cm² pieces; first fixation procedure was carried out for 6 h at 25° C in 2.5% glutaraldehyde or Karnovsky solution (Karnovsky 1965) with 0.1 M sodium-cacodylate buffer or 0.1 M phosphate buffer (pH 7.3, containing 10% sucrose). Afterwards the samples were washed three times for 10 min with the corresponding buffer solution (0.1 M) and stored for further processing.

In the second fixative (1% osmium-tetroxide with 0.1 M buffer solution) the samples were immersed for 1 h. Afterwards, the samples were separated for Transmission Electron Microscope (TEM) and for Scanning Electron Microscope (SEM).

For TEM, the samples, cut in 1mm², were dehydrated in a graded series of ethanol and embedded in Epon. Semithin sections (1 µm), stained with toluidine blue and viewed by light microscope, were used to provide an overview of the cell distribution within the adhesive area. Ultrathin sections (50-70 nm) were mounted on slot grids coated with formvar in dioxane, stained with uranyl acetate and lead citrate (Reynolds 1963) and photographed on a TEM Philips EM 208 and Zeiss EM 902.

Apart from the standard procedure the following modification was made: to improve the contrast 1% potassium ferricyanide was added during the second fixation to the 1% osmium-tetroxide solution.

For SEM, the samples were washed four times with aqua bidest, dehydrated in a graded series of ethanol, 100% acetone and immersed in hexamethyldisilazane. Afterwards the samples were dried on air overnight, mounted on stubs, coated with gold in a sputter coater (Polaron 5800) and examined by SEM Philips XL 20.

Results

Characterization of the adhesive system

The adhesive area in *S. tuberculata* is located on the ventral side of the mantle and the fourth arm pair. There are no morphological differences between the adhesive area on the arms and on the mantle. It is wrinkled and can be clearly separated from the ordinary mantle epithelium (Fig. 1). Apically, the adhesive system is characterized by densely arranged microvilli (3-4 µm high). In the interstitial cells the apically widened and less electron-dense parts of the microvilli are covered by glycocalix (Fig. 2). The connective tissue beneath the adhesive epithelium does not include chromatic elements (chromatophores, iridiophores and reflector cells) as in the other mantle. The adhesive epithelium (125 µm high) consists of a single layer of four glandular cell types (type 1, type 2, type 3 and goblet cells) and the non-secretory interstitial cells. All cell types can be distinguished by their morphology and secretory content.

Beneath the epithelium is the basement membrane (0.3-0.5 µm thick). Structurally the cell types 1-3 are elongate pear-shaped: at their base the cells are twice as broad as in the middle region and they taper towards the apical pole. In contrast the goblet cells are columnar shaped. The granules of cell types 1-3 are loosely packed. Next to the surface the secretory cells are connected to the neighbouring interstitial cells via zonulae adhaerentes (Fig. 3). In the glandular cells (types 1-3) the basal nucleus is oval or flat (goblet cells). In the interstitial cells the oblong nucleus is located in the middle region.

The synthesis of the secretory material in cell types 1-3 is carried out around the nucleus, where the cytoplasma is rich in rER (rough Endoplasmic Reticulum) and dictyosomes. The rER releases transition vesicles which migrate to the cis-sides of dictyosomes. On the trans-sides of the Golgi cisternae released vesicles fuse to larger granules and migrate to the apical pole.

Another common character of all three glandular cell types is the presence of tubular structures along the lateral cell membrane near the apical pole. Nerve fibres are present in the basal area of the adhesive as well as non-adhesive mantle epithelium and directly beneath the basement membrane.

Cell type 1 filled with membrane-bound granules (Ø 1-1.5 µm) (Fig. 2) is not restricted to the adhesive system but can also be found in the non-adhesive mantle epithelium.

Cell type 2 is full of small membrane-bound granules (Ø 0.5-1 µm), which are less electron-dense than the granules in cell type 1 (Fig. 4). Cell type 2 is also located in the mantle epithelium. In the adhesive system this cell type occurs almost always in conjunction with cell type 3.

Cell type 3 is narrower than cell types 1 and 2. Its granules are only 50-250 nm in diameter (Fig. 3). Via zonulae adhaerentes cell type 3 is always in conjunction with cell type 2 (Fig. 3). Cell type 3 is restricted to the adhesive epithelium only.

Goblet cells are mostly without content. They are also located in the ordinary mantle epithelium.

In the adhesive system and in the ordinary mantle the non-secretory **interstitial cells** are located between the glandular cells. Some interstitial cells

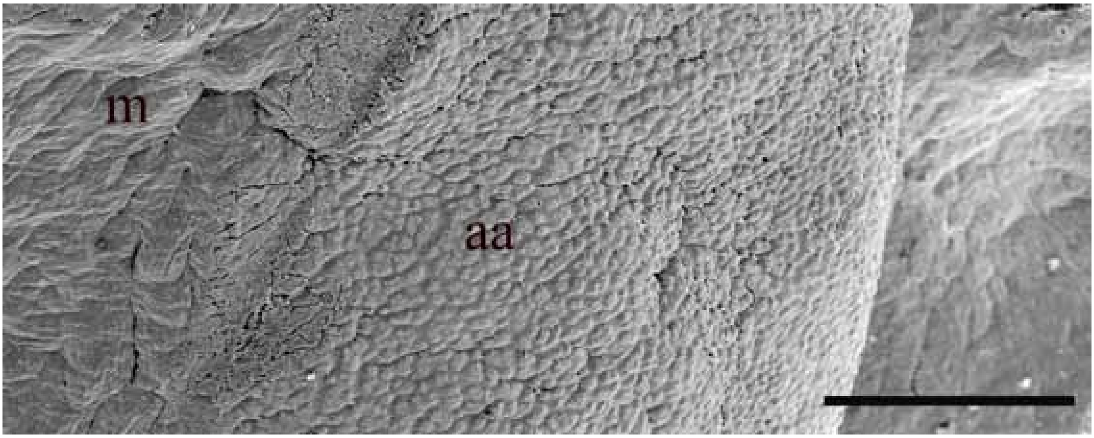

Fig. 1: The adhesive area (aa) can structurally easily be separated from the ordinary mantle (m) epithelium; scale bar = 500µm.

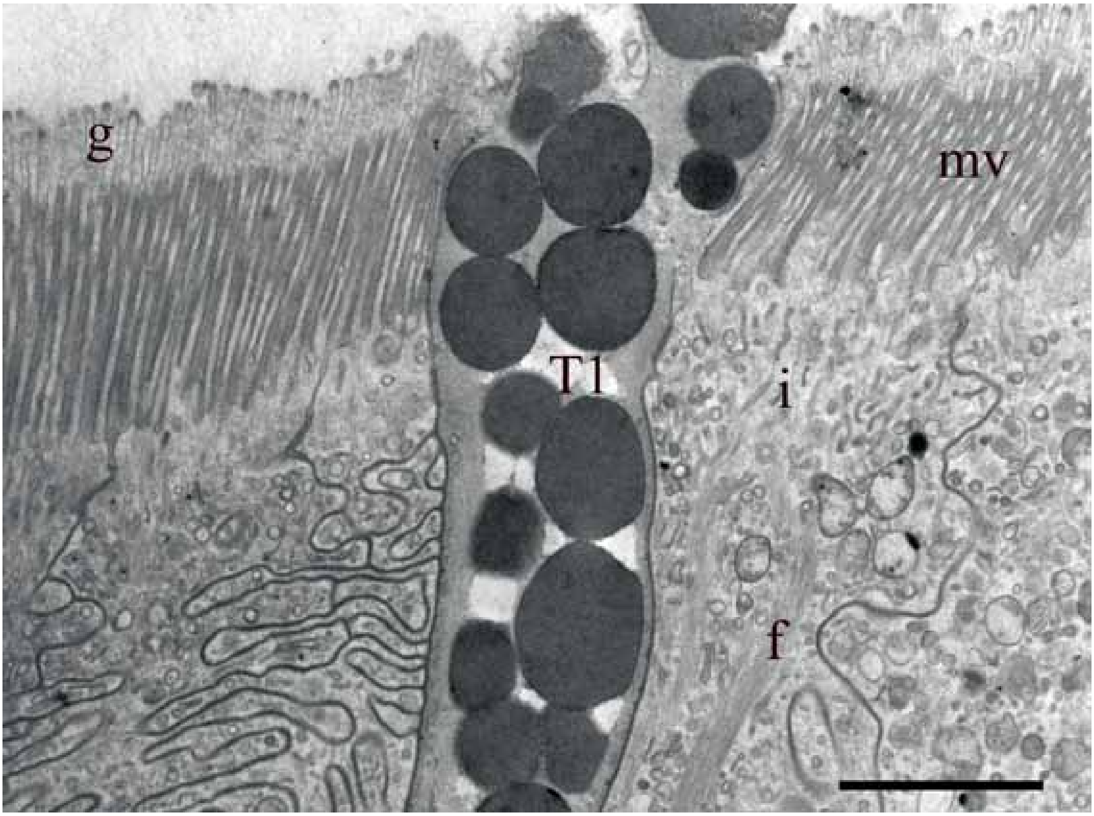

Fig. 2: Process of secretion in cell type 1 (T1). Its membrane-bound granules are loosely packed. In the interstitial cells (i) the apically widened and less electron-dense parts of the microvilli (mv) are covered by glycocalix (g); f = filaments; scale bar = 2 µm.

adjacent to the three granular cell types contain large bundles of filaments traversing from the cell base to the top of the cell. Further some irregularly distributed non-secretory cells in the ordinary mantle, in the adhesive area and on the arms have one or more cilia on the apical pole anchored with roots in the cell.

Process of secretion

All glandular cell types (cell types 1-3 and goblet cells) show high secretory activity. Cell types 1, 2 and 3 extrude their granules only partly, however the granules keep their form after release from the cell (Fig. 5). The conjugated cell types 2 and 3 always secrete simultaneously but the secretory material does not mix together.

Characterization of the non-adhesive mantle epithelium

The non-adhesive mantle epithelium is about 50 µm high. At the base of the epithelium is a basement membrane and all over the surface is a microvilli layer. The epithelium consists of the goblet cells and cell types 1 and 2 (less abundant than in the adhesive system) and the non-secretory interstitial cells. Some of the non-secretory cells have cilia as in the adhesive epithelium. Morphologically the cells correspond to those as described for the adhesive system.

Discussion

Sepia tuberculata adheres to hard substrates by mantle contractions to form a sucker-like cavity with the adhesive area (von Boletzky & Roeleveld

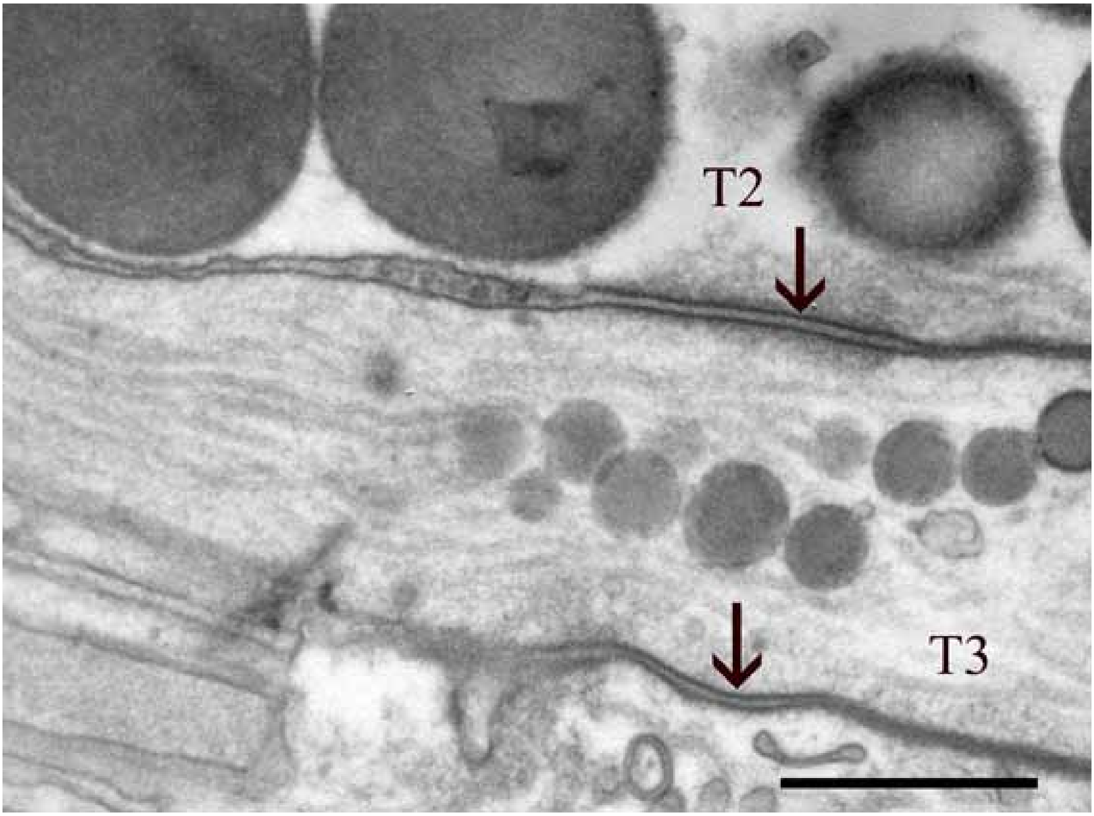

Fig. 3: Connection of cell type 3 with cell type 2 (T2) by zonulae adhaerentes (arrows); scale bar = 500 nm.

2000). The mechanical adhesion is supposed to be mediated by the distinct mantle musculature described by Sott (2000). In *Euprymna* an active deformation of the skin, that might be involved in deadhesion, is caused by the layers of mantle musculature (Singley 1982). In *Nautilus* is also an explicit musculature below the adhesive area (Kier 1987). In contrast, *Idiosepius* lacks a mantle musculature on its dorsal side below the adhesive organ (Cyran 2008).

Morphology of the adhesive system in Sepia tuberculata

The terminology of the cells proposed by Scott (2000) does not convene with the results of the present study: 1) the so-called columnar epithelial cells (Scott 2000) seem to correspond to the interstitial cells found in *Euprymna* (Singley 1982) and in *Idiosepius* (Cyran 2008). 2) The elongate "fine granular" mucus cells described by Scott (2000) were called cell Types 1-3 in this study. 3) The so-called ovate cells (Scott 2000) resemble the goblet cells of *Idiosepius* (Cyran 2008). Accordingly these cells are also termed goblet cells.

Although four gland cell types (cell types 1, 2, 3 and goblet cells) are present in the adhesive area, cell type 1, cell type 2, goblet cells and the non-secretory interstitial cells could also be found in the non-adhesive mantle epithelium. Only cell type 3 in conjugation with cell type 2 is specific for the adhesive system of *S. tuberculata*. This could be an indication for the involvement of these cells in the adhesive system. Anyhow these cell types are numerously dispersed in the adhesive epithelium.

The large bundles of filaments in the interstitial cells, extending from the cell base to the cell top, are also

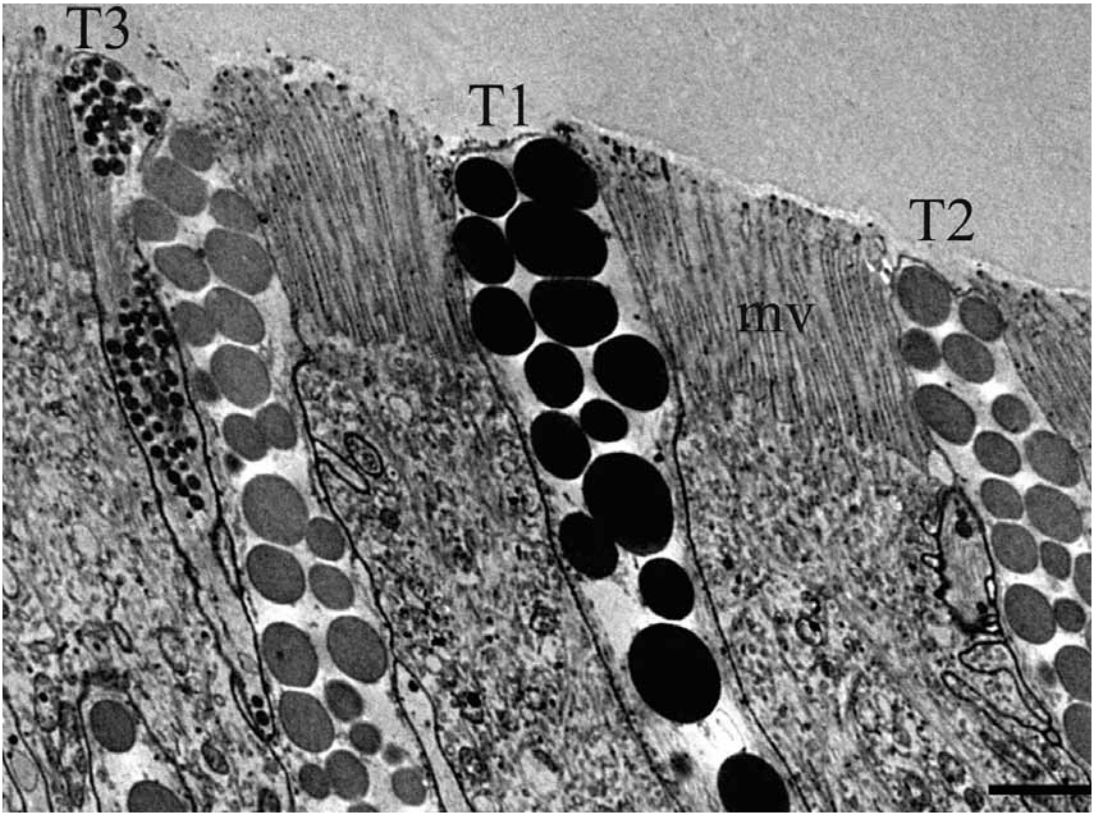

Fig. 4: Beginning secretion of cell types 1-3 (T1-T3). The granules of cell type 2 show a lower electron-density in comparison to the granules of types 1 and 3; mv = microvilli; scale bar = 2 µm.

an indication for mechanical adhesion as given for other cephalopods (Singely 1982; Cyran et al. 2008).

Von Boletzky and Roeleveld (2000) suggest that the neural mechanism underlying the specific use of the musculature for ventral adhesion must involve mechanoreceptors. They consider that mechanoreception is probably involved in recognizing differences of substrate properties (von Boletzky & Roeleveld 2000). The only evidence for mechanoreception found in the present study is the roots of cilia. The nerves found at the base of the adhesive area enhance this supposition.

Chemical nature of the adhesive system in Sepia tuberculata

All three glandular cell (types 1-3) show high secretory activity. The rER and the dictyosoms in cell types 1, 2 and 3 indicate the synthesis of protein and carbohydrate substances. Histochemical analysis by Scott (2000) verify that the elongate cells (filled with granules) are PAS positive, they contain neutral mucopolysaccharides. Scott does not distinguish the three different elongate cell types, as indicated in the present study. Therefore the elongate cells mentioned by Scott (2000) could be cell types 1, 2 or 3. Furthermore the secretion of both cell types is effected simultaneously. However, while in *Idiosepius* the glandular secretions merge together, in *Sepia* they seem to be still divided directly after secretion. Up to now it remains questionable if both secretions act combined as a two component system or antagonistic (duo gland system) as proposed for *Euprymna* (Singely 1982).

Goblet cells are partly filled with fine grained material but mostly empty, probably due to the preparatory process. Goblet cells are the mentioned

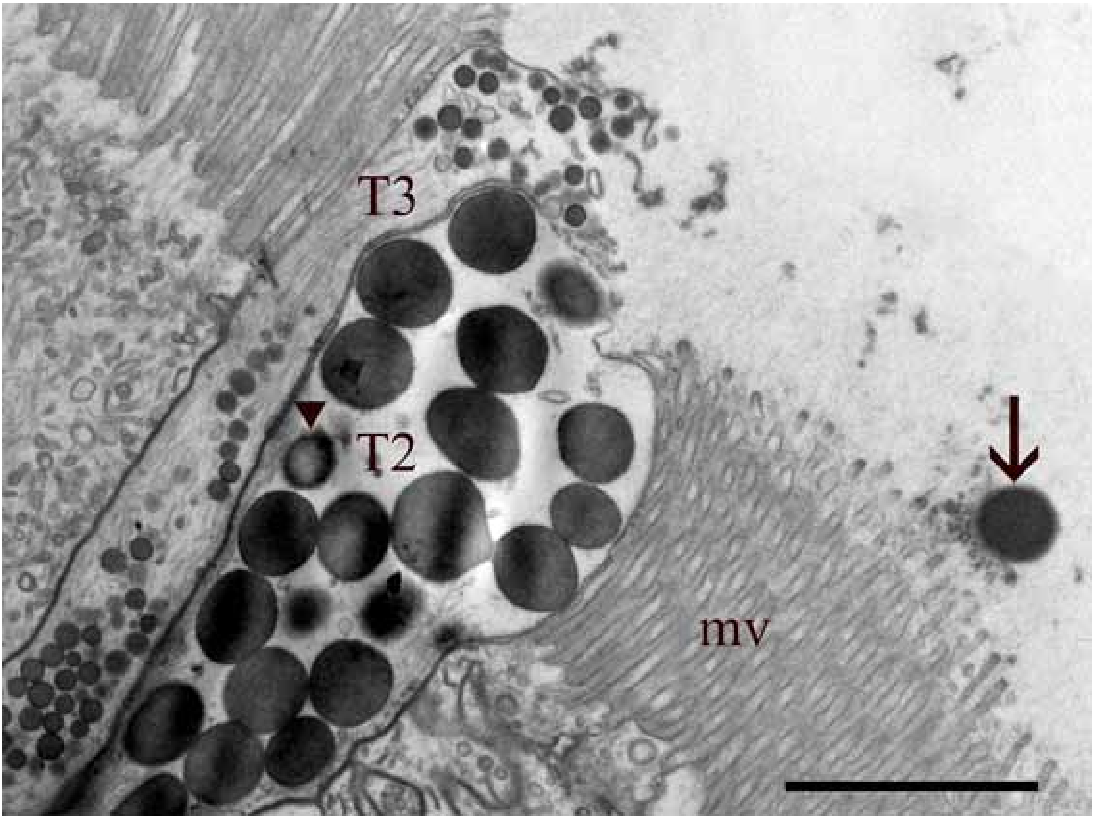

Fig. 5: Cell type 3 (T3) in comparison to cell type 2 (T2). The cell type T3 is narrower than cell type 2 and the granules are smaller. Granules of cell type 2 keep their structure after release of the cell (arrow); mv = microvilli; zonula adhaerens (arrowhead); scale bar = 2µm.

"ovate cells" (containing mucopolysaccharides) of the adhesive area in Scotts (2000) histochemical analysis. Scott assumes that the "ovate cells" might play a role in the adhesive behaviour of *Sepia tuberculata*. But the fact that goblet cells are also located in the ordinary mantle epithelium and even more numerous than in the adhesive epithelium indicates that they are neither involved in adhesion nor in release. Most likely the cells release mucus that is beneficial for adhesive and non-adhesive mantle epithelium. Scott (2000) mentioned a mucus layer covering the surface of the epithelium (stained PAS positive); this corresponds to the glycocalix of the microvilli from the interstitial cells. A glycocalix layer is also present on the adhesive epithelium of other cephalopods (Singely 1982; Cyran et al. 2008).

Conclusion

Despite *Sepia tuberculata* adhere mainly by mechanical mechanisms, the associated presence of cell types 2 and 3 exclusively in the adhesive area indicate their involvement in the attachment process. Scott (2000) observed bits of sands and other debris attached to the ventral mantle surface as well as to the ventral arms. This confirms that chemical substances, secreted from the two glandular cell types, might be adhesive. Which cell type synthesise the glue remains questionable in the present state. Scott (2000) does not differentiate the "elongate cell" type in her histochemical analysis, so either cell type 1, 2 or 3 could be meant.

However, this study points out similarities regarding the morphology of the adhesive organ of

Sepia tuberculata with those of the other cephalopod genera, further detailed histo- and biochemical analyses concerning the glue composition have to follow.

Acknowledgements

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Shell reduction and locomotory development in cephalopods: the recruitment of *engrailed* and *NK4* genes in *Sepia officinalis*

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Keywords: *Sepia officinalis*, cephalopod, *Engrailed*, *NK4*, shell differentiation, myogenesis, locomotory muscles, derived structures

Abstract

During evolution, shell regression among extant cephalopods allowed morphological and anatomical adaptations especially in the locomotory function. The structures belonging to the muscular hydrostat, i. e. the mantle, the arms and the funnel, which are associated to a specialized nervous system, are some of the numerous morphological peculiarities of cephalopods among other molluscs. Cephalopods showing morphological novelties that are instructive about the evolution of biological systems, we explored the role of homeobox genes known to be crucial in development, namely *engrailed* and *NK4*. Immunostaining results in *Sepia officinalis* suggest a role of engrailed in shell compartment delimitation but not in the mineralization function as was earlier proposed for other molluscs. *Engrailed* was also expressed in the mantle, arms and funnel. We observed *NK4* expression by whole-mount in situ hybridization in the same muscular structures. It seems that the emergence of cephalopods' morphological novelties required the secondary recruitment of these genes. This may have been necessary for the establishment of structures indispensable for an efficient locomotion in the absence of a protective shell.

Introduction

The majority of extant cephalopods have no external protective shell except Nautiloids. Coleoids possess an internal shell which is calcified and chitinous in sepiids (the cuttlebone) and only chitinous in squids (the gladius), whereas in octopods, the shell is reduced to a pair of chitinous stylets. During their evolution, the shell regression among extant cephalopods has allowed morphological and anatomical adaptations, especially concerning the locomotory function. Actually, the mantle, which is dedicated only to shell secretion in other molluscs, has changed in both structure and function. It has gained in associated muscles, which are strong enough to allow moving the animal by jet propulsion, and are therefore very efficient to achieve active predateion or to escape from other predators. The modified mantle associated with arms and funnel, probably derived from the unique molluscan foot (Naef 1923; Boletzky 1988a) are the main morphological novelties implied in the locomotory function in cephalopods.

Extant cephalopods live in a great diversity of environments reflecting their locomotory type. Indeed, pelagic forms such as *Loligo* move principally by jet propulsion whereas *Octopus*, a benthic form, uses also reptation. Our model species, *Sepia officinalis* shows intermediate characteristics linked to its lifestyle, at the interface of the benthic domain and the pelagic space. It presents giant fibers associated to a well developed chromatophore system, which is used for camouflage, and an internal calcareous shell which contains gas-filled chambers facilitating attainment of neutral buyoancy. We aimed to know how these structures are set up in an evolutionary perspective. Very few data are available concerning molecular pathways of cephalopod development. Thus, we focus on homeobox genes, which are known to be involved in cephalopod development, namely *engrailed* and *NK4* and investigate into their possible role in setting up locomotor structures. We have determined *engrailed* and *NK4* expression patterns in previous studies (Baratte, Andouche & Bonnaud 2007; Navet et al. 2008).

Among various roles identified for *engrailed* (segment polarity establishment: Fjose, Mcginnis & Gehring 1985, neurogenesis: Patel et al. 1989), it has been shown to be expressed at the margin of the future shell in a wide range of molluscs (e. g. a bivalve, *Transenella tantilla*, Jacobs et al. 2000, and a gastropod, *Ilyanassa obsoleta*, Moshel, Levine & Collier 1998).

NK4 is one of the numerous transcription factors implied in myogenesis in vertebrates. Its role in cardiac muscle development seems to be conserved throughout metazoan evolution. Indeed, *NK4* homologue genes have been implicated in cardiac cell determination in *Drosophila* (Bodmer 1993) and heart morphogenesis in vertebrates (Cripps & Olson 2002).

Here, we focus on *engrailed* and *NK4* expression patterns during *Sepia officinalis* development and related the recruitment of these genes to the emergence of morphological novelties involved in cephalopod locomotion.

Materials & Methods

Collection of *S. officinalis* embryos

During spring and summer (April to September), fertilized eggs were laid by captive *S. officinalis* females maintained in the biological stations of Luc-sur-mer (France) and Banyuls-sur-mer (France). Eggs were kept in artificial see water (Red Sea®) with aeration at room temperature. Development occurred in the chorion, which was surrounded by a tough nidamental membrane that surrounds the egg. In these conditions, the development was normal, as assessed by its time course and the morphological aspect of the embryos. From egg batches, individual eggs were detached and embryos were taken out by removing some of the nidamental envelopes, using forceps in sea water. Then, embryos were visually staged using Lemaire's (1970) system which has been defined for *S. officinalis*. As we focused on organogenesis, embryos at stages 15 to 30 were selected for whole-mount immunochemistry and *in-situ* hybridization (ISH). Embryos were fixed in 3,7% paraformaldehyde (PFA) in phosphate buffered saline (PBS). They were conserved in methanol at -20°C until use.

Study of *engrailed* expression patterns by Whole-mount immunochemistry

Engrailed has been cloned as previously described by Baratte, Andouche & Bonnaud (2007). The *engrailed* protein (*En*) was detected by using a monoclonal antibody Mab4D9 (Developmental Studies Hybridoma Bank, University of Iowa, USA) raised against a portion of the *Drosophila En* as the primary antibody (Patel et al. 1989). Whole-mount immunochemistry was performed according to the method described previously (Baratte, Andouche & Bonnaud 2007).

Study of *NK4* expression patterns by In situ hybridization

The full-length *NK4* cDNA was kindly provided by Dr Richard P. Harvey from the Developmental Biology unit of Victor Chang Cardiac Research Institute, Sydney, Australia.

RNA probes (1150 pb) were obtained with the Sp6-T7® kit from Roche as recommended by the manufacturer. Antisens probes were obtained with Sp6 polymerase. Sens probes, obtained with the T7 polymerase, were used as a control.

In situ hybridization (ISH) was done in tubes, each containing one embryo in large volumes of solution (approximately 2 ml), and kept under agitation. At least 3 embryos from each studied stage have been treated, and controls were done for each stage. *In situ* hybridization was achieved according to the method described previously (Navet et al. 2008).

Discussion of results

En has been recruited for shell sac boundary establishment

In early stages, the embryonic mantle invaginates and delineates a circular inner 'shell sac' (Boletzky 2006) (Fig. 1a). This cavity grows in size during stages 17 to 19, while its aperture at the mantle surface decreases in size and closes at stage 20 (Fig. 1b). Then the enclosed 'shell sac' starts producing both the organic matrix of the shell and a periostracum at stage 21. The aragonite mineralization starts at stage 24 (Fig. 1c) and continues as the first four chambers are set up progressively (Spiess 1972).

As we were expecting an *En* expression in nervous structures, we observed staining in several locomotory structures such as in the mantle and shell sac boundaries. As shown by Baratte et al. (2007), the *En* protein was first detected simultaneously at stage 16 in both the mantle and the whole shell sac area, as these two structures appeared (Fig. 1d). From stage 17, *En* immunostaining was restricted to both the shell sac and mantle edges (Fig. 1e). This is consistent with observations made in previous studies on other molluscs (Moshel, Levine & Collier 1998; Jacobs et al. 2000; Wanninger & Haszprunar 2001; Nederbragt, van Loon & Dictus 2002). Thus the *En* role as known in the molluscan external shell has been conserved in cephalopods, despite shell internalization. Wanninger & Haszprunar (2001) suggested that the lack of a mineralized shell in *Loligo* (squid) was a convincing explanation for a lack of an *En* cognate in *Loligo* species (Wray et al. 1995). The finding of *En* in a squid (Baratte, Andouche & Bonnaud 2007) and the previous characterization of *En* in *Euprymna scolopes* (with a rudimentary chitinous gladius) definitely eliminate this assumption. Moreover, *En* was no longer detected in the shell sac of *S. officinalis* embryos when shell formation started (at around stage 21). This provides evidence that *En* in molluscs is not required for the shell mineralization (i.e., the actual skeletogenesis, Jacobs et al. 2000) but rather for delimiting the shell compartment boundary.

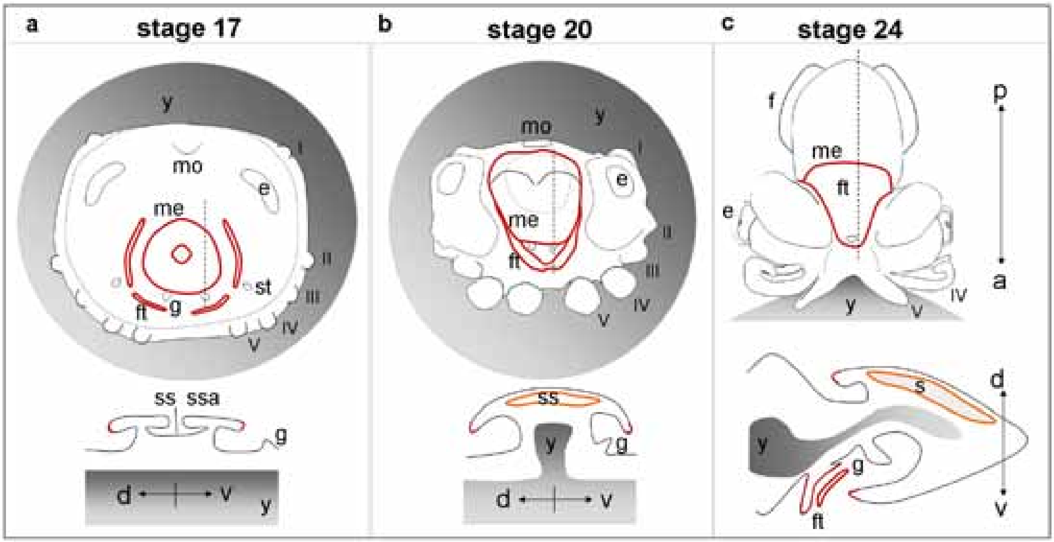


Fig. 1: Schematic representations of *S. officinalis* embryos throughout organogenesis, from stage 16 to 24 (hatching occurs at stage 30). Upper figures represent external views of embryos; lower figures represent transversal sections through the mantle region (dashed line in upper figures). In all figures, mantle edges and funnel are underlined with a red thick line and the shell with an orange line. a) Apical view of the embryo. From stage 14 to 19, the embryo is disc-shaped, lying on the spherical yolk mass. Future adult posterior structures are central (mantle) and future adult anterior structures (eyes, arms) are peripheral. The mantle is a placode and a central invagination predates the future shell sac (ss). b) Apical view of the embryo. From stage 20 to 22, the whole embryo grows in volume as organs develop. The shell sac is now closed. c) Ventral view of the embryo. From stage 23 (shown here is stage 24), the embryo eventually adopts the adult orientation: arms, funnel and head are gathered close to the yolk (anterior) while the mantle has a distal position (posterior). The shell has started mineralization and is now dorsally located. Abbreviations: I, II, III, IV and V: arm; a: anterior; d; dorsal; e, eye; f: fin; fp: funnel pouch; ft: funnel tube; g: gill; m: mantle; me: mantle edge; mo: mouth; s, shell; ssa: shell sac aperture; sse: shell sac edge; st: statocyst; p, posterior; v, ventral; y: yolk.

NK4 has been recruited for mantle muscle development

As we were looking for *NK4* expression in muscles of cardiac structures, we discovered that *NK4* was also expressed in locomotory muscles in *S. officinalis* (Navet et al. 2008). This was notable as the first case of *NK4* expression in a molluscan mantle.

NK4 expression was already observed at the earliest studied stage (early stage 15), before any visible structure emerged (Fig. 2a) in the presumptive territories of the mantle. Sections at these stages showed that *NK4* expression was exclusively mesodermal in all cases (Fig. 2d). This early expression of *NK4* suggests a role in muscle cell determination. From stage 20 the *NK4* staining in the mantle was maintained (Fig. 2c) until stage 23 when the staining decreased progressively (Fig. 2g). During development, a muscular reorganization leads to a progressive thinning down of the dorsal mesoderm layer. From stage 24, this layer is reduced to a thin membrane covering the shell whereas the ventral side of the embryo shows a large muscular layer. However, *NK4* is expressed in both the dorsal and ventral side, without any difference in the later stages of the embryos. Thus *NK4* would not seem to be involved in this late muscular reorganization but rather in the setting up of the mesodermal structures, as suggested by its very early expression in the mantle. This is emphasized by the fact that, in the later stages (28 to hatching), all specific staining disappeared.

Both *En* and *NK4* have been recruited for arm development

Embryonic arms, which are supposed to be foot-derived structures, emerge as a peripheral crown at stage 15 (Fig. 1a). As the whole embryo expands and raises above the yolk, arms become regularly

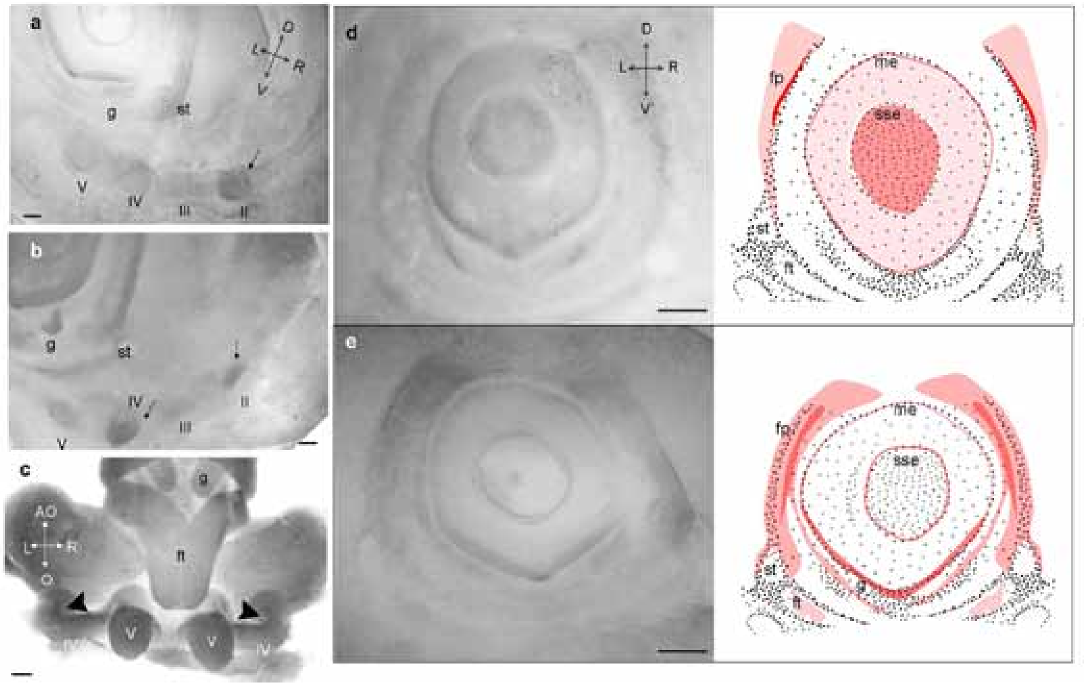


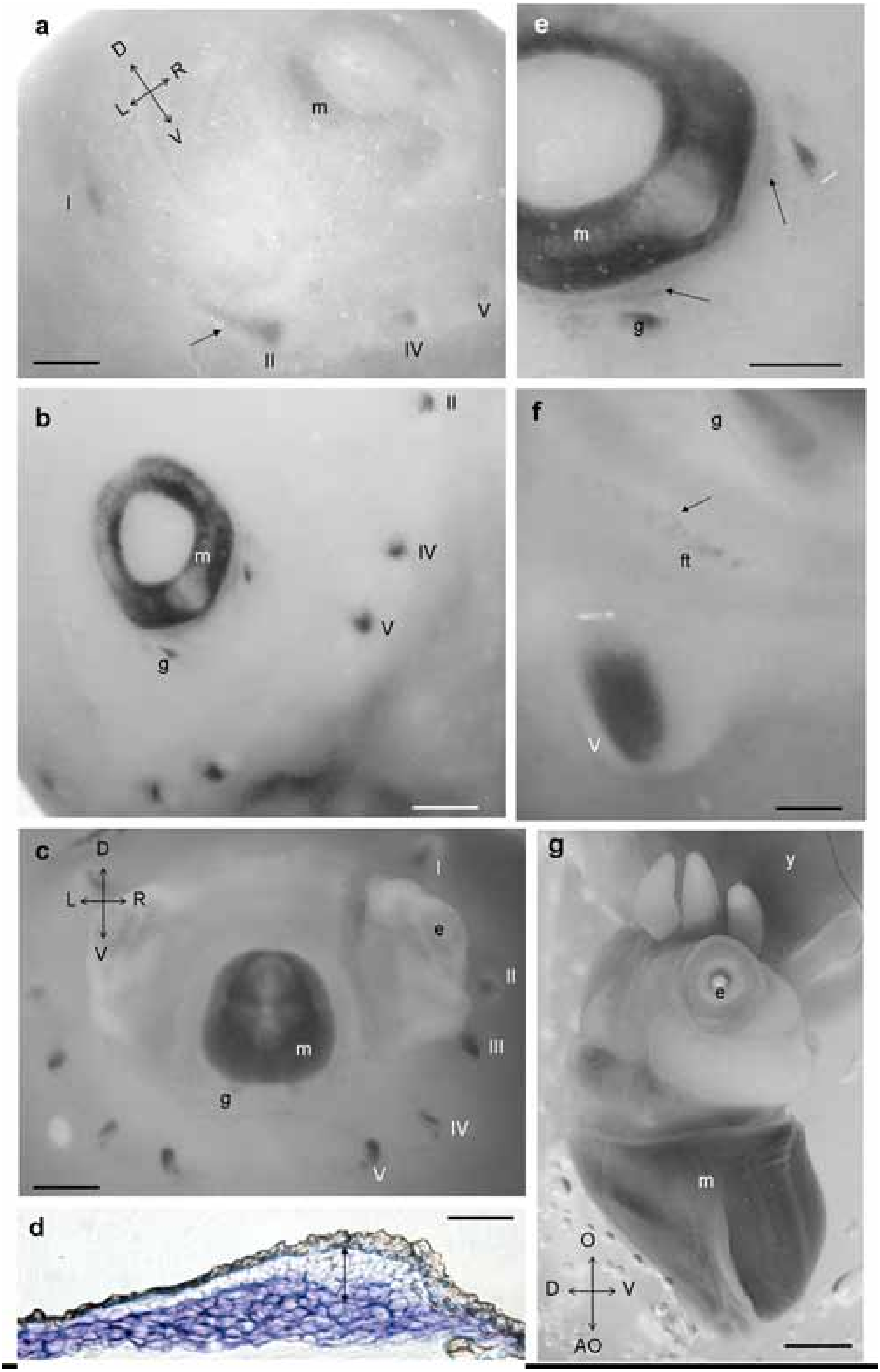
Fig. 2: Results of 4D9 immunostaining on *S. officinalis* embryos showing En expression. a to c: arms development, d and e: aboral pole of the embryo, showing both the developing mantle and shell. Same legends as in Fig. 1. Orientation: L: left; R: right; V: ventral; D: dorsal; AO: aboral; O: oral; scale bar = 100 µm. a) At stage 17, En is located in arm I (not shown) and arm IV (arrow). b) At stage 18 where arms I, II and IV are stained (arrows). c) At stage 24, arms IV exhibit En within an aboral line along the proximo-distal axis (arrowheads). d) At stage 16, En is located in the shell area (before invagination), in the mantle and in both funnel tube and pouch. e) At stage 17, En is located at the edge of the shell, at the edge of the mantle and in both funnel tube and pouch.

arranged around the mouth at the oral end of the animal. They first appear as small buds and then grow as cylinders with suckers located on their oral surface starting from stage 22. Suckers are present all along the arms, except for the so-called tentacles (derived arms IV) showing suckers at their extremities only.

Interestingly, we observed that both *En* and *NK4* were unexpectedly expressed in arm buds, whereas no *NK4* and *En* expression has ever been noticed in the molluscan foot before. We also noted that their expression followed a precise sequence during development.

Concerning *En*, it began to be expressed in the prospective dorsal arms (I and II) at stage 17 (Fig. 1a), soon followed by arms IV from stage 18 (Fig. 1b). At late stages 18 and 19, only arms II and IV expressed *En* with high intensity, whereas arms III and V were less strongly colored (Fig. 1c). From stages 19 to 23, no expression was detected; then,

Fig. 3 (right): *NK4* expression in early stages of *S. officinalis* organogenesis. Whole-mount *in situ* hybridization. Same legends as in Fig. 1. a) At stage 15 (no visible morphogenesis). *NK4* is expressed in the future mantle and arms, an extension adjacent to arm II prospective area is visible (arrow) ; scale bar = 500 µm. b) At stage 16, with arm buds emerging. Arm III is not yet visible. Gills show *NK4* staining; scale bar = 500 µm. c) At stage 20, *NK4* staining is restricted to the dorsal side of arm buds. Scale bar: 250 µm. d) Paraffin section of the mantle. The epithelium (double arrow) does not express *NK4*, which is exclusively mesodermic; scale bar = 25 µm. e) Detail of the ventral region of the mantle, stage 16. Arrows point to the prospective branchial heart area; scale bar = 250 µm. f) Detail of the funnel tube expressing *NK4*, stage 18; scale bar = 100 µm. g) At stage 26, only the mantle expresses *NK4* (left lateral view); scale bar = 1 mm.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a thin and linear *En* staining briefly appeared along the aboral side of arms IV at stage 24, which became restricted to the distal part, the future 'club', at stage 25 (Fig. 1d).

A sequential expression in the arms was also observed for *NK4*. Indeed, during early stages (15/16), all the prospective arm areas were *NK4* stained, except for arm III, which appeared later than the others (Fig. 2a and 2b). The intensity of expression was not the same in each arm, being in particular weaker in arm I. At stage 15, a weak but noticeable expression was observed as an anterior extension of arm II (Fig. 2a); it could correspond to migrating cells, as it was never observed on these arms in later stages. At stage 17, all arm buds were clearly visible and stained, including arm III. The staining remained until stage 20 (Fig. 2c). From stages 20 to 23, as the arm buds extended, *NK4* expression was restricted to the distal end in the arms and the expression was progressively less intense. *NK4* was not expressed concomitantly in every arm. Its expression seems to follow the different arms in their respective setting up as it appears at the same time as the development of arm III. However, no difference was ever noticed concerning arms IV, the future tentacles, and the other arms.

Both *En* and *NK4* have been recruited for funnel development

Also deriving from the molluscan foot, the adult funnel is an unpaired organ made of two structures, a tube and a pouch, which are developing from distinct areas in the embryo. Moreover, their embryological origins are different, the funnel tube rudiments belonging to the cephalopodium whereas the funnel pouch has a palliovisceral origin (Boletzky 1988b). The funnel tube arises from two bands that separate from the arm crown at stage 16, close to arms IV and V (Fig.1a). At stage 20, they join one another and form a cylinder that eventually closes at stage 22 (Fig. 1b). The funnel pouch, which essentially provides the retracting muscles, develops from two narrow bands lying along the lateral mantle edges that later connect to the funnel tube.

From stages 16 to 18, both tube and pouch areas showed *En* expressing cells (Fig. 1d and 1e). Then, the funnel pouch differentiated and *En* stopped being expressed. A sparse but clear *NK4* staining was also noticed in the funnel tube primordia (a structure already visible from stage 18 (Fig. 2f), but the funnel pouch primordia remained negative: these differences can be explained by their two distinct embryological origins. Indeed, the funnel tube rudiments belong to the cephalopodium, whereas the funnel pouch has a palliovisceral origin (Boletzky 1988b). At stage 21 to 22, as the two funnel tube primordia got in contact with one another, gills and funnel tube were no longer *NK4* - positive.

Conclusions

We show in this study two important genes to be recruited, and they appear correlated to morphological innovations in cephalopods. First, *NK4* is expressed during early development in *S. officinalis* not only in cardiac tissue (as is the case in most species) but also in muscle containing structures such as the mantle and foot's derived structures, e. g. the funnel, and the arms. This is also the case for the *En* protein which is not only expressed in mantle and shell sac edges but also in the funnel and arms.

Recruitment is correlated with subtle variations that may explain structural differentiations. The sequential expression of *En* in the five pairs of arms reminds one of the unexpected expression patterns of *Hox* genes as found in *Euprymna scolopes* (Lee et al. 2003). *Hox* genes encode a family of transcriptional regulatory proteins with a highly conserved role in axial patterning in Bilateria. Some data show that *Hox* orthologues have been recruited multiple times and could be at the origin of new cephalopod structures (Lee et al. 2003).

Expression of *NK4* was observed principally in locomotory muscles in *S. officinalis*. Locomotory muscles in cephalopods can be functionally compared to skeletal muscles of vertebrates or body wall muscles of *Drosophila*, which do not express *NK4*. Thus it seems that *NK4* has been recruited to participate in the setting up of these structures. Alternatively, these recruitments could be interpreted as a molluscan synapomorphy implicated in the foot morphogenesis. To our knowledge, no data are currently available regarding the expression of *NK4* homologues in other molluscan species. This could be an interesting perspective to explore.

Finally, it seems that *En* and *NK4* are involved in the formation of the muscular mantle, arms and funnel in *S. officinalis*. These structures belong to the hydrostat and are derived characters among molluscs. *En* and *NK4* could have been recruited in the cephalopod lineage for the development of these structures concomitantly with the internalization of the shell. These characteristics allow an efficient locomotion necessary to capture prey and to escape from predators in the absence of a protective external shell. This contributes to make the cuttlefish a better predator. This capacity is also directly related to the perception of the environment. This depends on the nervous system which is particularly complex in cephalopods. These new abilities would not have been possible without an already well developed and efficient nervous system. As a protective shell certainly became more obtrusive and less essential, the reduction of the shell and the subsequent mantle development clearly stand as an evolutionary win-win strategy. How the nervous system development, skeletogenesis and myogenesis in cephalopods' embryogenesis are connected is therefore a key question in any attempt to explain their evolutionary history.

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The Coleoidea of the upper Kimmeridgian Nusplingen Lithographic Limestone (Upper Jurassic, SW Germany) – diversity, preservation and palaeoecology

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Keywords: Coleoidea, belemnites, vampyromorphs, plattenkalks, Jurassic, Germany.

Abstract

The upper Kimmeridgian Nusplingen Lithographic Limestone, located in the south-western part of the Swabian Alb, is an important window into Late Jurassic sea life. Seven coleoid taxa have been recorded yet from this locality. Some of them are exceptionally preserved with their stomach contents, inc sacs and even their jaw apparatuses. Phosphatization of muscle tissue, better known from the Solnhofen area in Franconia, is rare, due to predation and special early diagenetic pathways. Nevertheless, the finds from Nusplingen permit not only the reconstruction of some previously unknown anatomical details of the animals but also close insights in the palaeoecological context (e.g. food-chains, habitats).

Introduction

The Nusplingen Lithographic Limestone represents the only fossiliferous example of laminates in the Upper Jurassic of Swabia. It is located in the south-western part of the Swabian Alb, c. 10 km north of the Danube Valley (Fig. 1), and covers an area of only c. 1.5 km^2. Excavations by a team of the Stuttgart Natural History Museum started in 1993 in the Egesheim quarry (lower part of section) and 1994 in the Nusplingen quarry (upper part of section), and are still ongoing in the latter quarry.

The Nusplingen Lithographic Limestone was deposited in an about 80 metres deep lagoonal environment surrounded by shallow water areas and sponge-microbial mounds which were partly (tectonically) elevated above sea level as small islands. These shallow water areas and islands were completely eroded in post-Jurassic times. In the Nusplingen lagoon, oxygen depletion caused the deposit of bituminous laminates, occasionally separated by decimetres-thick turbidite beds and a few bioturbated layers (Dietl et al. 1998). The existence of shallow water areas and islands is deduced from characteristic invertebrate

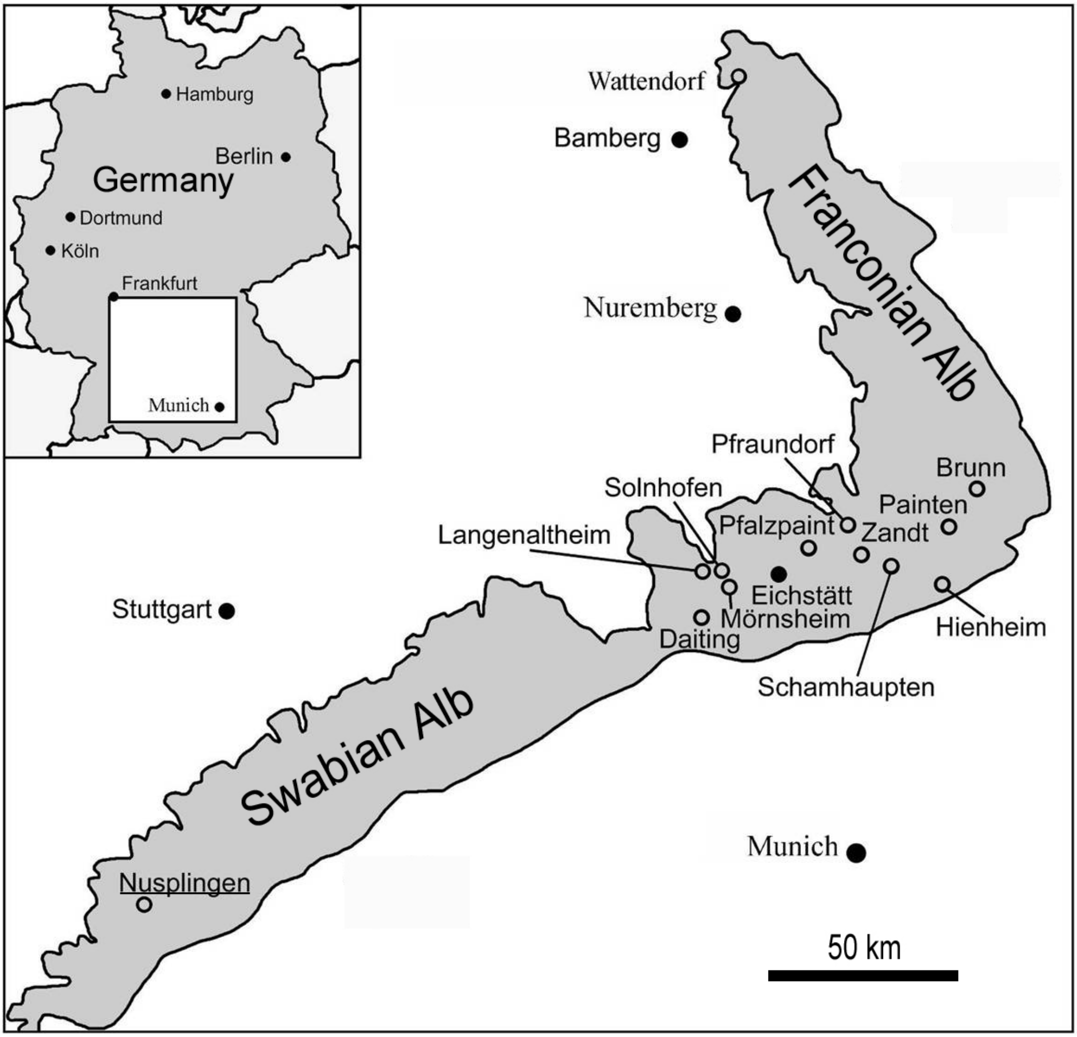
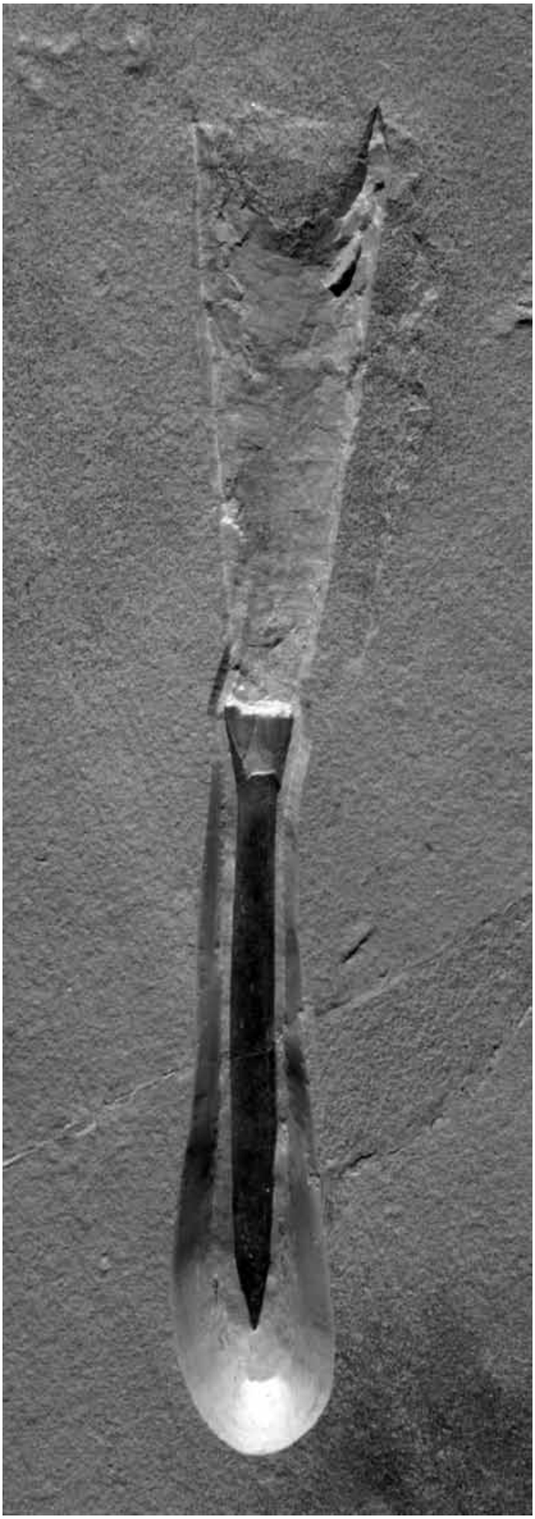


Fig. 1: Location of the Nusplingen Lithographic Limestone and other important plattenkalk sites in southern Germany (modified from Fürsich et al. 2007).

fossils (brachiopods, Dietl & Schweigert 2000; bivalves, Scholz, Schweigert & Dietl 2008) found in turbidite layers or as an input by predators, besides numerous land-plants and diagenetic observations in the vicinity of the fossil site. More details on the geological setting and the sections of this Fossil-Lagerstätte may be obtained from Dietl et al. (1998).

In contrast to the classic lower Tithonian Solnhofen Limestones of Franconia, the ammonite fauna of the Nusplingen Lithographic Limestone indicates a slightly older age, late Kimmeridgian, Ulmense Subzone (Schweigert 1998; 2007), and the Nusplingen lagoon was probably located in a more pelagic setting. The Nusplingen Lithographic Limestone is famous for its exceptional preservation of vertebrates such as fishes, sharks, marine crocodiles, pterosaurs, and land plants, although invertebrates like decapod crustaceans and especially cephalopods are much more common (Dietl & Schweigert 1999, 2001, 2004). Until 2009 a total of c. 355 fossil taxa is recorded.



Materials & Methods

The studied coleoid material from the Nusplingen Lithographic Limestone comes, with one exception, from the excavations of the Stuttgart Natural History Museum (SMNS) and is housed in the collections of this institution. One historical finding, a big *Leptotheuthis*, is in the permanent exhibition of the Institut für Geowissenschaften of Tübingen University. Material for comparison was studied in the Jura-Museum Eichstätt (JME) and in the Bayerische Staatssammlung für Paläontologie und Geologie in Munich.

The recently recovered specimens were extracted from the rock mechanically, occasionally with the control by ultraviolet illumination to detect possible phosphatic matter.

Observations

Coleoid diversity

In contrast to a rather high-diverse coleoid fauna found in the Solnhofen limestones of Solnhofen and Eichstätt (Frickhinger 1994, 1999; 18 nominal taxa according to Fuchs 2007), only seven taxa of coleoids (and some additional parataxa) are recorded from the Nusplingen site. Contrary statements by Bülow-Trummer (1920), who mentioned much more taxa coming from the Nusplingen site, are erroneous. Fuchs (2007) argued that the lower number of taxa in Nusplingen may result from the comparatively small area of investigation.

Interestingly, in contrast to the Solnhofen Lithographic Limestones in Franconia, belemnite rostra represented by *Hibolithes semisulcatus* Münster (Fig. 2) are very common in all beds of the Nusplingen section except of the turbidites. In addition, a specimen of a second belemnite taxon was recorded in the 2007 excavation campaign. This is the minute *Rhaphibelus acicula*

Fig. 2: *Hibolithes semisulcatus* (Münster). Specimen SMNS 67275, upper Kimmeridgian, Nusplingen Lithographic Limestone, *Beckeri* Zone, Nusplingen quarry, Bed C, rostrum with preserved phragmocone; length of rostrum 118 mm.

(Münster), previously only known from the lower Tithonian Lithographic Limestones in the vicinity of Eichstätt (Riegraf 1981). The sole specimen from Nusplingen (Fig. 3) expands the known stratigraphical range of this species down to the late Kimmeridgian.

Among belemnoteuthids, we tentatively assign some poorly preserved phragmocones lacking a calcitic rostrum to *Pavloviteuthis kapitzkei* Engeser (Fig. 4). The latter species was originally described from late Kimmeridgian coralliferous limestones of E Swabia (Engeser 1995) which are coeval with the Nusplingen Lithographic Limestones. Exactly the same form as in Nusplingen, however, also occurs in the lower Tithonian of Solnhofen (unpublished material in Coll. M. Wulf, Rödelsee). Some hooklets found within a phosphatic coprolite are partly assigned to the parataxon *Deinuncus* (cf. Kulicki & Szaniawski 1972) but it is unclear whether they also belong to *Pavloviteuthis* or to another still unknown form (Fig. 7b).

Besides these mentioned belemnoids there are three other species recently assigned to Vampyromorpha by Fuchs (2006) and Fuchs, Engeser & Keupp (2007): *Leptotheuthis gigas* v. Meyer, *Plesioteuthis prisca* (Rüppell), and *Trachyteuthis nusplingensis* Fuchs, Engeser & Keupp (see Fuchs, Klinghammer & Keupp 2007). When recently the illustrated specimens of *Trachyteuthis* from Nusplingen were recognized as a separate taxon which seems to be restricted to this site (Fuchs, Engeser & Keupp 2007), the question arose whether this occurrence is either due to evolutionary or to regional reasons. In other words, did the long-known species *Trachyteuthis hastiformis* (Rüppell) from the early Tithonian Solnhofen limestones evolve from the late Kimmeridgian *Trachyteuthis nusplingensis*, or does the latter just represent a local race? *Trachyteuthis nusplingensis* exhibits a well-limited central median field with a finely granulate dorsal surface (Fig. 5a). In contrast, *Trachyteuthis hastiformis* shows a more spindle-like elevation on the central median field with a coarser granulation. A re-study of all prepared specimens of *Trachyteuthis* from Nusplingen and new material from the 2008 excavation campaign indicates that some specimens of *Trachyteuthis nusplingensis* have a less distinct central field and a somewhat coarser granulation, but are still closer to *T. nusplingensis* than to *T. hastiformis*. These cf.-specimens (Figs. 5b, 6) occur already in the lowermost beds of the Nusplingen section, but they become predominant in the highest autochthonous parts of the section. Thus, true *T. hastiformis* does not occur in Nusplingen, whereas in contrast *T. nusplingensis* is extremely rare in Franconia, although the total

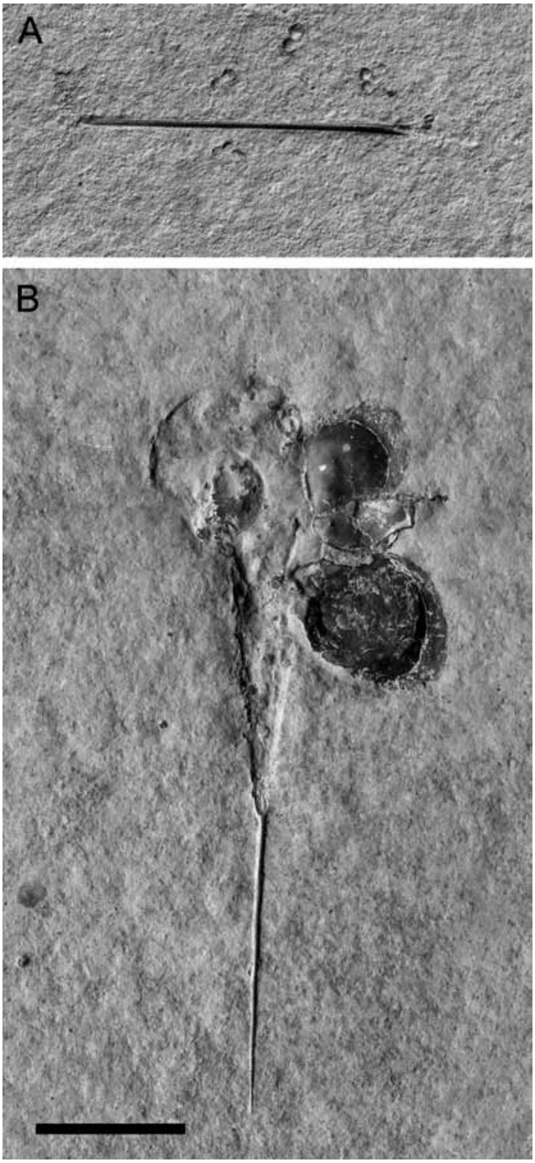


Fig. 3: *Rhaphibelus acicula* (Münster). A) Specimen SMNS 67539, upper Kimmeridgian Nusplingen Lithographic Limestone, *Beckeri* Zone, Nusplingen quarry, Bed C. B) Specimen JME SOS 5589a, lower Tithonian Eichstätt Formation, *Hybonotum* Zone, Wintershof near Eichstätt; scale bar = 20 mm.

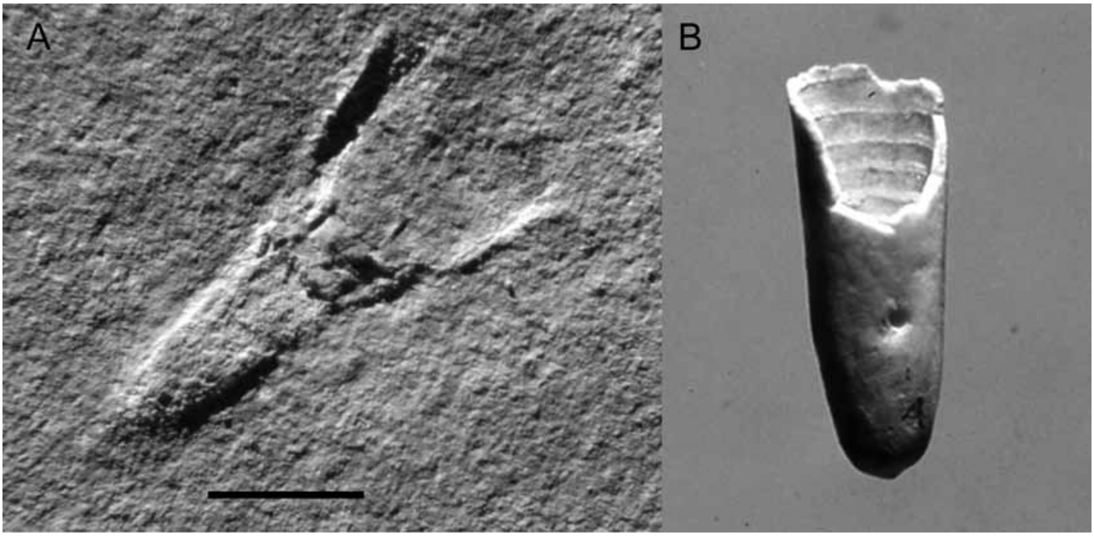


Fig. 4: *Pavloviteuthis kapitzkei* Engeser. A) Specimen SMNS 64877, upper Kimmeridgian, Nusplingen Lithographic Limestone, *Beckeri* Zone, Nusplingen quarry, Bed G. B) Holotype (SMNS 62433/1), Mergelstetten Formation, coraliferous beds, upper Kimmeridgian, *Beckeri* Zone; scale bar = 5 mm.

number of recovered *Trachyteuthis* specimens is much bigger there than in Nusplingen. Besides a questionable example illustrated by Münster (1846) we know of only a single still unpublished specimen that resembles the cf.-specimens from Nusplingen. The latter, however, comes from the uppermost Kimmeridgian site Schamhaupten. Additional material from other localities in Franconia is needed to decide whether *T. nusplingensis* was just a phyletic forerunner of *T. hastiformis* or an independent taxon with ecologically differing requirements.

Preservation of coleoids and implications for palaeoecology

The various modes of preservation of the belemnite *Hibolithes semisulcatus* (Münster) in the Nusplingen Lithographic Limestone were studied in detail by Schweigert (1999a). Besides belemnite rostra (Fig. 2) also their isolated hooks (Onychites) are rather common. Their obvious changes in shape during ontogeny led Engeser (1987) to assume a higher belemnite diversity than suggested from the quite uniform rostra shape alone. Most recently, we recovered a unique specimen of a belemnite with the inc sac, beaks and tentacles preserved, which will be published elsewhere due to its outstanding importance (Klug et al. 2009).

From the above mentioned vampyromorph coleoids not only the gladii but also the mandibles are known, in the cases of *Leptotheuthis gigas* v. Meyer and *Trachyteuthis nusplingensis* Fuchs, Engeser & Keupp even as *in-situ* finds (Klug et al. 2005). Also in a single newly recovered specimen of *Plesioteuthis prisca* (Rüppell) from a bituminous bed of the Nusplingen quarry not only the gladius and the ink but also one of its jaws is preserved (Klug, Schweigert und Dietl, this volume). Another remarkable example from the uppermost Kimmeridgian of Painten in Franconia shows the jaw apparatus, the inc sac, the gills, and even the cirri along the arms.

In Nusplingen, the phosphatic mantle tissue of coleoids is only preserved in a few specimens, among them a large *Leptotheuthis gigas* showing its arms with cirri, the beaks, the eye, and the funnel (see Klug et al. 2005: fig. 10). This rarity is somewhat strange, because either the ink sac or the leaked ink is almost always present, pointing to a rapid burial. Unfortunately the old *Leptotheuthis* find is in a rather poor preparation state. The only newly recovered find of this species from the Nusplingen Lithographic limestone is a carcass from the Egesheim quarry which is badly preserved due to the onset of decay.

Interestingly, like in many belemnites rostra (Hölder 1955; Schweigert 1999a, b) also the bulk of vampyromorph gladii from Nusplingen show

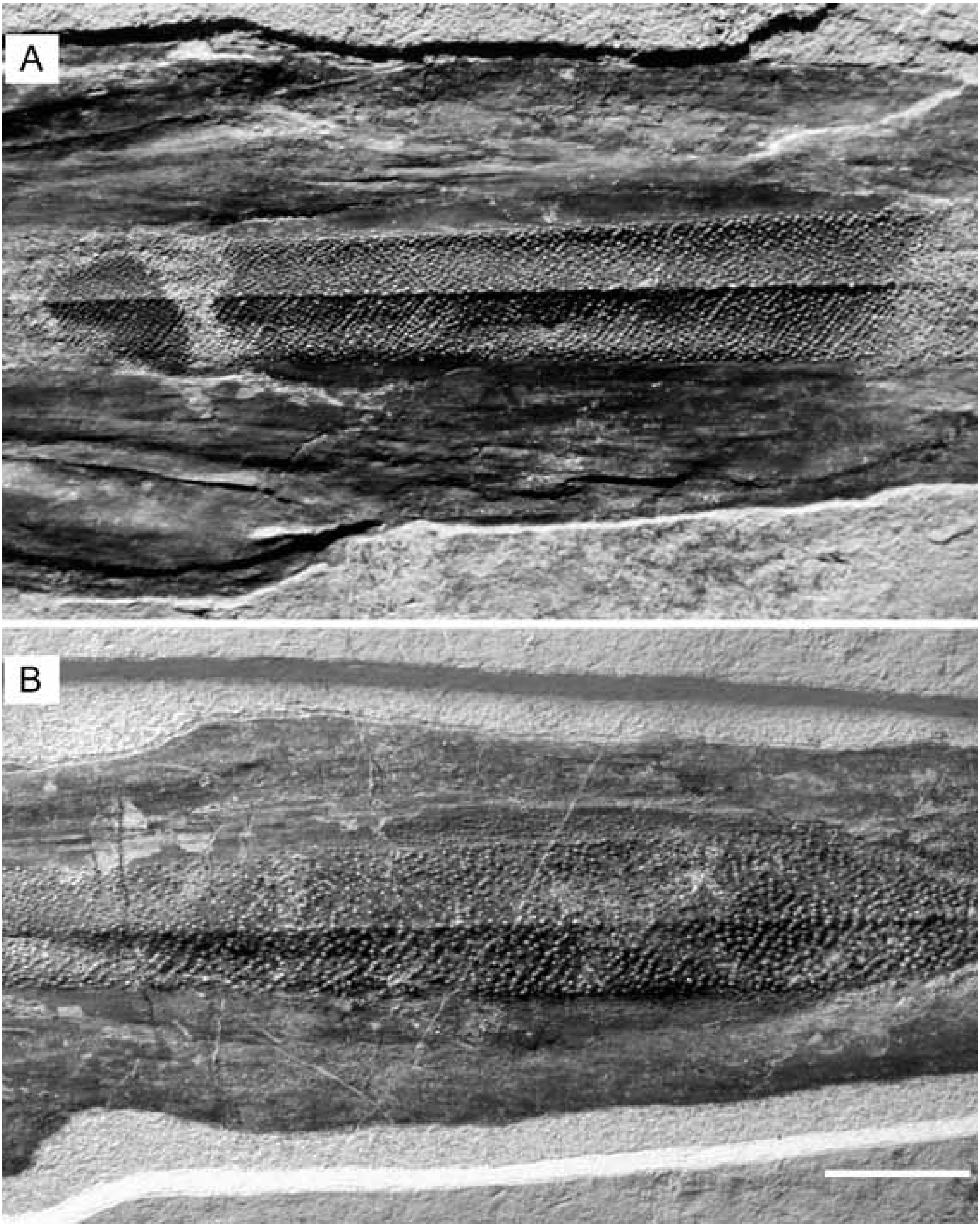


Fig. 5: A) *Trachyteuthis nusplingensis* Fuchs, Engeser & Keupp. Specimen SMNS 65345, Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Nusplingen quarry, Bed L, detail of the central median field showing the dorsal pustulation. B) *T.* cf. *nusplingensis* Fuchs, Engeser & Keupp. Specimen SMNS 63257, Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Egesheim quarry, Bed Pk4, note the differences in postulation; scale bar = 20 mm.

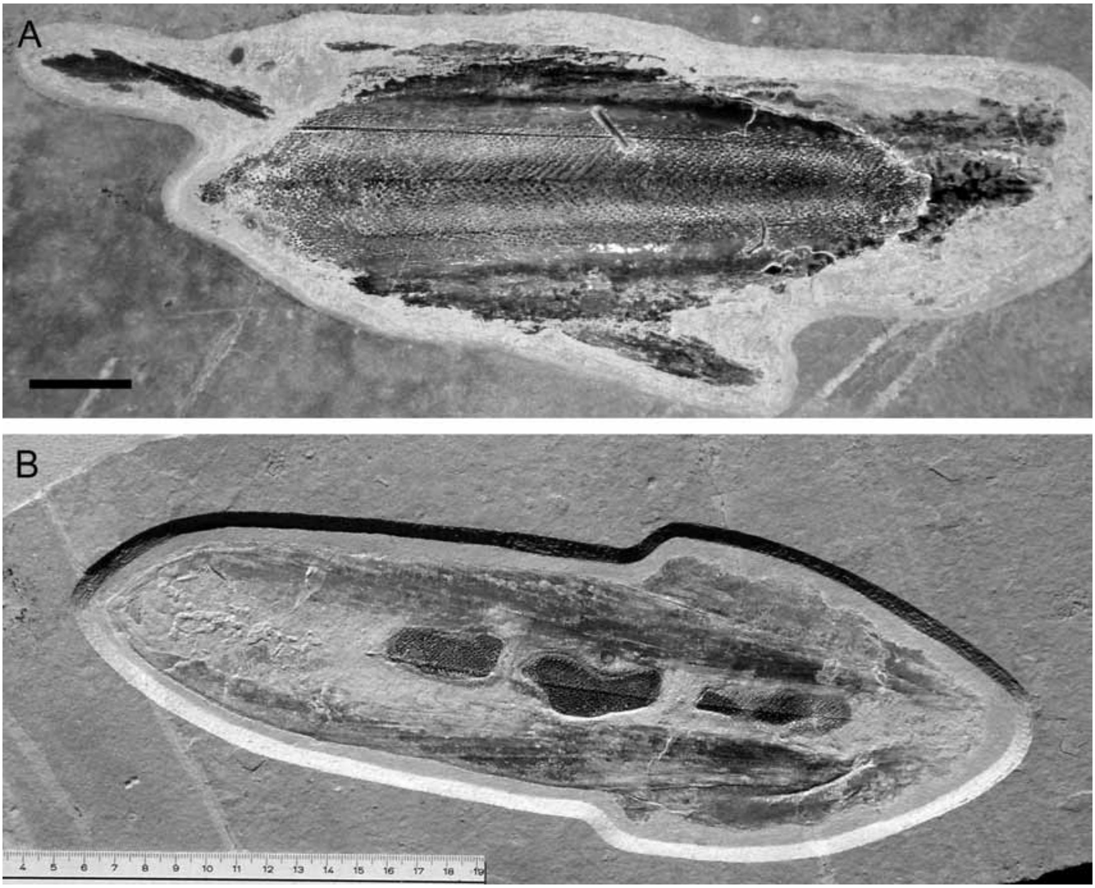


Fig. 6: *Trachyteuthis* cf. *nusplingensis* Fuchs, Engeser & Keupp, Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Nusplingen quarry. A) Specimen SMNS 67540, strongly bitten gladius, Bed G; scale bar = 20 mm. B) Specimen SMNS 67500, gladius with bite marks along posterior margin and remains of phosphatic muscle tissue covering the surface, Bed C.

evidences of predation (Fig. 6). In none of these specimens the beaks are preserved. Moreover, in several examples of *Plesioteuthis* and the *Leptotheuthis* mentioned above (but not yet in belemnites) the prey is preserved as stomach or crop contents. It consists of fish remains (bones and scales), in one example of *Plesioteuthis prisca* together with small lamellaptychi (the lower beaks of oppeliid ammonites). Lying directly on the ventral side of a newly recovered *Trachyteuthis* sp. a larger fragment of a lamellaptychus may represent a rest of its prey. According to these observations some finds of coprolites containing bitten aptychi and fish scales may originate from coleoids.

Conversely, belemnite rostra or hooklets assignable to belemnites are sometimes found in coprolites or as regurgitates (Schweigert 1999a; Figs. 7, 8). A broken belemnite rostrum was found in the *in-situ* preserved stomach content of the marine crocodile *Cricosaurus suevicus* (E. Fraas) from the 2004 excavation campaign. Surely, coleoids were also consumed by other predators such as fish or bigger sharks, like in the Early Jurassic Posidonia Shale (e.g. Delsate, Fuchs & Weis 2008), but there is no evidence from our sampled fossils yet that confirms this assumption. In one specimen of *Plesioteuthis prisca* the gladius shows healed breakages, leading to the conclusion that this specimen escaped from a predator (Dietl & Schweigert 2001: fig. 135.4).

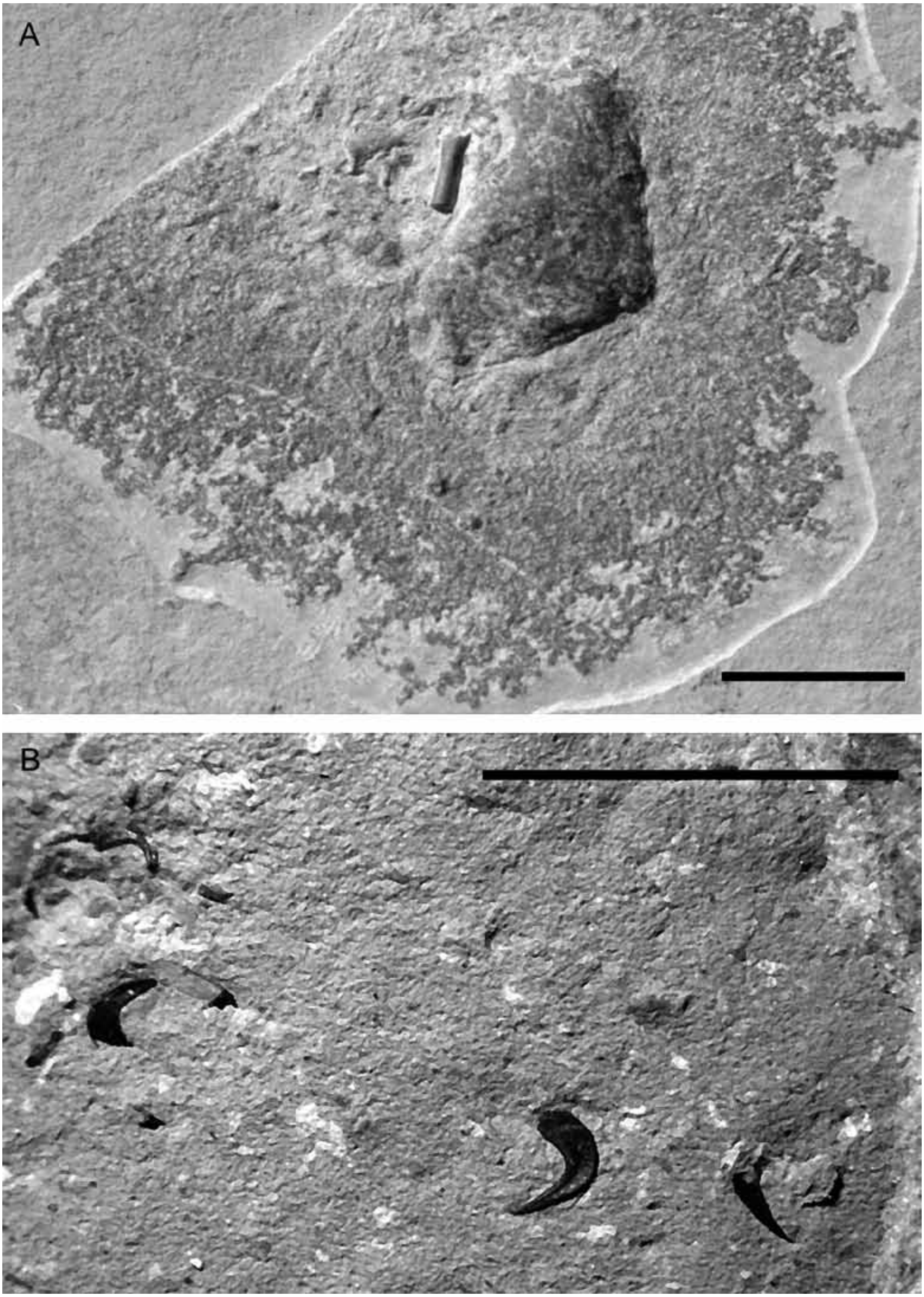


Fig. 7: A) Phosphatic coprolite with fragment of a belemnite rostrum. Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Nusplingen quarry, Bed G, SMNS 67541; scale bar = 20 mm. B) *Deinuncus*-type hooklets on the surface of a phospatic coprolite; Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Nusplingen quarry, Bed G, SMNS 65516; scale bar = 5 mm.

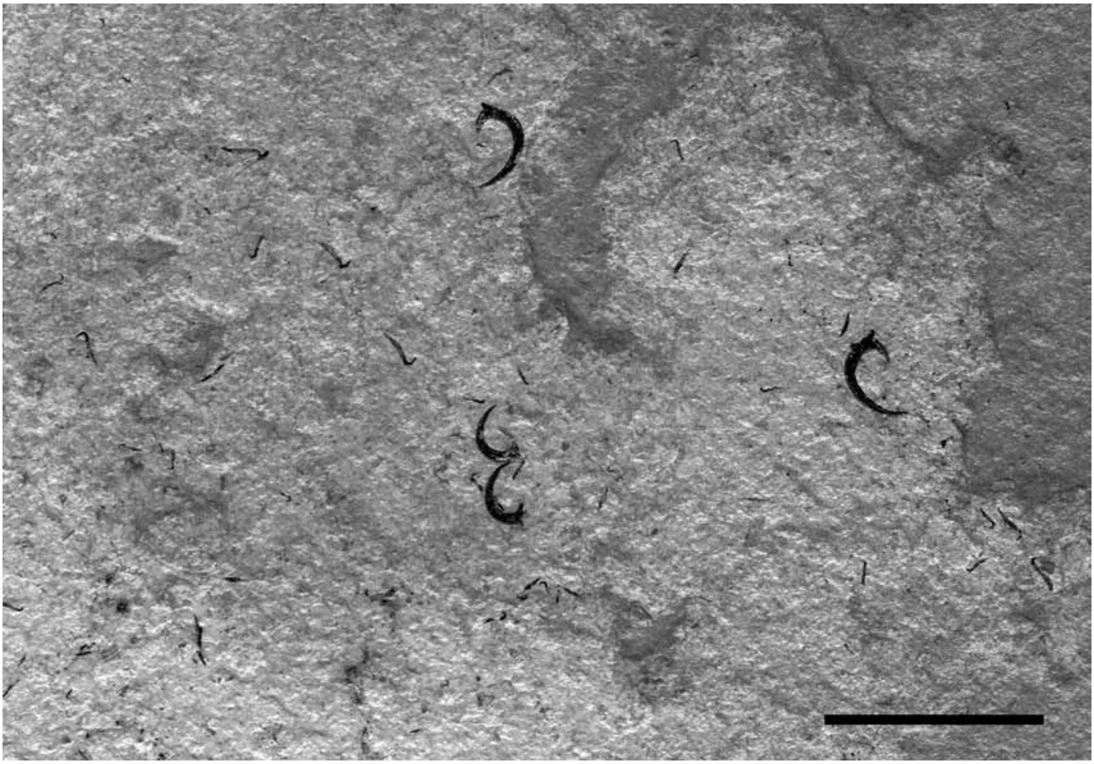


Fig. 8: Regurgitate with remains of at least two specimens of belemnites indicated by their bigger *Onychites* hooks and tiny tentacle hooklets. Nusplingen Lithographic Limestone, upper Kimmeridgian, *Beckeri* Zone, Nusplingen quarry, Bed F, SMNS 63725; scale bar = 20 mm.

Although both belemnites and vampyromorph coleoids are rather common in the Nusplingen Lithographic Limestone there is one remarkable difference. Belemnites occur in all ontogenetic stages, from very small juveniles with a length of the rostrum of c. 10 mm to adult ones with a length of over 150 mm. In contrast, there are no records of juveniles of vampyromorphs at all. After more than 15 years of excavations we can exclude a collecting bias. This leads to the conclusion that belemnites lived permanently in the Nusplingen lagoon whereas the vampyromorphs obviously inhabited this site only in the adult stage for feeding, but their reproduction area was located somewhere else.

Acknowledgements

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Naefia sp. from the Cenomanian of south-eastern India

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Abstract

Since Blanford (1865) figured a *Naefia*-like phragmocone from the Cenomanian of south-eastern India, the definite existence of *Naefia* from the Cenomanian of India has not been confirmed. Longiconic phragmocones with comparatively high chambers collected from an outcrop near Odiyam (Ariyalur district, Tamil Nadu, south-eastern India) have now proofed to represent *Naefia* sp. The collection site belongs to the Cenomanian Kunnam Member of the Karai Formation. The phragmocones are poorly preserved why systematically relevant characters are scarce. A specific determination is therefore problematic. However, one specimen exhibits a maximum phragmocone diameter of 13 mm, the largest diameter recorded in the genus *Naefia*. Paleobiogeographically, the specimens represent the first evidence of Cenomanian *Naefia* outside the paleopacific and the northern hemisphere.

Introduction

The extinct genus *Naefia* is typified by a longiconic phragmocone with comparatively long chambers and has been first described from the Maastrichtian of Chile by Wetzel (1930). Increased research activities in the past 30 years of coleoid research rapidly improved our knowledge about the stratigraphic and geographic occurrence of *Naefia* (Doyle 1986; Stilwel & Zinsmeister 1987; Hirano, Obata & Ukishima 1991; Doguzhaeva 1996; Hayakawa & Takahashi 1993; Ifrim, Stinnesbeck & Lopez-Olivia 2004). According to the current state of the art, *Naefia* was worldwide distributed and appeared during the early Cretaceous and disappeared in the latest Cretaceous. Although morphological investigations on *Naefia* have been also considerably increased (Biro-Bagoczky 1982; Doyle 1986; Hewitt, Yoshike & Westermann 1991; Doguzhaeva 1996; Bandel & Stinnesbeck 2006; Fuchs 2006a+b; Fuchs & Tanabe in press), observations and/or their interpretations are still controversial resulting in different systematics. Previous classifications of *Naefia* therefore differ between belemnoid, spirulid and transitional affiliations. Apart from its true affiliation, the character complex observed in *Naefia* (and

Groenlandibelus) seems to link belemnoids and Cenozoic spirulids (Fuchs & Tanabe in press).

Long before Wetzel (1930), Forbes (1846) published the first phragmocones of *Naefia*. His poorly preserved specimens have been recorded from the Campanian - Maastrichtian Valadavur beds of Pondicherry (south-eastern India). However, Forbes (1846) was not able to realize the peculiarity of these longiconic phragmocones and considered them as "Belemnites" (see Doyle 1986). Although Wetzel (1930) mentioned similarities between *Naefia neogaiea* from the Quiriquina Formation (Chile) and the coeval phragmocones from Pondicherry, he excluded the specimens of Forbes (1846) from his newly erected genus *Naefia*. Hence, Doyle (1986) is credited to have first recognized and described *Naefia* from the Campanian - Maastrichtian of India.

Although pre-Campanian *Naefia* has been first mentioned by Hirano, Obata & Ukishima (1991) from the Cenomanian Mikasa Formation of Hokkaido (northern Japan), Blanford (1865: pl 2, fig. 8) and Foote (1879: 159-160) seem to have much earlier recorded *Naefia*-like phragmocones from the Cenomanian of Odiyam (Ariyalur region, south-eastern India). This assumption is based on phragmocones from the Odiyam region collected during 1981 and 1983 by one of us (A.V.). Since these phragmocones clearly confirm the existence of *Naefia* from the Cenomanian of India, it is the aim of the present article to provide a full description for the first time.

Geological setting

21 phragmocones were collected along a road 1.2 km west of the village Odiyam (Odiyam: 11^{0} $13^{'}$ 00" N, 78^{0} $59^{'}$ $30^{''}$ E; c. 14 km northwest of Ariyalur, Tamil Nadu district, south-eastern India; see Fig. 1). The collection site belongs to the Karai Formation of the Uttattur Group (Fig. 2) and more precisely to the Kunnam Member (in contrast, *Conoteuthis azizi* n. sp. described by Fuchs et al. in this volume originates from the slightly older Odiyam Member). The thickness of the Kunnam Member is about 125 m and consists of mudstone, siltstone and fine-grained sandstone with calcite and siderite cements, which is variably fossiliferous sometimes ranging to coquinite. The shell limestone of the Kunnam Member is ammonite rich, hard, well cemented and greyish brown in nature. It yielded unusual concentrations of heteromorph ammonites and many belemnite phragmocones (no belemnite rostra have been collected). As postulated in Sundaram et al. (2001) and followed by Kendrick & Vartak (2007), the Kunnam member is more arenaceous than the Odiyam Member, which is also exposed around Odiyam. Vartak (1990) recorded heteromorph ammonites as *Turrilites acutus*, *Hypoturrilites tuberculatus*, *Hypoturrilites gravesianus*, *Scaphites obliquus, Scaphites daileyi, Sciponoceras baculoide* and *Sciponoceras roto*. Kendrick & Vartak (2007) suggested a Lower or Middle Cenomanian age (R. A. Henderson, personal communication December 1998) for the shell limestone.

Systematic Paleontology

Subclass COLEOIDEA Bather, 1888
Order SPIRULIDA Pompeckj, 1912
Family GROENLANDIBELIDAE Jeletzky, 1966

Type genus: *Groenlandibelus* Jeletzky, 1966

Genera included: *Groenlandibelus* Jeletzky, 1966 and *Naefia* Wetzel, 1930

Genus *Naefia* Wetzel 1930

Type species: *Naefia neogaeia* Wetzel, 1930, by original designation (type specimens GPIK 121a+b are deposited at the Geologisches und Paläontologisches Institut, Kiel, Germany).

Species included: *Naefia neogaeia* Wetzel, 1930 from the Campanian-Maastrichtian of Chile (Wetzel 1930; Biro-Bagoczky 1982; Stinnesbeck 1986; Bandel & Stinnesbeck 2006), Mexico (Ifrim, Stinnesbeck & Lopez-Olivia 2004), Antarctic Peninsula (Wetzel 1930; Stilwell & Zinsmeister 1987), South India (Doyle 1986), California (Hewitt, Yoshike & Westermann 1991); *Naefia matsumotoi* Hirano, Obata & Ukishima, 1991 from the Cenomanian -Campanian of Japan (Hirano, Obata & Ukishima 1991; Hewitt, Yoshike & Westermann 1991), and *Naefia kabanovi* Doguzhaeva, 1996 from the Aptian of the north-western Caucasus (Doguzhaeva 1996).

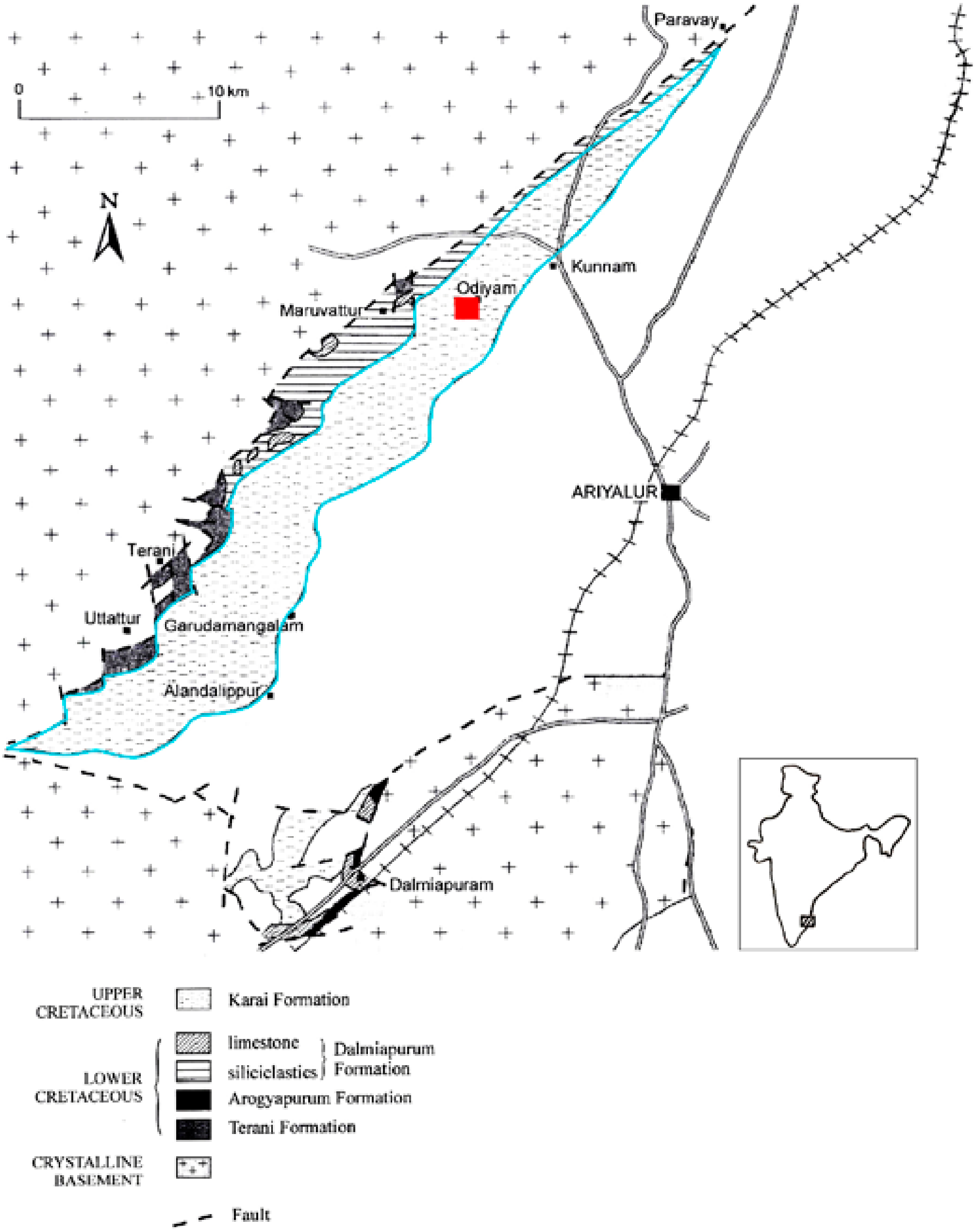

Fig. 1: Map of India with south-eastern India in inset. The outcrop area of the Karai Formation is indicated by the checked pattern (modified after Sundaram et al. 2001).

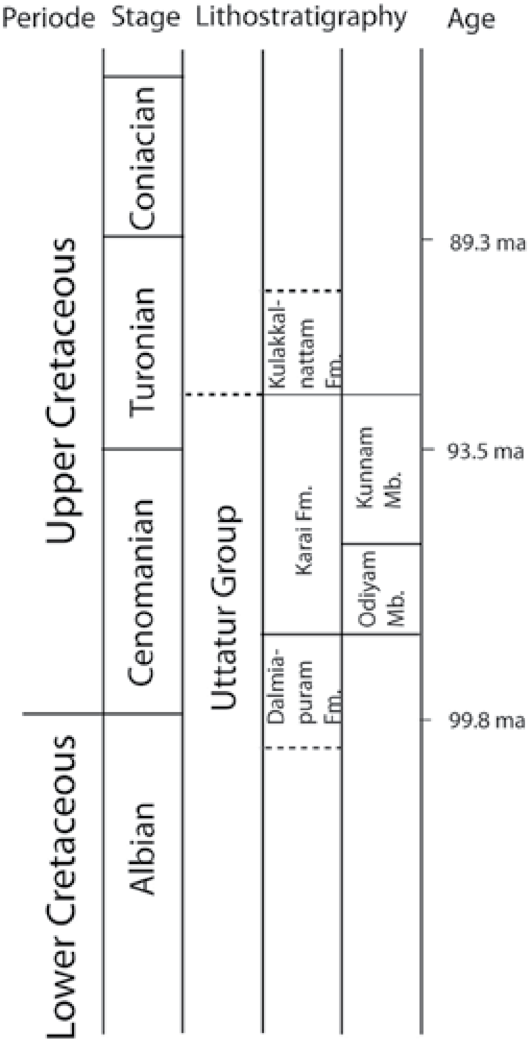

Fig. 2: Stratigraphic position of the Karai Formation.

***Naefia* sp.**
Fig. 3A-E

Material: 21 specimens (MACS G1712-1732); the specimens are housed in the Agharkar Research Institute (ARI), Pune, India.

Preservation: Phragmocones are exclusively fragmentary, i.e. the material consists mostly of isolated sometimes slightly compressed chambers consisting of an aragonitic septum and the internal mould (steinkern) of the corresponding chamber. Only a few specimens preserved more than one chamber. While the guard-like sheath is always missing, the conotheca is sometimes present. It is assumed that paper-thin shell layers can be lost during preparation.

In general, the preservation is the same as recently described in *Naefia* shells from Cenomanian – Campanian deposits of Hokkaido (northern Japan) by Fuchs & Tanabe (in press).

Locality: Odiyam, west of Ariyalur, Tamil Nadu district, south-eastern India

Horizon: Uttatur group, Karai Formation, Kunnam member (Late Cretaceous, Cenomanian)

Description: Uncompressed specimens show that the phragmocones are circular in cross-section with a marginal siphuncle in ventral position (Fig. 3B, F). The maximum diameter of the largest specimen (MACS G1712) is 13mm (Fig. 3A). Specimens never include more than three chambers. Early ontogenetic chambers (including the protoconch) as well as a body chamber are still unknown. Although statements about a precise apical angle are ambiguous, it must have been conspicuously small (10-15°). The chamber height / chamber diameter ratio of the phragmocones is approximately 0.3. The length of the mural parts compared to the chamber height is difficult to determine owing to the poor preservation. Except a slight ventral lobe, the sutures are simple and not inclined, i.e. rectangular to the longitudinal axis (3A, C-E). Ventral septal necks are retrochoanitic as is visible on internal moulds (Fig. 3E). Only one specimen preserves parts of the lateral conotheca (Fig. 3D). Evidence about the dorsal or dorsolateral conotheca is still absent. Anterior projecting growth lines that could refer to the proostracum morphology are therefore not observable. The surface of the internal moulds is more or less smooth, except a longitudinal imprint in the mid-dorsal line probably reflecting soft part attachments on the inner surface of the conotheca. The guard-like sheath reported from other species of *Naefia* is missing in the entire material.

Comparisons: As can be judged from the overall phragmocone morphology, the studied specimens are most similar to *Groenlandibelus* and *Naefia*. Both genera are well characterized by comparatively long chambers and an extremely longiconic phragmocone (i.e. small apical angle).

A character that is simply visible even in incomplete specimens and that unambiguously

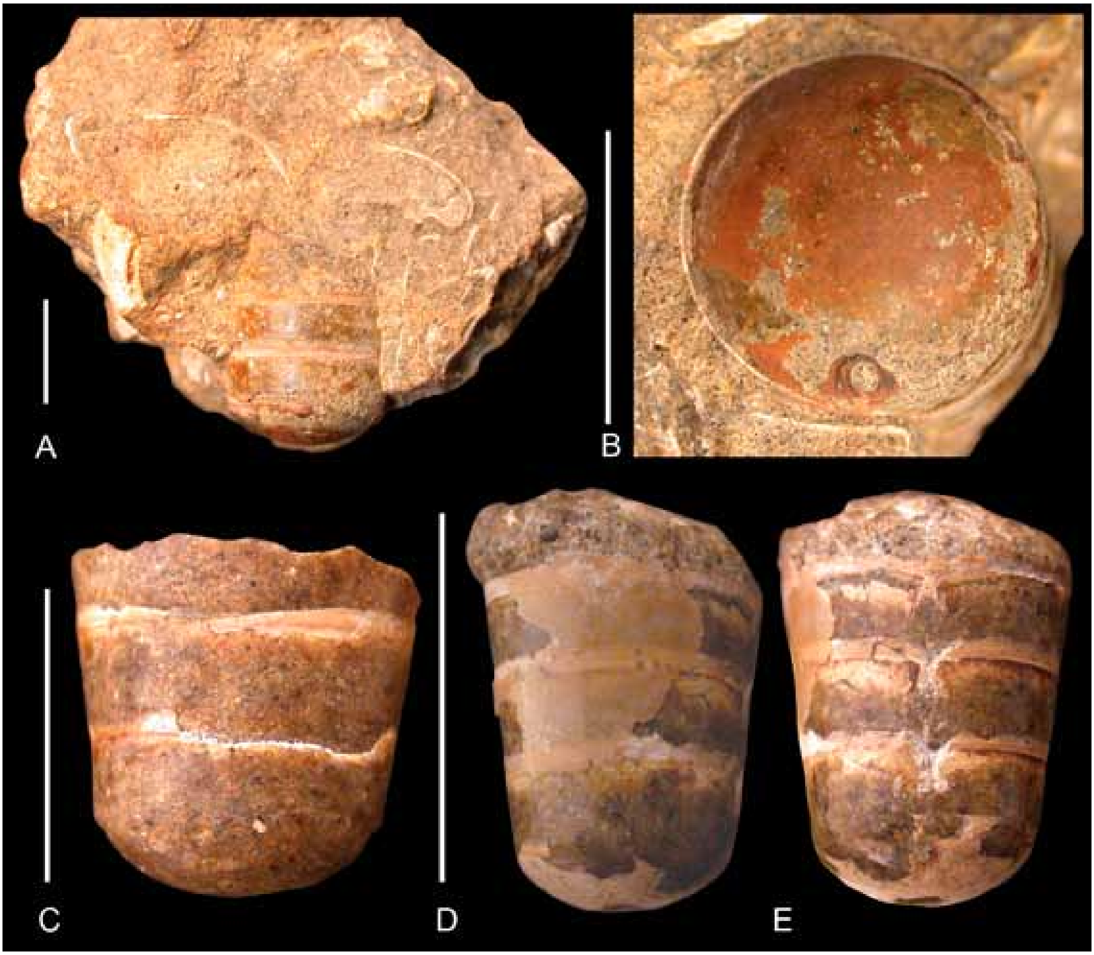

Fig. 3: *Naefia* sp., Odiyam (Arialur region, south-eastern India), Karai Formation, Cenomanian. A) Specimen MACS G1712, lateral view. B) Same specimen in apical view to show the circular cross-section. C) specimen MACS G1713, lateral view. D) Specimen MACS G1714, lateral view to show the patch of preserved conotheca. E) Same specimen ventral view to show the retrocoanitic septal necks. Scale bars = 10 mm

separates the two genera is the orientation of the suture lines. *Groenlandibelus* is well known to possess slightly oblique suture lines in lateral view (Jeletzky 1966: 90, pl. 18, fig. 3C, D, pl. 20, fig. 1A; Fig. 4A-B). *Naefia*, in contrast, exhibits straight sutures as can be observed in the type material of *N. neogaiea*, *N. matsumotoi* or *N. kabanovi* (Hirano et al. 1991: pl. 1, Doguzhaeva 1996: Text-fig. 4, Bandel & Stinnesbeck 2006: pl 1, fig. 1, Fuchs & Tanabe in press). The present specimens thus belong to the genus *Naefia*.

A precise comparison on the species level is unfortunately not possible owing to the lack of adequate characters (i.e. shape of the proostracum). The present specimens are therefore determined as *Naefia* sp.

Remarks: Doyle (1986) interpreted the type material of Forbes (1846) from the Campanian – Maastrichtian Ariyalur Group of Pondicherry (south-eastern India) as *Naefia* cf. *neogaiea*. According to him, the "septal sutures are oblique". In the light of this character, Doyle's observation would rather justify affinities with *Groenlandibelus* (see comparison above). Whereas his figures 1 and 3 actually show obliquely oriented sutures, at least figure 2 rather offers straight sutures ("The

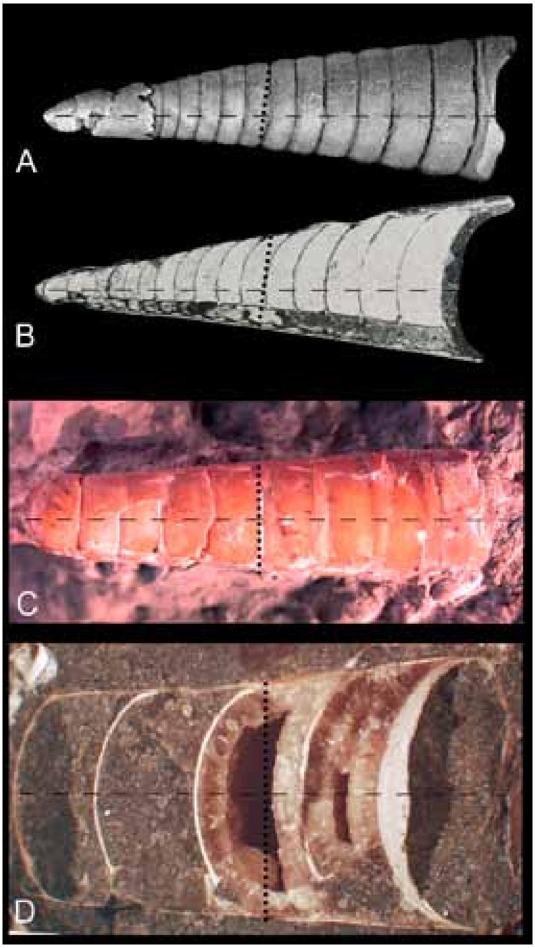

Fig. 4: Orientation of septa in the phragmocones of *Groenlandibelus rosenkrantzi (*Birkelund, 1956) (A-B) and *Naefia matsumotoi* Hirano, Obata & Ukishima, 1991 (C-D). A) Lateral view of the phragmocone to show slightly oblique suture lines (reproduced from Jeletzky 1966: pl. 18, fig. 3B). B) Longitudinal thin section to show the degree of septum inclination (reproduced from Jeletzky 1966: pl. 20, fig. 1A). C) Lateral view of the phragmocone to show straight suture lines (specimen PM7689, paratype). D) Longitudinal cross section to show septa that are oriented rectangular to the longitudinal phragmocone axis. Dashed line = longitudinal phragmocone axis; dotted line = orientation of the septum.

suture lines are less oblique in this specimen than the last,…"). For that reason, the determination of Doyle (1986) appears ambiguous and therefore needs re-investigation.

Discussion

Blanford (1865) and possibly also Foote (1879) already referred to peculiar "Belemnites sp." from Cenomanian rocks near Odiyam. The assumption whereupon their phragmocones rather belong to *Naefia* sp. has been confirmed by the help of the new material. The present article therefore introduces the first Cenomanian *Naefia* outside the western paleopacific and the northern hemisphere. Unfortunately, the preservation of the specimens is not good enough to compare the phragmocones with other species of *Naefia*. However, it is worthwhile to note, that specimen MACS G1712 exhibits the largest known chamber diameter of 13 mm. Fuchs & Tanabe (in press) recently reported a phragmocone of *Naefia matsumotoi* from the Santonian of Hokkaido (Japan) with a preserved length of 26.1 mm and a maximum diameter of 12.3 mm. Fuchs & Tanabe (in press) estimated a maximum phragmocone length of 60-65 mm for this specimen (without proostracum!). Hence, the phragmocone of MACS G1712 should have been slightly longer.

As *Groenlandibelus* and other species of *Naefia*, the present individuals have been found in sediments that indicate a neritic shallow water paleoenvironment. Hewitt, Yoshike & Westermann (1991: 52) estimated a maximum depth range of 580 meters for *Naefia matsumotoi* on the basis of the septal construction. Accordingly, they reconstructed an epipelagic life style for *Naefia*. Since *Naefia* could dive substantially deeper than the shelf dwellers belemnites and Mesozoic nautiloids, they furthermore suggested a post-mortem drifted of the phragmocones from a more oceanic environment to the shelf regions.

Acknowledgements

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Molecular phylogenetic relationships of the genus *Idiosepius* (Cephalopoda; Idiosepiidae)

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Keywords: Coleoidea, *Idiosepius*, COI; COIII; 12S; 16S; holotype samples

Abstract

The family Idiosepiidae comprises the smallest of the cephalopods and is represented by a single genus with eight species. Morphological examinations of the valid species and of several populations indicate that the taxonomic position of some species is doubtful. To provide further information on the phylogenetic relationships of *Idiosepius*, four mitochondrial loci (12S rRNA, 16S rRNA; cytochrome *c* oxidase subunit I and III) were analyzed using maximum parsimony and Bayesian analysis methods. The molecular phylogenetic analysis supports a recent morphological study, revealing that the genus can be differentiated into five species: *I. biserialis*, *I. notoides*, *I. paradoxus*, *I. picteti* and *I. pygmaeus*. Populations of *I. biserialis* from African and Indo-Pacific regions are geneticaly distinct. Less geographical variability was detected in the other *Idiosepius* species. Moreover, an apparent pattern of genetic differentiation can be verified in *I. biserialis* from Japan, which are distinct from Africa and the Pacific populations and do not conform to the morphological data, and in *I. paradoxus* from Okinawa, which agree with currently unpublished morphological data.

Introduction

The genus *Idiosepius* Steenstrup, 1881 comprises the smallest cephalopods, with a mantle length less than 6 mm in females and 3 mm in males (Jackson & Choat 1992; Martinez & Moltschaniwskyj 1999; von Boletzky 2003). A conspicuous morphological character of this family is the adhesive organ (also known as adhesive gland) which is restricted to the posterior part of the dorsal mantle side and fin region (Steenstrup 1881; Sasaki 1921; von Byern et al. 2008).

To date, *Idiosepius,* is the only genus of the family Idiosepiidae, comprises eight species (Jereb & Roper 2005): The 2-rowed species *Idiosepius biserialis*; and *I. thailandicus* and the 4-rowed species *I. macrocheir*; *I. minimus*; *I. notoides*; *I. paradoxus*; *I. picteti* and *I. pygmaeus.*

The systematic key, proposed by Nesis (1982), included only six species because the taxonomic position of *I. minimus* within the genus remained doubtful and *I. thailandicus* (Chotiyaputta, Okutani & Chaitiamvong 1991) was described later. Morphologically, the species are characterized by the sucker arrangement on the tentacular club (two or four rows) and the number of suckers on each ventral arm (both are hectocotylized).

Simultaneous description of *I. biserialis* (Hylleberg & Nateewathana 1991) next to *I. thailandicus* in Thai waters raises questions about the validity of both species. They resemble each other closely, have a biserial sucker arrangement on the tentacle clubs and differ only marginally in size.

Previous examinations evaluated the extent of morphological variations in all *Idiosepius* species (von Byern & Klepal 2006; Nürnberger, von Byern & Klepal 2006b). The morphological data indicate that the current systematic positions of *I. macrocheir* and *I. thailandicus* are uncertain; both species could be summarized to *I. biserialis* (von Byern & Klepal 2009). Based on the number of suckers on the tentacle club, *I. macrocheir* belongs to the 2-rowed rather than to the 4-rowed group. *Idiosepius thailandicus* does not differ morphologically from *I. biserialis* (Thailand, Indonesia, Japan or Moçambique), either in mantle length, number of suckers on the extremities or in the arrangement of pegs on suckers.

The aim of this study was to determine whether the molecular phylogenetic relationships of all seven *Idiosepius* species (except *I. minimus*) supported these morphological characters.

Materials & Methods

In total, 90 specimens of 7 taxa (*I. biserialis* (n=35); *I. thailandicus* (n=6); *I. pygmaeus* (n=21); *I. paradoxus* (n=20); *I. notoides* (n=5); *I. macrocheir* (n=2) and *I. picteti* (n=1) were obtained for the analysis. A detailed description of the collected specimens and their locations with latitude and longitude data can be found elsewhere (von Byern & Klepal 2009).

DNA extraction, amplification and sequencing

Living specimens were fixed in 90% ethanol. Total DNA from fresh and museum material was isolated from small pieces of tissue (mantle or extremities) with the DNeasy Tissue Kit (Qiagen, Valencia, USA). Once isolated, 1-10 ng DNA was used for polymerase chain reaction (PCR), performed with MangoTaq DNA Polymerase (Bioline, Massachusetts, USA). Four different loci were analyzed, i.e. partial sequences of the genes for 12S rRNA (12S), 16S rRNA (16S), cytochrome *c* oxidase subunit I (COI) and cytochrome *c* oxidase subunit III (COIII). The PCR protocol for 12S amplification included an initial denaturation for 2

Abbreviations used

I. bis. Ind	*I. biserialis* from Indonesia
I. bis. Inhac	*I. biserialis* from Inhaca Island, Moçambique
I. bis. Inham	*I. biserialis* from Inhambana, Moçambique
I. bis. Jap	*I. biserialis* from Japan
I. bis. Mon	*I. biserialis* from Monque, Moçambique
I. bis. Thai	*I. biserialis* from Thailand
I. bis. Type Hylle	Type material of *I. biserialis* (Hylleberg & Nateewathana 1991)
I. mac.	*I. macrocheir*
I. not. Tas	*I. notoides* from Tasmania
I. para. Nag	*I. paradoxus* from Nagoya, Japan
I. para. Okin	*I. paradoxus* Okinawa, Japan
I. para. Seto	*I. paradoxus* Seto, Japan
I. para. Ushi	*I. paradoxus* from Ushimado, Japan
I. pic.	*I. picteti*
I. pyg. Bang	*I. pygmaeus* from Klong Bangrong, Thailand
I. pyg. Mud	*I. pygmaeus* from Klong Mudong, Thailand
I. pyg. Ind	*I. pygmaeus* from Indonesia
I. thail.	*I. thailandicus*

min at 94° C, then 25s at 94° C, 30s at 46° C and 40s at 72° C for 35 cycles; for 16S, initial denaturation for 2 min at 94° C, 20s at 94° C, 20s at 50° C, 40s at 72° C for 15 cycles and 20s at 94° C, 20s at 43° C, 40s at 72° C for 20 cycles; for COI and COIII genes, initial denaturation for 2 min at 94° C, 2 min at 94° C, 10s at 42-45° C (with a temperature gradient of 1.8° C), 1.45 min at 72C for 36 cycles.

PCR products were purified with the peqGold Cycle-pure Kit (Peqlab, Erlangen, Germany) and sequenced on an Applied Biosystems automated capillary sequencer (Mod. 3130xl) using Applied Biosystems Big Dye v3.1 (Foster City, CA, USA).

Phylogenetic analysis

Sequences were aligned with ClustalX Version 1.83 (Thompson et al. 1997) – there were no gaps or ambiguities in the alignments of any sequences. All variable sites were reconfirmed by visual inspection of the sequence data and verified on both strands.

A total of 86 aligned sequences of *Idiosepius* were used for an initial Neighbor-Joining (NJ) analysis with *Sepia officinalis* (AB 193808.1; AB 193804.1; AB 240155.1) as outgroup. *Idiosepius notoides* appeared as a sister group to the other members of the genus in all analyses. We therefore arbitrarily selected this clade as the outgroup for subsequent analyses to avoid long branch attraction artefacts due to the long branches of *Sepia* (Felsenstein 2004). After eliminating identical sequences from the data set, there were 46 unique *Idiosepius* sequences.

Maximum parsimony (MP) analyses of the aligned data were conducted in PAUP (4.0b10) (Swofford 2002), applying the branch and bound search. Branch support was assessed by bootstrapping (1000 replicates) using the heuristic search option and TBR branch swapping. All characters of the alignment were treated as unordered and unweighted. Maximum likelihood (ML) searches of the individual genes were started with the NJ tree and subsequent TBR branch swapping, using the model and parameters returned by MODELTEST 3.06 (Posada & Crandall 1998). A concatenated Bayesian analysis with MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was performed with 1 million generations.

Results

In total, 386 base pairs (bp) of 12S, 505 bp of 16S, 640 bp of COI and 657 bp of COIII were used to estimate *Idiosepius* phylogenetic relationships. Similar tree topologies (Fig. 1) resulted from phylogenetic analyses using NJ, MP, ML and Bayesian inference.

Consistent with expectations, many of the recognized species appeared monophyletic. Four well-supported clades were consistently recovered. Of the species with 2 rows of suckers *I. biserialis* (clade 1) is robustly subdivided into two populations: the African group consisting of *I. biserialis* from the different locations in Moçambique, and *I. macrocheir.* The Indo-Pacific group comprises all specimens from Thailand, Indonesia and *I. thailandicus*. The specimens of *I. paradoxus* from Okinawa Island (Japan) form a sister group to the clade containing *I. thailandicus, I. macrocheir* and *I. biserialis* (Africa and Indo-Pacific).

The group with 4 rows of suckers is represented by *I. pygmaeus* (clade 2), *I. notoides* (clade 3) and *I. paradoxus* (clade 4) from Nagoya, Ushimado, Seto Island (Japan) and including the japanese group of *I. biserialis*. The position of the type specimen of *I. picteti* is unstable and varies between the gene loci and analysis methods (Fig. 2).

Phylogenetic analyses of the protein-coding genes in the African population of *I. biserialis* indicate a slight divergence for *I. macrocheir*, which cannot be observed on the rRNA level (data not shown). In the Indo-Pacific population, a moderate genetic distance of *I. biserialis* Indonesia is given. Clade 2 shows a geographical variability of the individuals from Indonesia. *Idiosepius notoides* show no signs of genetic variability because the specimens were all collected in the same location.

Discussion

Phylogenetic analyses of partial 12S, 16S, COI and COIII genes clearly revealed four different clades within the genus *Idiosepius*. Overall, the analyses of the four mitochondrial loci provide support for the results on the individual and species level for *Idiosepius*. The methodology was also valid as for the other cephalopod species (Söller et al. 2000;

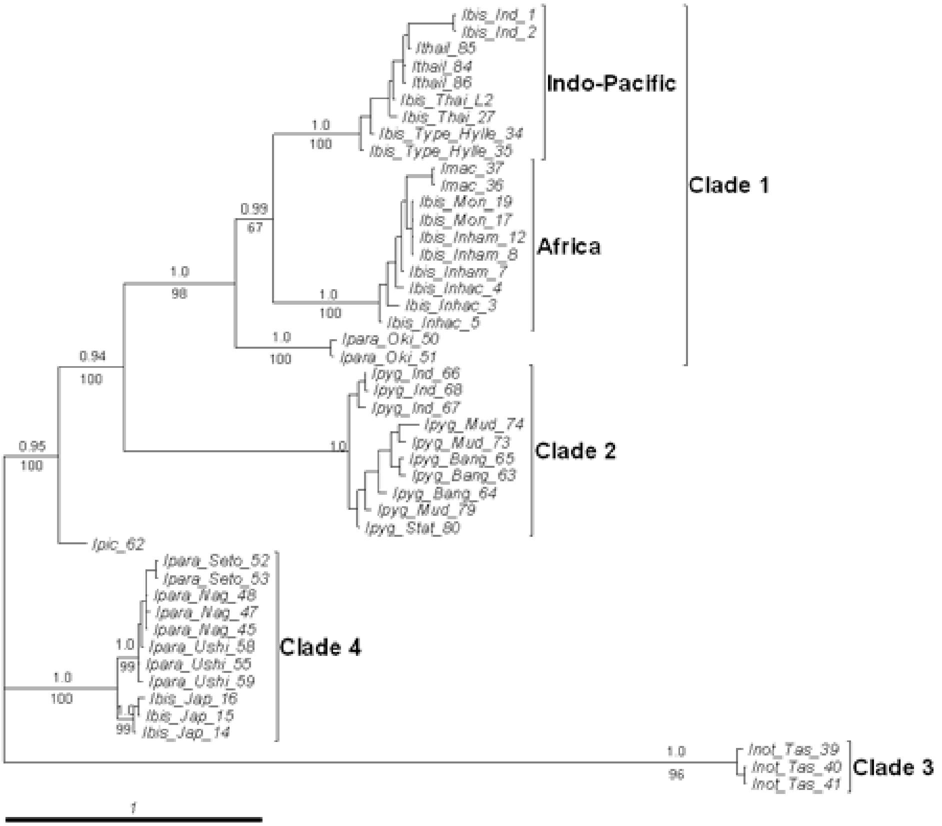

Fig. 1: Bayesian phylogenetic tree depicting relationships of *Idiosepius* individuals. Posterior probabilities and parsimony bootstrap values are above and below the branches, respectively.

Shaw 2002; Lindgren et al. 2005; Carlini, Kunkle & Vecchione 2006; Yoshida, Tsuneki & Furuya 2006).

Together with the previous morphological characterization (Nürnberger, von Byern & Klepal 2006b; von Byern & Klepal 2009), the current phylogenetic analyses provide a clear characterization of the genus *Idiosepius*. The data suggest to reduce the number of species in the genus *Idiosepius* to five: *I. biserialis* (= *I. thailandicus*, = *I. macrocheir*), *I. notoides*, *I. paradoxus*, *I. pygmaeus* and *I. picteti*.

According to the sucker number on the tentacles, the female holotype *I. macrocheir* corresponds morphologically to *I. biserialis*. Similarly, detailed examinations of *I. thailandicus* (von Byern & Klepal 2006; Nürnberger, von Byern & Klepal 2006a) enabled no clear distinction from *I. biserialis*. Both species exhibit the same number of suckers on tentacles and arms, and the suckers are similar in structure and size. Furthermore, cross-mating studies – as done by Nabhitabhata, Suwanmala & Tasanasuwan (2006) – contradict a species differentiation of *I. thailandicus* and *I. biserialis*. The present phylogenetic analyses clearly confirm that *I. macrocheir* and *I. thailandicus* were incorrectly assigned as single species. In fact, they should be summarized under *I. biserialis*.

The present phylogenetic analyses fail to provide a clear relationship of *I. picteti* to *I. pygmaeus* or any other *Idiosepius* species as proposed morphologically (Nürnberger, von Byern & Klepal 2006b). The reason for the incomplete allocation possibly reflects damage of the four gene loci caused by the long storage (more than 100 years) and/or fixation (10% formalin). It is currently impossible to verify with certainty whether *I. picteti* represents a single

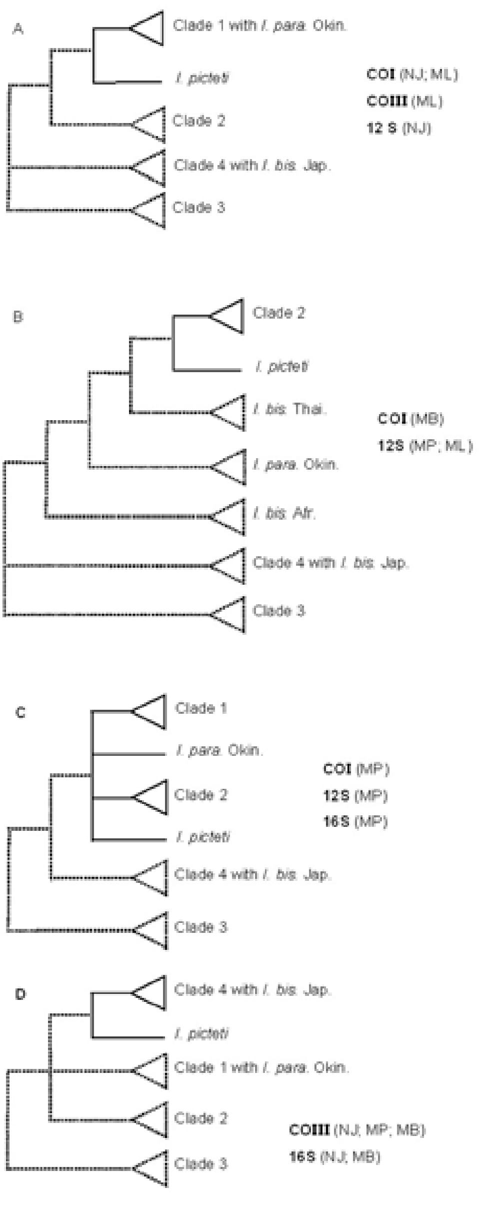

Fig. 2: Cladograms (A-D) representing the systematic position of *I. picteti* in the phylogenetic analysis using all four gene loci and NJ, MP, ML and Bayesian inference.

species or belongs to another 4-rowed species; therefore its present systematic status will remain valid.

The molecular biological analyses clearly indicate a geographically related genetic variability of *I. biserialis* into an African and Indo-Pacific population. While no differences can be determined morphologically, the present results point to limited gene flow between these geographically widely separated populations.

Specimens of *I. biserialis* from Japan, morphologically clearly classified to the group with 2 rows of suckers (von Byern, Nürnberger & Shigeno 2005), appear to be more closely related to *I. paradoxus* which has 4 rows of suckers. This phylogenetic distinctiveness to *I. biserialis* from Africa and Indo-Pacific cannot be attributed to geographic diversification since the individuals of Japan should then still be more close to the individuals of Clade 1. Unfortunately, little is known about the ecology and distribution of *I. biserialis* from Japan; the animals were recently discovered in southern Japan (von Byern, Nürnberger & Shigeno 2005). Larger studies involving more specimens from different locations are necessary to morphologically and genetically elucidate the relationship between *I. biserialis* and *I. paradoxus* and reveal the reason for this congruence.

The taxonomic status of *I. paradoxus* from Okinawa also remains questionable on both the morphological and genetic level. The provided samples were unexceptional females and provide therefore no clear species classification based on the systematic key of Nesis (1982).

Thus, the animals remain characterized as *I. paradoxus* based solely on the geographical assignment of Okinawa Island to Japan. The current phylogenetic analyses, however, point to a sister-taxon relationship to the tropical species *I. biserialis* than to the cool water species *I. paradoxus*. It would, however, currently be inappropriate to speculate whether the individuals from Okinawa are related to *I. paradoxus* or any other species of *Idiosepius*. Firstly, more detailed morphological examinations, in particular of males, are crucial to verify their taxonomic status.

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Stable isotopes during ontogeny of *Spirula* and derived hatching temperatures

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Keywords: Cephalopoda, *Spirula*, oxygen isotopes, carbon isotopes, embryonic development

Abstract

The mesopelagic recent cephalopod *Spirula* Lamarck, 1799 seems to be a suitable model organism for approaching the embryonic development of the extinct ammonoids. As no spawned egg masses of *Spirula* were found, artificial fertilization provides an opportunity to obtain information about the embryonic development. The critical point is the correct rearing temperature for the fertilized eggs which is unknown like all other spawning conditions of *Spirula*. As the animal has an internal calcareous shell, hatching temperature was estimated with the help of isotope analysis at approximately 8°C. This study was a first-time possibility to use *Spirula* shells from a relatively fresh caught animal for this kind of analysis. However, temperature curves received from stranded shells proved comparable to the curves of the dissected shells.

In addition to this, numerous tests were run to provide insights on internal isotope variation ranges within a single septum, within a single chamber and between septum and chamber. Standard deviations from a mean value in $\delta^{18}O$ of one single septum were found to account for temperature variations of ~0.5°C. The error interval of the analysed chamber wall is up to four times higher. Therefore, it is advisable to prefer material from septa, and either analyse more than one shell or run more than one carbonate sample from each septum.

Introduction

With its unique internal chambered shell, the mesopelagic recent decabrachian *Spirula* is one of the most unusual of all recent cephalopods.

There are many morphological hints indicating that *Spirula* could be a useful model organism for approaching the embryonic development of the extinct ammonoids (for discussion see Keupp 2000; Warnke & Keupp 2005). Jacobs & Landman (1993) stated that a coleoid model should be preferred in interpretations of the ammonoid mode of life. Usually *Nautilus* is used as a model. The sister taxon relationship of coleoids and ammonoids appears to be well supported by various synapomorphies. These are for example the assumed number of arms (Berthold & Engeser 1987), the number of radular teeths (Lehmann 1967), or the position of the retractor muscle. Reproduction behaviour of ammonoids may have been similar to that of coleoids. Ammonoids, in contrast to

Nautilus, may have been semelparous (producing large quantities of small eggs in a single breeding season) like the majority of coleoids. *Nautilus* produces only a few large eggs over a period of several years (Jacobs & Landman 1993). However, the external structure of the early *Spirula* shell, starting from a spherical initial chamber, resembles very much that of Ammonoidea and contrasts with *Nautilus* or *Sepia* (Bandel & Boletzky 1979; Bandel 1982; Tanabe, Fukuda & Obata 1980; Doguzhaeva 1996). Furthermore, the general mode of mineralization of the embryonic shell of *Spirula* and ammonoids is the same. It does not show growth lines because it was initially entirely organic and then rapidly mineralized (Engeser 1996). Possible laid egg masses of ammonoids (e. g. Lehmann 1966) have not been confirmed but the diameter of the ammonitella which is the initial shell of hatchling ammonites measures between 0.5 and 2 mm (Landman 1988; House 1996; Landman, Tanabe & Shigeta 1996). The egg size is comparable to the size of *Spirula* which measures about 2 mm. Mature ovarian eggs measure between 1.7 mm (Chun 1910; Naef 1923) and 1.9 mm (Warnke & Keupp 2005). However, due to the small egg size Bandel (1982) assumed the same mode of hatching for ammonoids and *Spirula* with small shells and fully functional buoyancy control.

Although *Spirula* is widely distributed near the continental slope in the neighbourhood of oceanic islands in tropical and subtropical waters at temperatures above 10°C (in 400 m depth) (Bruun 1943; Clarke 1969a; Lu et al. 1992) material of *Spirula* is rare, though the shells of the animals are washed ashore in large numbers. *Spirula* is difficult to access since it has no commercial value and is hard to catch. The adult animals were assumed to migrate daily within the water column between depths of 100 m at night and 1750 m during the day (Bruun 1943). The youngest specimens found with less than 0.5 cm in length are concentrated between 1000 m and 1750 m (Bruun 1943). The lower limit was determined by finding the pressure resistance of dead *Spirula* shells. Bruun (1943) analysed the capture of the Danish deep sea "Dana Expedition" in the 20ies. Because no closing nets were used, Bruun (1955) himself formulated doubts concerning the lower depth limit. However, Clarke (1969b) conducted a study on the base of closing nets catches and found the centre of *Spirula* distribution in 200-300 m in darkness and 600-700 m in daylight. The embryonic development is still unknown as no laid eggs have been found. Therefore, it is not known what spawned egg masses look like or where in the water column spawning takes place (Nesis pers. comm.). It is assumed that the eggs are laid near the bottom (Bruun 1943; Nesis 1987).

To use *Spirula* as a suitable recent model organism for understanding the embryonic development of ammonoids, artificial fertilization will help to provide information (Boletzky 1989, 1998).

Artificial fertilization is possible using freshly caught animals and running fresh seawater, e. g. as described by Arnold and O´Dor (1990). The critical point is the correct rearing temperature for the fertilized eggs. As mentioned above, the problem is that the depth where spawning takes place within the water column is only presumed (Nesis, pers. comm.). Therefore, it is the aim of this study to estimate the hatching temperature with the help of isotope analysis.

In this study the septa of *Spirula* caught alive near the Canary Islands (Fig. 1) and stranded shells were analysed for isotope ratios and compared with each other. The oxygen isotope ratio ($\delta^{18}O$) of carbonates in combination with a known $\delta^{18}O$ of sea water provides information on temperature and depth of *Spirula* egg masses during septal carbonate precipitation.

As the well-preserved material of *Spirula* is pretty scarce, compared to the countless shells stranded on oceanic beaches (Dauphin 1979a, 1979b) the isotope data of stranded shells and shells dissected out of the animal from the same geographical region were compared to find out if there is any post-mortem effect in isotope ratios. In addition to this isotopic variations within outer shell and septa were analysed and discussed.

Materials & Methods

We examined two shells from live-caught *Spirula* (A, B) and three stranded *Spirula* shells (C, D, E), each with a total number of 30-33 septa. Alive *Spirula* were caught with a pelagic net near the Canary Islands (Fig. 1). *Spirula* A (2005/08/06) was caught near longitude 27.53°N and latitude 13.73°W at night within the uppermost 250 m of the water column. The whole water depth at

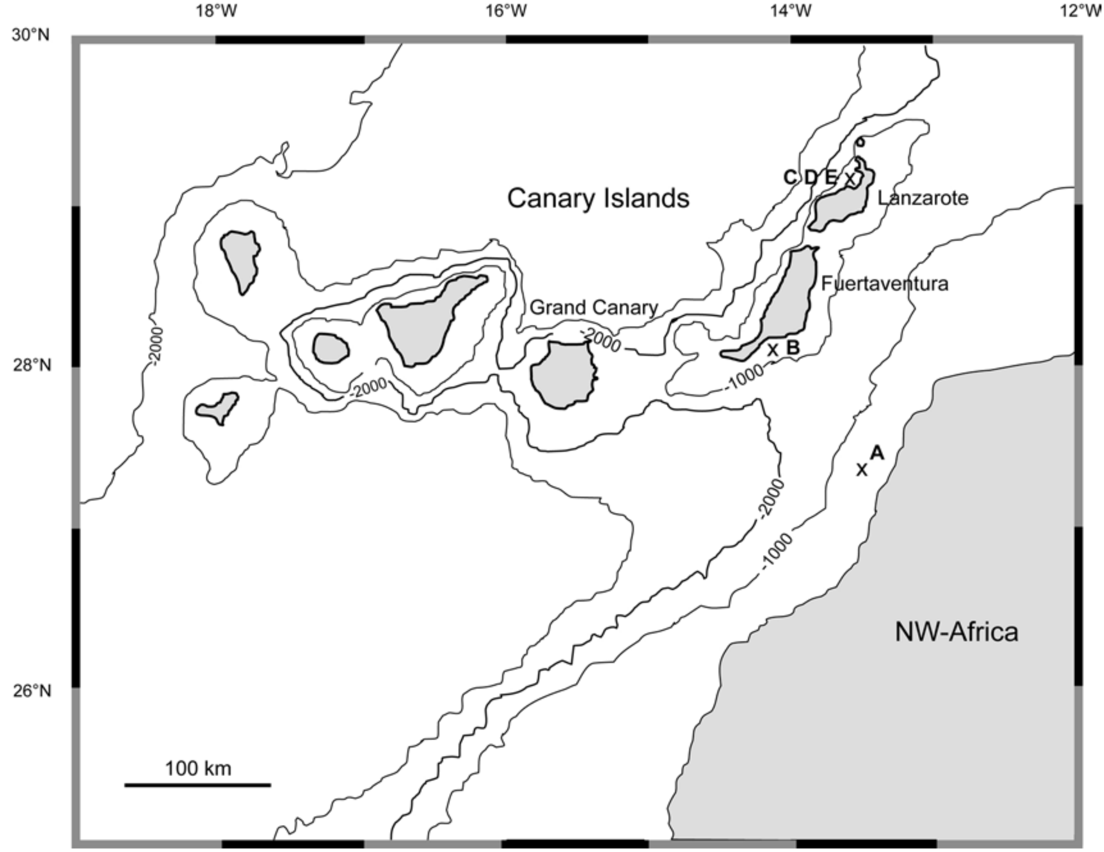


Fig. 1: Map of the Canary Islands, live-caught specimens were captured at A and B, and C D E indicates locality where stranded shells were collected (map slightly modifed, copyright by V. Diekamp, Marum, Universität Bremen).

that position was about 1100 m. An Isaacs-Kidd midwater trawl (mash size of 6 to 10 mm, mouth opening of 6 m^2 and a length of 10 m) was used. *Spirula* B (2002/03/16) was caught as bycatch of a commercial trawl near longitude 28.00°N and latitude 14.27°W. Additional material was collected from Playa da Famara (Lanzarote) near longitude 29.12°N and latitude 13.55°W (leg. G. Schreiber, Münster, Germany).

Prior to the here presented analysis various tests were run on a number of stranded shells from this region. Shells were selected according to full ontogenesis and cleaned in destilled water in an ultrasonic bath. Organic rests on both live-caught specimens were removed in a cold 3.5% H_2O_2 bath over night. The shells were attached to a glass slide using a two-component epoxy metal (iron fibre) resin for fixation during extraction of septa. The resin is of such high viscosity that intrusion of the substance into the chambers of the shell is avoided. No material that had been contaminated with resin was selected for analysis. A blast stylus W 224 (Krantz) was used to remove the outer shell and extract material from individual septa (Fig. 2). From specimens A, B and C a full set of septa was sampled, from D and E only septum 1-11. The carbonate was collected in TabTop Microcentrifuge tubes 1.5 ml (Roth, Karlsruhe-Germany) and sent to the Leibniz laboratories at Christian-Albrechts-Universität in Kiel for analysis. Measurement of oxygen and carbon isotope ratios was conducted in a Finnigan Mat-252 mass spectrometer after reacting the material with 100% phosphoric acid at 75°C in an online carbonate preparation line

(Carbo-Kiel-single sample acid bath). All ratios are reported per thousand relative to V-PDB (Peedee belemnite) measured against standard NBS-19 with an assigned $\delta^{18}O$ value of -2.20‰ and $\delta^{13}C$ value of +1.95‰. The analytical precision is better than ±0.04‰ (1σ) for $\delta^{18}O$ and ±0.03‰ for $\delta^{13}C$ values.

For additional information on internal isotope variation ranges within a single septum, carbonate along one of the latest precipitated septa was randomly extracted 11 times in the same way as for all other septa. All samples are chosen with attention to a full set of septal layers as temporal inconsistencies during precipitation of single layers were reported from *Nautilus* septa (Oba et al. 1992). A total of 11 randomly chosen samples from the outer shell wall of the preceding chamber was analysed to compare internal variations (Fig. 2). These isotope ratio measurements were run at the Freie Universität Berlin on a Thermo Electron Mat-253 isotope ratio mass spectrometer (IRMS) and Gasbench II carbonate device (e.g. Spöttl & Vennemann 2003). For isotope analysis about 200 µg carbonate was loaded into Exetainers and reacted with 100% H_3PO_4 at 70°C. All isotope data are reported as $\delta^{18}O$ and $\delta^{13}C$ relative to V-PDB. International reference materials NBS-18 ($\delta^{18}O$=-23.0‰; $\delta^{13}C$=-5.01‰) und NBS-19 ($\delta^{18}O$=-2.20‰; $\delta^{13}C$=+1.95‰) have been used for external calibration of the analysed samples to the PDB scale. The precision of the method has been calculated from a total of 20 measurements of Laaser Marmor (LM) inhouse reference material which averages at 1.51±0.04‰ for carbon und -5.17±0.06‰ (1σ standard deviation) for oxygen isotopes. The precision for $\delta^{18}O$ is better than ±0.03‰ and better than ±0.02‰ for $\delta^{13}C$.

So, considering those factors, it is possible to reconstruct temperatures from oxygen isotope ratios and convert those temperatures into information about the depth in which the animal lived at that time. The individual isotopic profile for this stretch of the Atlantic will be considered. For temperature reconstruction we apply

$$T(°C) = 19.7 - 4.34*(\delta_{carbonate} - \delta_{water}) \qquad (1)$$

a variation of the temperature equation proposed by Grossman & Ku (1986). $\delta_{carbonate}$ is the $\delta^{18}O$ value of the carbonates reported relative to PDB and δ_{water} is the $\delta^{18}O$ value of the surrounding water relative to SMOW (standard mean ocean water). The δ_{water} value was adopted from two profiles from nearby locations at 20.53°N and 18.13°W, and 20.53°N and 18.57°W (Fig. 1) that record temperature and isotopic variations within the water column close to the shelf of the African west coast (Bigg & Rohling 2000; Pierre, Vangriesheim & Laube-Lenfant 1994). The different $\delta^{18}O$ profiles for water in this region reach a steady value of ~+0.3‰ below the fluctuating upper layer. This value for δ_{water} was inserted as a basis for calculations.

Results

The $\delta^{18}O$ curves all start at a value of approximately +3‰, hold that value along 6 septa, then continuously decrease to about +2‰ until septum 17-19 before they slowly rise again to ~+3‰ (Fig. 3). The curves show highest deviations from an average course during the drop in values. The minimum $\delta^{18}O$ values show only small variations and individual curves don't diverge much from each other. Towards the end of the precipitation period and with rising $\delta^{18}O$ variations within individual curves slightly increase again. $\delta^{18}O$ values converted to temperatures using equation (1) result in a curve stretching from 7°C to ~12°C (Fig. 4). The course of carbon isotope ratios (Fig. 5) starts below +2‰, rises by ~0.5‰ in the 2nd septum, and continuously drops thereafter to values below -1.0‰ between septum 19 and 20 with individual curves varying stronger than for oxygen isotope ratios. The drop coincides with decreasing $\delta^{18}O$ values and the declining trend of both curves reverses more or less simultaneously. $\delta^{13}C$ values rapidly increase to ratios around 0‰, and rise only slightly for the rest of the shell growth. This last relatively stable part can be interrupted by single sharp but short excursions to lower values that always affect 1 or 2 septa. The carbon isotope ratios from individual shells show strongest deviations from a common course between septum 10 and 16 as well as from 19 to 22.

The expected variations within one septum account for ±0.05‰ (1σ) in $\delta^{18}O$ and ±0.05‰ in $\delta^{13}C$. The variations within material from the outer shell show a range of ±0.09‰ (1σ) in $\delta^{18}O$ and ±0.19‰ in $\delta^{13}C$.

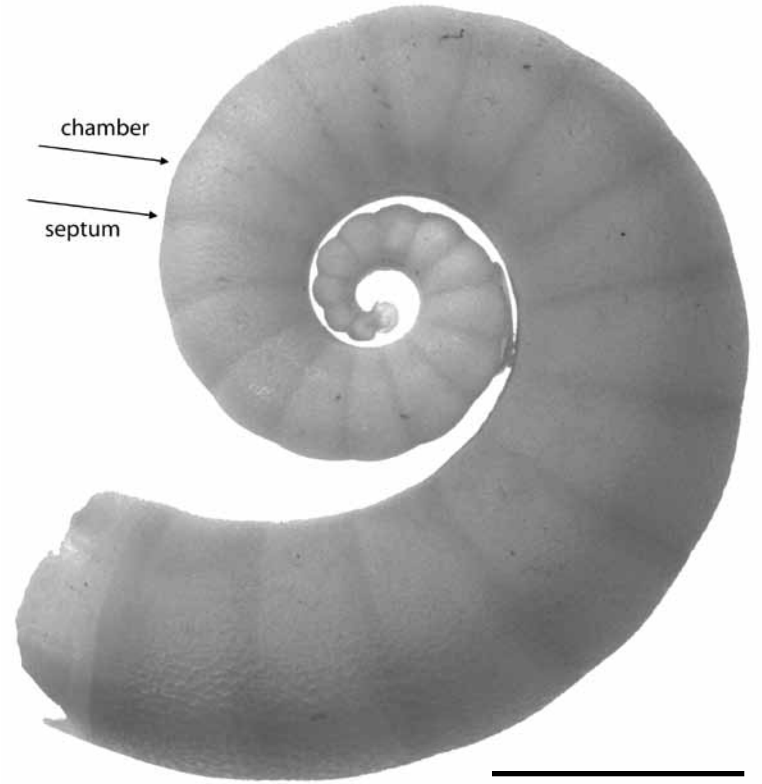


Fig. 2: Stranded shell of *Spirula spirula* from the Canary Islands (coll. Kerstin Warnke, Freie Universität Berlin, branch palaeontology). The arrows indicate the locations of chamber and septum. The protoconch is slightly damaged; scale bar = 5 mm.

Discussion

Oxygen Isotope Ratio Variations: The aim of this isotope study was to gain additional information on the live cycle of *Spirula* with special attention to temperature during embryonic development and hatching. Oxygen isotope ratios of carbonates precipitated in seawater mainly reflect changes in temperature. The isotope ratio of the water might also be influenced by fractionation effects due to evaporation which mostly affect the upper part of the water column, and is neglectable for deeper water. There is no vital effect known for oxygen isotopes in cephalopods (e.g. Wefer & Berger 1991), therefore, all changes in oxygen isotope ratios (Fig. 3) in the aragonitic shell can be directly connected to variations in temperature. The use of H_2O_2 to remove organic material is known to cause fractionation effects of varying extent (e.g. Keating 1996; Cochran, Rye & Landman 1981) and is, therefore, handled with care. Cochran, Rye & Landman (1981) found $\delta^{18}O$ values in *Nautilus* to decrease by 1‰ and $\delta^{13}C$ values to increase by 0.46‰ after treatment with cold bleach. The resulting isotope ratios lie very close to all other curves which lead to the conclusion that such a fractionation effect might exist but is kept very low when carbonates are treated at low temperatures and bleach is sufficiently diluted. A small rest of fractionation effect has an effect on individual temperature reconstruction but as all samples experience the same fixed offset, overall variation ranges are not influenced. Resulting errors in temperature should be well below 1°C which does not change habitat depths significantly.

Standard deviations from a mean value in $\delta^{18}O$ of one single septum can account for temperature variations of ~0.5°C. Considering vertical diurnal migrations of several 100 m in depth some variations in isotopic composition of the

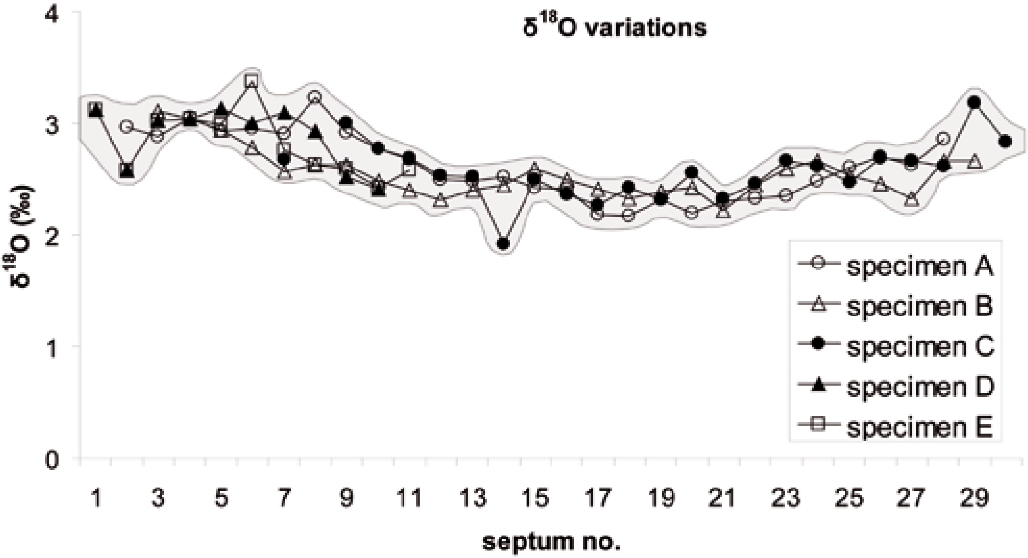


Fig. 3: Variation of oxygen isotope ratios ($\delta^{18}O$ in ‰ vs. PDB) during ontogeny of live-caught specimens and stranded shells.

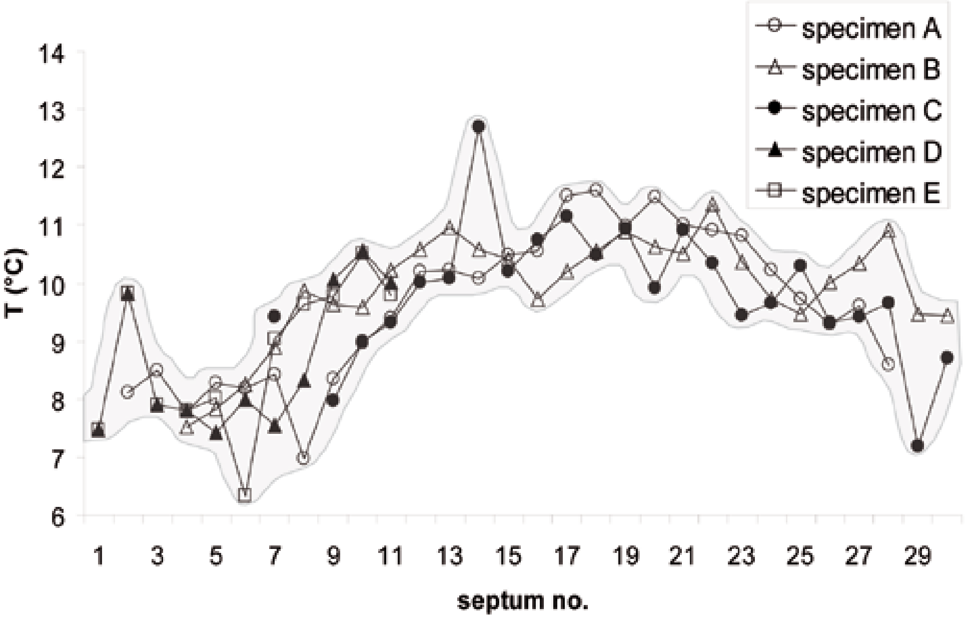


Fig. 4: Reconstructed temperature for live-caught specimens and stranded shells.

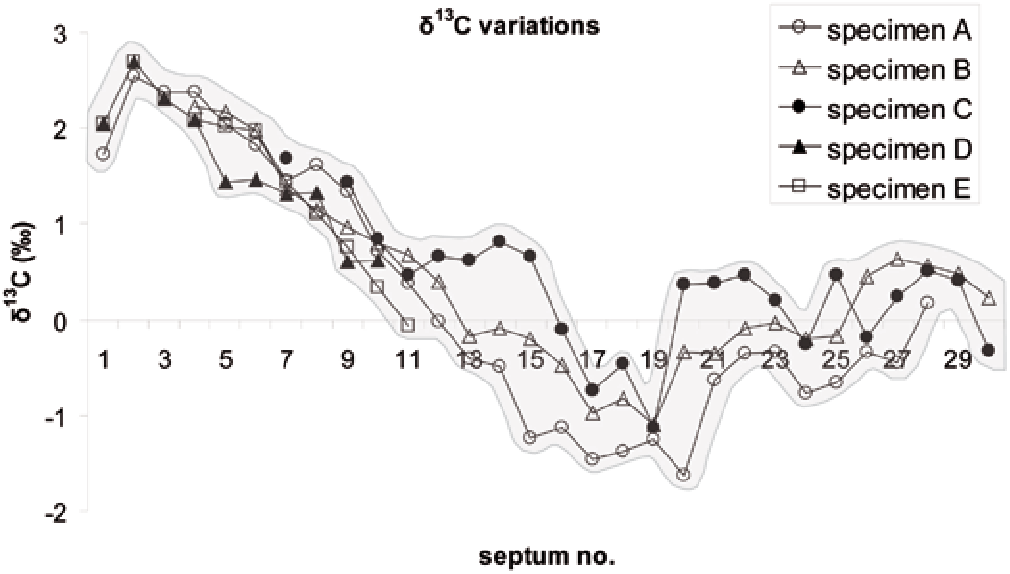
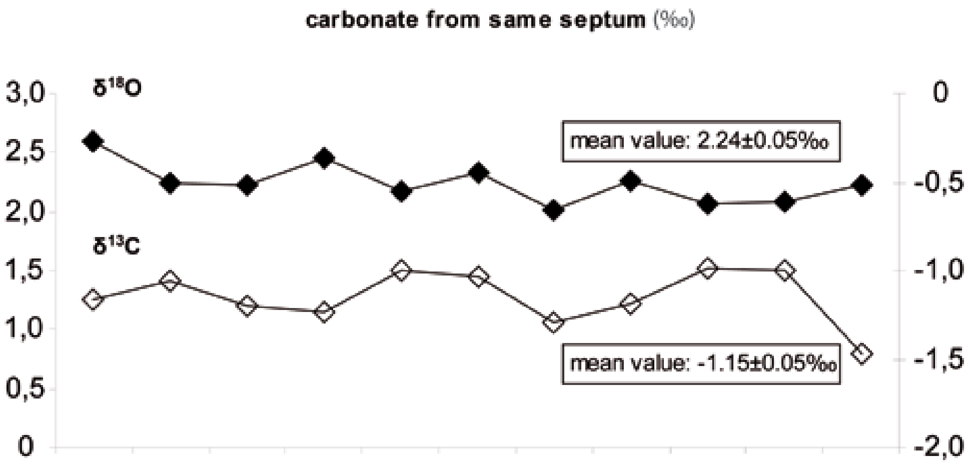


Fig. 5: Variation of carbon isotope ratios ($\delta^{13}C$ in ‰ vs. PDB) during ontogeny for live-caught specimens and stranded shells.

septum are to be expected. Especially the course of $\delta^{18}O$ that only ranges over ~1‰ in total can be changed by such a fact. The total of isotope curves produced by shells from this geographic region for this report, therefore, gives a good average course. The carbonate from the shell wall can also be used for isotope analysis but contain higher variation ranges as they are precipitated over an even longer time period. The error interval is up to four times higher (Fig. 6). This is caused by the fact that *Spirula* has only two shell layers (Mutvei 1964, Dauphin 1976) and the "outer prismatic layer" was subsequently secreted with a temporal delay at the penultimate chamber (Appellöf 1893). It is, therefore, advisable to prefer material from septa, and either analyse more than one shell or to run more than one carbonate sample from each septum to receive a good average (see also Price et al. 2009).

Fractionation effects in stranded shells due to contact with meteoric water are also excluded here as the curves from these shells and those from live-caught *Spirula* are, too, similar in appearance, and, therefore, the exposure was apparently not long enough (Fig. 3).

The resulting living depth: The $\delta^{18}O_{water}$ of depth profiles from this region are lower than expected compared to a global map by Bigg & Rohling (2000) showing the middle Atlantic region with highest $\delta^{18}O_{water}$ values at ~1‰. Isotopic water profiles were selected due to the close proximity to the shelf slope and the relatively low $\delta^{18}O_{water}$ and temperature gradients compared to open sea profiles. The difference is caused by upwelling deep water. Calculated temperatures were compared to temperature-depth curves (Bigg & Rohling 2000; Pierre, Vangriesheim & Laube-Lenfant 1994). Correlation of reconstructed temperatures with temperature measurements from the fishing area led to a hatching depth of about 800 m in which youngest *Spirula* remains for another approximately five septa (one septum equals about two weeks) before it continuously migrates upwards to an average depth of 350-400 m during the following half year. The decrease of $\delta^{18}O$ in later septa reflects the migration into warmer, shallower water (Fig. 3+4). Afterwards a slow downward trend sets in until an average depth of 550-600 m is reached at the end of observed shell precipitation. With decreasing depth $\delta^{18}O_{water}$ increases to ~+0.5‰ which leads to an increase in maximum temperatures of 1°C.



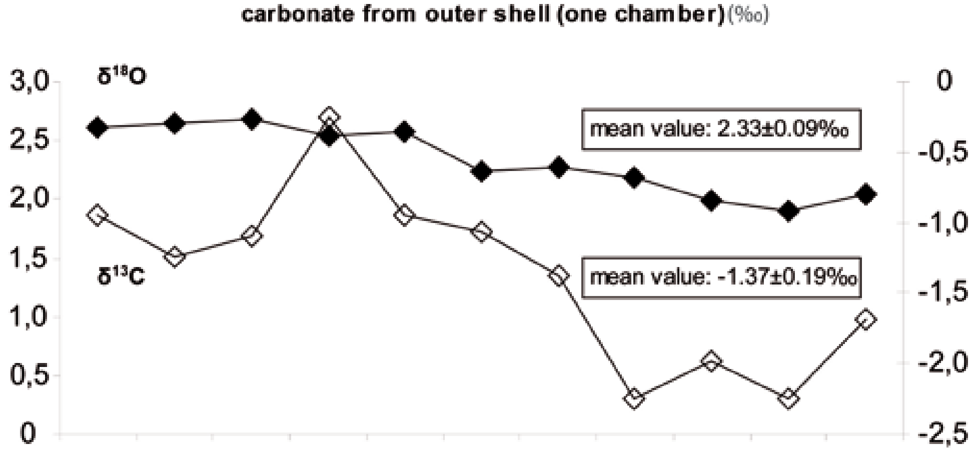


Fig. 6: Variation of oxygen and carbon isotope ratios within a single septum (top) compared to variation of oxygen and carbon isotope ratios within the outer shell of one chamber (bottom). Apparent trends are coincidence as samples are chosen randomly.

Applying this correction even underlines the observed migration pattern.

Carbon Isotope Ratio Variations: The discussion of $\delta^{13}C$ signals in biogenic carbonates is accompanied by many unknown factors (e.g. McConnaughey et al. 1997). Temperature-dependent $\delta^{13}C$ variations in carbonates precipitated by molluscs are still discussed (e.g. Fischer et al. 1999; Wefer & Berger 1991). The levels of such effects on *Spirula* are poorly known but would not exceed ~0.1‰ per °C (see Wefer & Berger 1991; Fischer et al. 1999). The calculated temperature differences account for ~5°C or ~6°C after corrections of $\delta^{18}O_{water}$ according to measurements. Therefore, such an effect might be responsible for a change of ~0.6‰ in $\delta^{13}C$ which does not cover the full range of $\delta^{13}C$ variations seen in our *Spirula* specimens.

Latest investigations on *Spirula* discuss the influence of metabolic effects on recorded carbon isotope ratios (Price et al. 2009). Vital effects on carbon isotopes are known for cephalopods and reach values of up to -7‰ (Wefer & Berger

1991). The influence of dietary carbon on $\delta^{13}C$ in carbonates is not easy to estimate as it is difficult to detect and varies with the amount and $\delta^{13}C$ value of the food (Tanaka, Monaghan & Rye 1986). There is no significant signal that could be interpreted as a change in diet as it might be produced by a change from egg yolk to sea animals after hatching (Fig. 5). Therefore, it is not yet possible to detect an exact time of hatching from isotope ratio variations.

The shells all originate from a region of the Atlantic that lies within the area of upwelling of cold and nutrient rich deep water along the African west coast. Surface waters are moved northward by the Canary current that also drives upwelling along the close-by stretch of African coast. The oxidation of organic materials at the bottom results in low $\delta^{13}C$ values of upwelling waters. A ^{12}C enrichment of upper parts of the water column can be explained by this phenomenon and could account for relatively low $\delta^{13}C$ levels during *Spirula*'s stay in warmer waters. A low $\delta^{13}C$ would consequently also be expected for deep waters. The fact that $\delta^{13}C$ of the carbonates is clearly higher for the period spent in deep waters leaves at least two possible explanations: 1.) the $\delta^{13}C$ signal of the water is of no significant influence, 2.) the animals live in intermediate depths within the water column far enough above the much deeper ground where the deep water signal does not reach them.

Resulting temperature and depth: The calculated water temperature indicates that young *Spirula* lives in average depths of 800 m not taking diurnal vertical migrations into account. Bruun (1943) found youngest *Spirula* with 2 chambers in about 1000 m depth. Clarke (1969b) caught youngest *Spirula* (smaller than 0.67 cm mantle length) between 600 and 1000 m irrespective of day or night time. Therefore, he assumed that hatchlings do not migrate immediately but adopt the diurnal migratory behaviour during early growth.

The trend to downward migration of the adults seen in the presented study and other recent reports (Price et al. 2009) suggests that for spawning *Spirula* probably returned to waters of a similar temperature to those in which they hatched. A very short excursion (only for a few days at maximum) only to lay eggs in deeper waters is possible and would consequently leave no obvious trace in the isotope signal (Fig. 4). A return to those deep waters and spawning shortly before death while no more shell was precipitated is a thesis.

Our data does not allow us to decide whether the eggs were attached to the ground or laid as pelagic egg masses in the water column.

Concerning the reconstructed water temperature from oxygen isotopes of the septa there is no signal indicating the hatching event (Fig. 4).

In summary, it was possible to obtain the temperature during embryonic development and, therefore, it is possible to execute artificial fertilization. However, to receive additional information on spawning itself it is necessary to conduct further deep sea expeditions.

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An overview of coleoid Cephalopods from Paleogene and Neogene aged rocks of Southern North America

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Keywords: Paleogene, Neogene, *Belemnosella*, *Belosaepia*, *Beloptera*?, *Anomalosaepia*, *Oligorostra*, *Oligosella*, *Amerirostra*

Abstract

To date studies of coleoid cephalopods from Paleogene and Neogene age rocks of southern North America have yielded guard-like sheaths of one genus of belemnosellids, from the Eocene of Mississippi, Alabama and Louisiana. Two different genera of belosaepiids are present, *Belosaepia* from Alabama, Louisiana, Texas, and *Anomalosaepia* from Louisiana and North Carolina and two different genera of spirulids, *Amerirostra* from the Miocene of Mexico and *Oligorostra* from the Oligocene of Alabama. One other guard-like sheath of uncertain family affinity (*Oligosella*) from the Oligocene of Alabama has also been described. Workers have also recovered phragmocone steinkerns of *Beloptera*? sp. and *Anomalosaepia* sp. from the Eocene of North Carolina. This is a survey of what is currently known about Paleogene and Neogene coleoid cephalopods from the southeastern Atlantic and Gulf regions of North America through 2008. It is meant to encourage future research on Oligocene and younger coleoids from North America to compare with those from Europe and to assist in determining phylogenetic linkages with the modern coleoids.

Introduction

Documentation of Paleogene and Neogene coleoids from the southeastern Atlantic and Gulf regions of North America began in 1860 when Gabb described *Sepia* (*Belosepia*) *ungula* from the Eocene of Texas and has since been primarily focused on systematic descriptions of guard-like sheaths, phragmocone steinkerns and their phylogenetic linkages.

Since 1860 research on Paleogene and Neogene coleoid cephalopods from southern Atlantic and Gulf regions of North America has occurred as a series of fits and starts. Meyer & Aldrich (1886) described *Belemnosis americana* from the Eocene of Mississippi and Berry (1922) named *Spirulirostra americana* from Miocene sediments of the Isthmus of Tehuantepec, Mexico (Fig.1). Palmer (1937) recognized and described several belosaepiids: *Belosaepia ungula* Gabb, 1860 from the Cook Mountain Formation, Wheelock, Texas (Figs 1; 2); *B. uncinata* and *B. veatchi* from the Lower Claiborne Group, Columbus, Louisiana (Figs 1; 2); *B. alabamensis, B. alabamensis voltzi,* and *B. harrisi*, all from the Lower Claiborne Group at Claiborne Bluff, Alabama; and *B. saccaria* from the Lower Claiborne Group, Lisbon Landing, Alabama (Figs 1; 2). Palmer (1937) also erected the new genus *Advena*, with the newly described species *Advena floweri* from the

Gosport Sand, Claiborne, Alabama as type species for the genus and included *Belemnosis americana* (Meyer & Aldrich, 1886) from Clabornian, Cook Mountain Formation, Watubee, Clarke County, Mississippi in the genus. Palmer (1940) re-named the genus *Advena* to *Anevda* because *Advena* was preoccupied by a gastropod genus; however Stenzel (1941) recognized the subjective synonomy of *Anevda* with *Belemnosella* Naef, 1922.

Published research on Paleogene and Neogene coleoid cephalopods from southern North America went through a twenty-year hiatus, until Jeletzky (1966) focused on Tertiary coleoids for the Treatise volume he was preparing. In this publication Jeletzky considered *Belemnosella americana* (Meyer & Aldrich, 1886) and *Belemnosella floweri* (Palmer, 1937) to be a morphological variants within the same species *Belemnosella americana*. Allen (1968) named a new species of *Belemnosella*, *Belemnosella palmerae*, from the Moody's Branch Formation, down river from Montgomery Landing, Louisiana and recognized *Belemnosella floweri* (Palmer, 1937) from the same locality (Figs 1; 2). Allen (1968) also named three new species of *Belosaepia*: *B. vokesi* from the Gosport Sand, Monroe County, Alabama; *B. stenzeli* from the Cook Mountain Formation, Winn Parish, Louisiana, and *B. jeletzkyi* also from the Cook Mountain Formation, Winn Parish, Louisiana (Figs 1; 2). With more specimens available for study Jeletzky (1969) retained *Belemnosella floweri* as a valid species. Jeletzky (1969) also moved *Spirulirostra americana* Berry, 1922 from the Miocene of Mexico to *Amerirostra americana* because of differences in phragmocone, the guard-like sheath, and other differences from the European and Australian *Spirulirostra*.

Carter et al. (1988) made a brief mention of a phragmocone steinkern from the Castle Hayne Limestone, New Hanover County, North Carolina (Figs 1; 2). At the time Carter et al. (1988) called their specimen ?*Belemnosella* but did not formally describe it. Garvie (1996) described a new species, *Belosaepia penna*, from the Reklaw Formation, Joe Taylor Branch Creek, Bastrop County, Texas (Figs 1; 2).

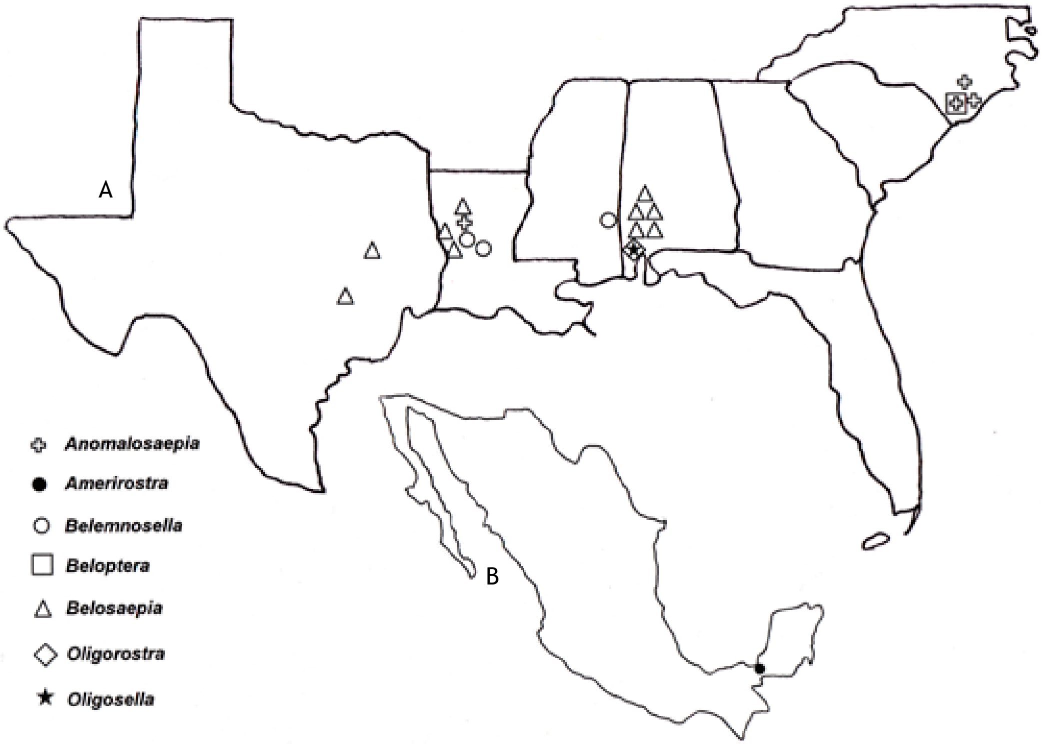


Fig. 1: A) Generalized map of southern North America showing where Paleogene and Neogene coleoids have been found. B) Generalized map of Mexico showing where Miocene coleoids have been found.

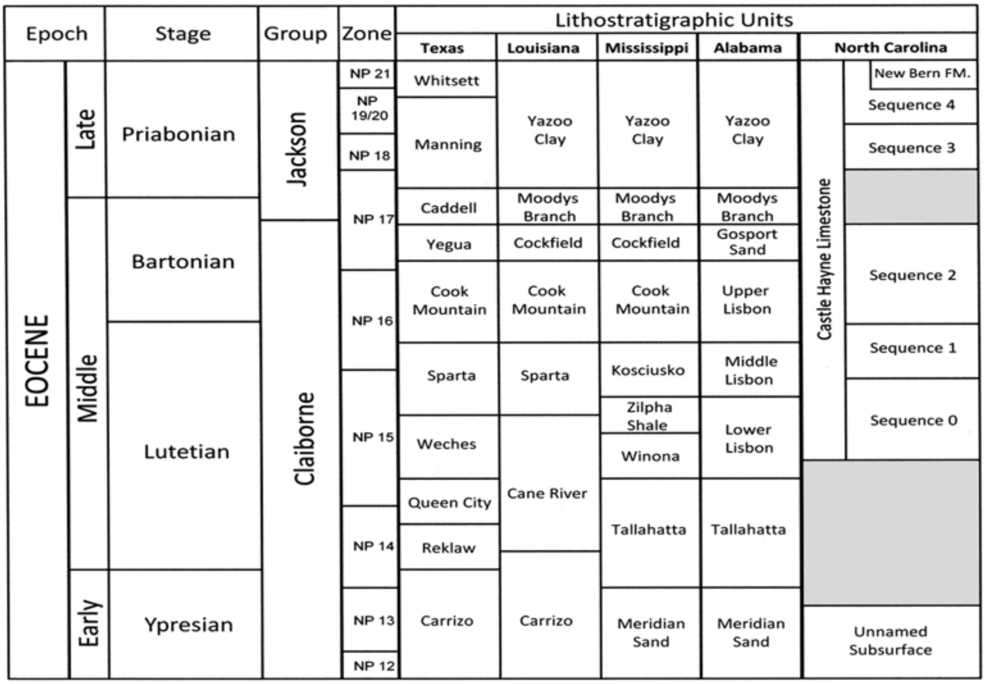


Fig. 2: Correlation chart of Eocene stratigraphic units of the southern United States. Chart compiled from information in Gaskell (1991); Harris & Zullo (1991); Harris, Zullo & Laws (1993); Rosen, Bowen & Thies (1994); Dockery (1996); Falls & Prowell (2001) and Zachos & Molineux (2003).

More recently, Weaver & Ciampaglio (2003), named a new genus of belosaepiid, *Anomalosaepia*, and four new species *A. alleni*, *A. mariettani*, *A. vernei*, and *A. andreanae* from the Castle Hayne Limestone, North Carolina. Based on similarities of their guard-like sheaths, Weaver & Ciampaglio (2003) also moved *Belosaepia jeletzkyi* Allen, 1968 from the Cook Mountain Formation, Louisiana into the genus *Anomalosaepia,* creating a new combination *Anomalosaepia jeletzkyi* (Allen, 1968). Weaver, Ciampaglio & Chandler (2007), based on new material, formally described as *Beloptera*? sp. phragmocone steinkerns from the Castle Hayne Limestone, North Carolina and included the specimen illustrated by Carter et al. (1988) as *Belemnosella?* sp. in *Beloptera*? sp. From the same locality, Weaver, Ciampaglio & Chandler (2007) also described other phragmocone steinkerns with high-angled septae as *Anomalosaepia* sp.

Lastly, Ciampaglio & Weaver (2008) described and named the first Oligocene coleoids from North America as a spirulid, *Oligorostra alabami,* and *Oligosella longi*, with unknown family affinity, from the Chickasawhay Limestone of Alabama (Fig. 1).

Though the number of species of Eocene *Belosaepia* from southern North America is comparable to those from Europe, considerably more research is needed on Paleogene and Neogene coleoid cephalopods to fill in geographic, stratigraphic and phylogenetic gaps.

Materials & Methods

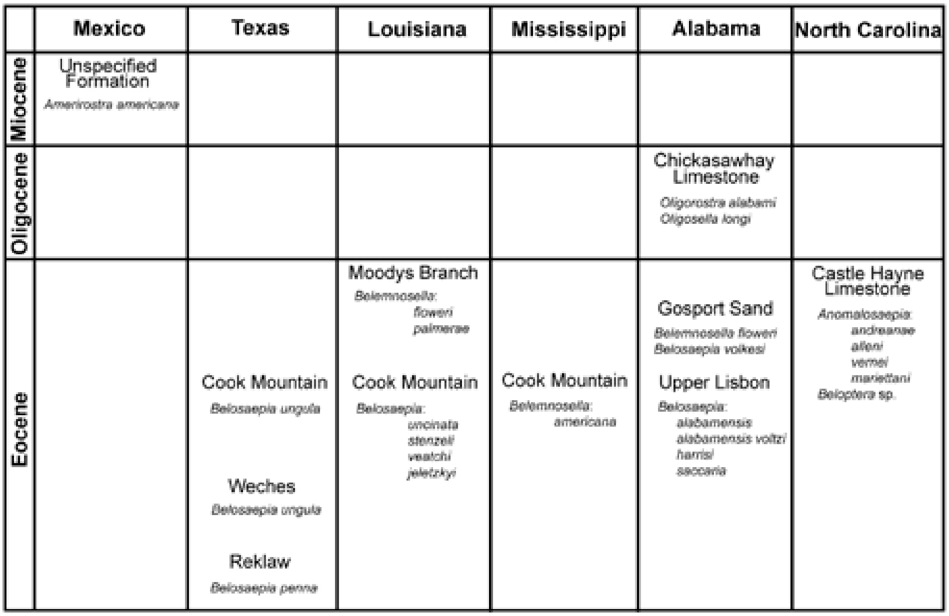
Through loans from the United States National Museum (USNM) and the Paleontological Research Institution (PRI), as well as through a survey of the collections at the North Carolina Museum of Natural Sciences (NCSM), published type specimens of Paleogene and Neogene coleoid cephalopods from the southeastern Atlantic and Gulf regions of North America were examined and photographed. A thorough survey of the literature was conducted and all known species of southern North American Paleogene and Neogene coleoid cephalopods are compiled here (Fig. 3).

Over the course of our research we have examined guard-like sheaths of: 1 specimen of *Belemnosella americana* (Meyer & Aldrich, 1886); 2 specimens of *B. floweri* (Palmer, 1937); 1 specimen of *B. palmerae* Allen, 1968; 1 specimen of *Belosaepia alabamensis* Palmer, 1937, 1 specimen of *B. alabamensis voltzi* Palmer, 1937; 1 specimen of *B. harrisi* Palmer, 1937; 1 specimen of *B. penna* Garvie, 1996; 2 specimens of *B. saccaria* Palmer, 1937; 2 specimens of *B. stenzeli* Allen, 1968; 4 specimens of *B. uncinata* Palmer, 1937; 1 specimen of *B. ungula* Gabb, 1860; 2 specimens of *B. veatchi* Palmer, 1937; 1 specimen of *B. vokesi* Allen, 1968; 67 specimens of *Anomalosaepia alleni* Weaver & Ciampaglio, 2003; 43 specimens of *A. andreanae* Weaver & Ciampaglio, 2003; 1 specimen of *A. jeletzkyi* (Allen, 1968); 63 specimens of *A. mariettani* Weaver & Ciampaglio, 2003; 70 specimens of *A. vernei* Weaver & Ciampaglio, 2003; 4 specimens of *Oligorostra alabami* Ciampaglio & Weaver, 2008; 14 specimens of *Oligosella longi* Ciampaglio & Weaver, 2008 and 3 specimens of *Amerirostra americana* (Berry, 1922). All of these specimens were well preserved showing very little weathering, though most specimens were broken anteriorly, and some were broken at the tip of the apical spine.

We also examined phragmocone steinkerns of 1 specimen and 1 image of *Beloptera*? sp. Weaver, Ciampaglio & Chandler, 2007 and 3 specimens of *Anomalosaepia* sp. Weaver, Ciampaglio & Chandler, 2007. These steinkerns were also well preserved.

Eocene and Oligocene paleoenvironments

Sea level rise during the middle Eocene, coupled with a productive, relatively warm-water, environment, allowed for the development of limestone facies along the Southeast Atlantic Coast (Gibson 1970; Otte 1986; Harris & Laws 1997). The depositional basin was formed by



	Mexico	Texas	Louisiana	Mississippi	Alabama	North Carolina
Miocene	Unspecified Formation *Amerirostra americana*					
Oligocene					Chickasawhay Limestone *Oligorostra alabami* *Oligosella longi*	
Eocene		Cook Mountain *Belosaepia ungula* Weches *Belosaepia ungula* Reklaw *Belosaepia penna*	Moodys Branch *Belemnosella:* *floweri* *palmerae* Cook Mountain *Belosaepia:* *uncinata* *stenzeli* *veatchi* *jeletzkyi*	Cook Mountain *Belemnosella:* *americana*	Gosport Sand *Belemnosella floweri* *Belosaepia volkesi* Upper Lisbon *Belosaepia:* *alabamensis* *alabamensis voltzi* *harrisi* *saccaria*	Castle Hayne Limestone *Anomalosaepia:* *andreanae* *alleni* *vernei* *mariettani* *Beloptera sp.*

Fig. 3: Stratigraphic representation of coleoid species from southern North America through time. Information compiled from Gabb (1860); Meyer & Aldrich (1886); Berry (1922); Palmer (1937 & 1940); Stenzel (1941); Palmer & Brann (1965); Jeletzky (1966 & 1969); Allen (1968); Carter et al. (1986) Gaskell (1991); Harris & Zullo (1991); Harris, Zullo & Laws (1993); Rosen, Bowen & Thies (1994); Dockery (1996); Garvie (1996); Falls & Prowell (2001); Weaver & Ciampaglio (2003); Zachos & Molineux (2003); Weaver, Ciampaglio & Chandler (2007) and Ciampaglio & Weaver (2008).

differential movement of fault-bounded crustal blocks, relative movement of which also controlled thickness and distribution of carbonate lithofacies (Jones 1983). Depositional environments formed an open, relatively warm-water embayment that paralleled the present-day shoreline (Jones 1983; Otte 1986) and outer shelf deposits most likely correspond to lithofacies seen in the present day Southeast Atlantic Coastal Plain.

The Eocene of the Gulf Coastal Plain contains basal fossiliferous, transgressive, marine sands and marine clays. Deposition of the Gulf Coastal Plain lithological units represents a significant sea-level-rise event across the Gulf Coastal Plain. The resulting marine transgression extended the shoreline from southern Alabama and Mississippi to north of Memphis, Tennessee, and deposited marine strata in the Desha Basin of Arkansas (Dockery 1996).

Analysis of Oligocene lithostratigraphic units indicate that depositional conditions in the west-central Gulf were dominated by deltaic and marginal marine settings, while south-central and southeastern Gulf regions were under the influence of a stable carbonate platform (Tew 1992).

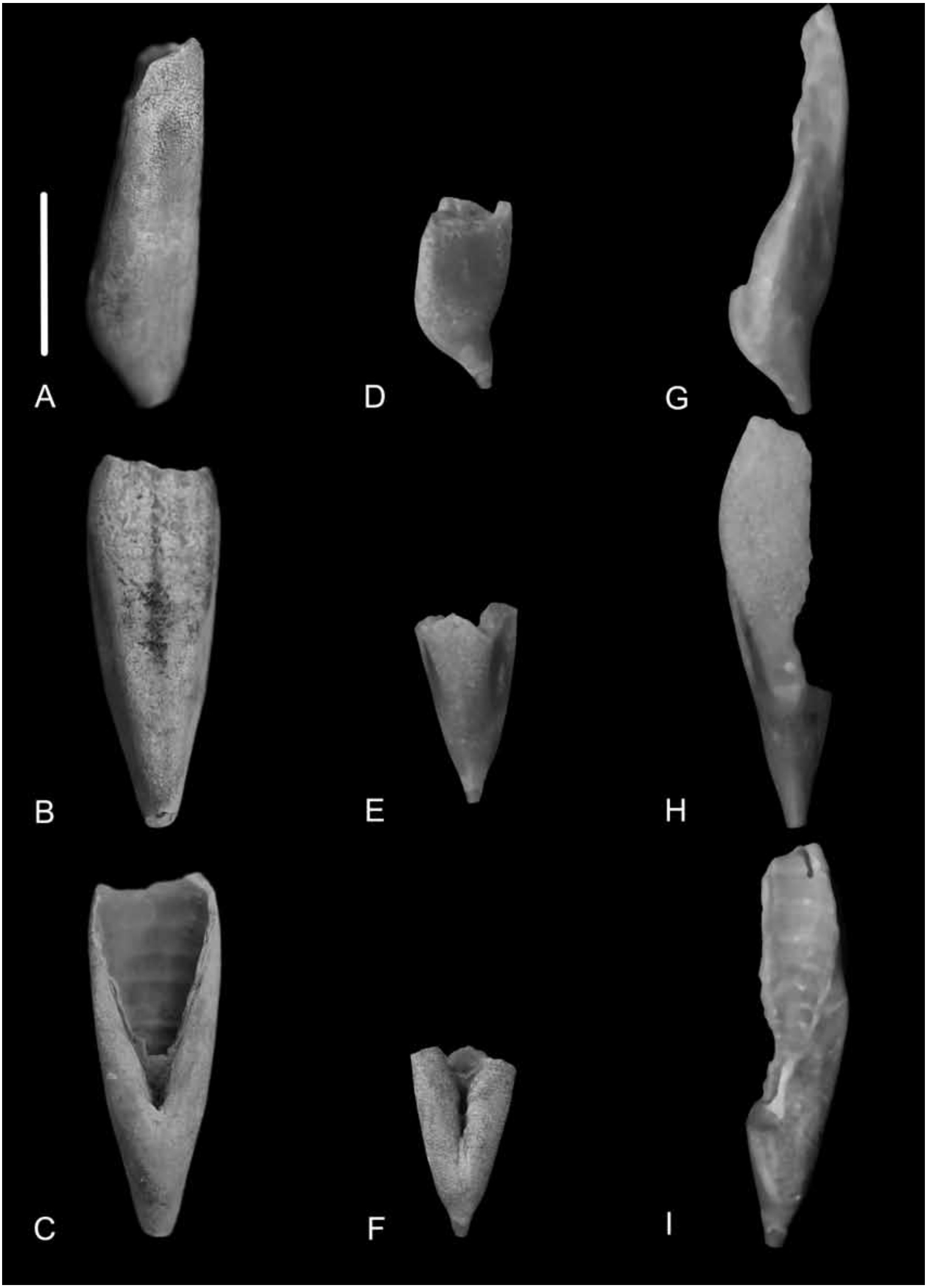
Eocene coleoids

Eocene aged coleoids are the most studied Paleogene coleoids from North America. They have been described from North Carolina, Mississippi, Alabama, Louisiana and Texas. Eocene coleoid cephalopods from North America divide into two main groups, belemnosellids from Claibornian sediments of Alabama, Mississippi and Louisiana and two genera of belosaepiids, *Belosaepia* from Alabama, Louisiana and Texas, and *Anomalosaepia* from North Carolina and Louisiana. There has also been a phragmocone steinkern of *Beloptera?* described by Weaver, Ciampaglio & Chandler (2008) from North Carolina (Fig.3).

Three species of *Belemnosella* have been described based on their guard-like sheaths (Fig. 4): *B. americana* from Mississippi (Meyer & Aldrich 1886; Jeletzky 1966, 1969), *B. floweri* from Alabama (Palmer 1937, 1940; Stenzel 1941; Allen 1968; Jeletzky 1966, 1969) and *B. palmerae* from Louisiana (Allen 1968). The genus *Belemnosella* is characterized by having a long, straight phragmocone, which may be feebly endogastrically incurved in the protoconch and earliest two or three camerae (Jeletzky 1969). *Belemnosella americana* (Meyer & Aldrich, 1886) is the type species for the genus (Jeletzky 1969). Differences between the three species of *Belemnosella* can be seen in figure 4. *Belemnosella floweri* (Palmer, 1937), differs from *B. americana* (Meyer & Aldrich, 1886) in having a greater enlargement of the ventral area anterior to apical spine, more depressed (carina-like) adoral-most part of the sheath, a regularly rounded, more boss-like and better defined ventral callus, and a more rugose dorsum. Based on the original descriptions given by Allen (1968), *B. palmerae* Allen, 1968 differs from *B. floweri* (Palmer, 1937) in having a much longer more evenly tapered and slightly dorsally curved sheath. Allen (1968) does remark that this species may turn out to be conspecific with *B. floweri* (Palmer, 1937).

The most studied group of Eocene coleoid cephalopods from southern North America is the belosaepiids. To date guard-like sheaths of ten different species, including one subspecies, of *Belosaepia* (Fig. 5) and five different species of *Anomalosaepia* (Fig. 6) have been recovered from southern North America. Belosaepiids, as a family, have dorsally incurved phragmocones, dorsally strongly mineralized sheaths (Engeser 1990), angled septae, ventral plates, and a distinct apical spine. Differences between the species of *Belosaepia* relate to the overall size, shape or ornamentation of the guard-like sheath or variations of the ventral plate (Palmer 1937; Allen 1968; Garvie 1996). Species recovered thus far include *B. ungula* Gabb, 1860, *B. uncinata* Palmer, 1937, *B. veatchi* Palmer, 1937, *B. alabamensis* Palmer, 1937, *B. alabamensis voltzi* Palmer, 1937, *B. saccaria* Palmer, 1937, *B. harrisi* Palmer, 1937; *B. vokesi* Allen, 1968, *B. stenzeli* Allen, 1968 and *B. penna*

Fig. 4: The three species of *Belemnosella* from the Eocene of the southern United States. A-C) *Belemnosella americana* (Meyer & Aldrich, 1886), USNM 638750, Cook Mountain Formation, Mississippi lateral, dorsal and ventral views. D-F) *B. floweri* (Palmer, 1937), PRI 27548 Moodys Branch Formation, Louisiana lateral, dorsal and ventral views. G-I) *B. palmerae* Allen, 1968, PRI 27549, Moodys Branch Formation, Louisiana lateral, dorsal and ventral views; scale bar = 10 mm.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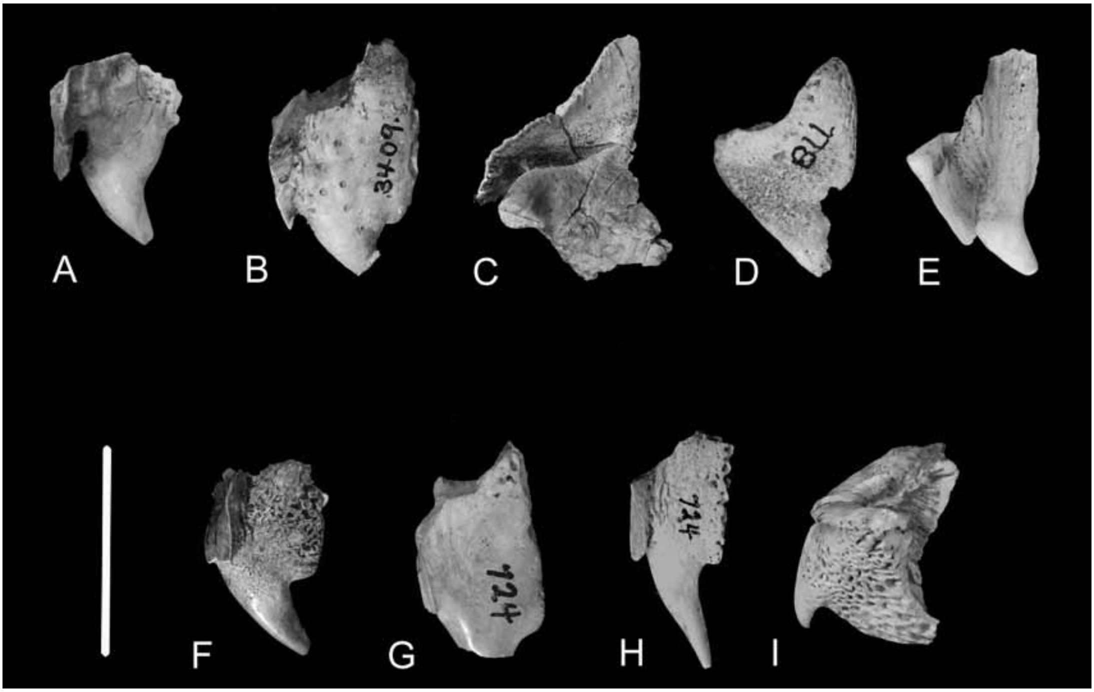

Fig. 5: Lateral views of species of *Belosaepia* from the Eocene of southern North America. A) *Belosaepia alabamensis* Palmer, 1937; Upper Lisbon Formation, Alabama, PRI 3403. B) *B. harrisi* Palmer, 1937; Upper Lisbon Formation, Alabama, PRI 3409. C) *B. penna* Garvie, 1996; Reklaw Formation, Texas, PRI 8450. D) *B. saccaria* Palmer, 1937; Upper Lisbon Formation, Alabama, PRI 9083. E) *B. vokesi* Allen, 1968; Gosport Sand, Alabama, PRI 27550. F) *B. ungula* Gabb, 1860; Weches Formation, Texas, PRI 3045. G) *B. uncinata* Palmer, 1937; Cook Mountain Formation, Louisiana, PRI 3047. H) *B. veatchi* Palmer, 1937: Cook Mountain Formation, Louisiana PRI 3041. I) *B. stenzeli* Allen, 1968; Cook Mountain Formation, Louisiana, PRI 27551; scale bar = 10 mm.

Garvie, 1996 (Fig. 5). The type species *B. sepioidea* (Blainville, 1827), a European species, has yet to be recognized from North America. It is possible that further examination of these species, currently being conducted by Yancey, Garvie & Wicksten (2008), may reveal conspecific taxa and ultimately reduce the number of North American species.

The other belosaepiid genus described from southern North America is *Anomalosaepia* Weaver & Ciampaglio (2003). This genus differs from *Belosaepia* in having a smooth guard-like sheath, a laterally curved ventral plate and a slit-like aperture at terminus of the apical spine (Fig. 6). Weaver & Ciampaglio (2003), described this genus from the Eocene of North Carolina, and moved *Belosaepia jeletzkyi* Allen, 1968 into this genus. Currently there are five species of *Anomalosaepia* known to occur in Eocene sediments of southern North America; *A. alleni*, *A. mariettani*, *A. vernei*, *A. andreanae* from North Carolina and *A. jeletzkyi* from Louisiana. It is possible upon re-examination that some of the differences described by Weaver & Ciampaglio (2003) as specific variations may ultimately turn out to be due to dimorphism or other taphonomic factors. Weaver, Ciampaglio & Chandler (2007) also described a phragmocone steinkern of *Anomalosaepia* sp. (Fig. 7) from the Eocene of North Carolina. Their generic determination was based on the curvature of the phragmocone and the high angle of the septae, which they deemed much too high for these steinkerns to belong to *Belosaepia*.

The phragmocone steinkerns illustrated by Carter et al. (1988) as *Belemnosella*? sp. were described by Weaver, Ciampaglio & Chandler (2007) as *Beloptera?* sp. (Fig. 7). As more specimens became available, Weaver, Ciampaglio & Chandler (2007) determined that these phragmocone steinkerns, due to their orthoconic shape, slight recurvature of their apical end and siphuncular ridge, accurately

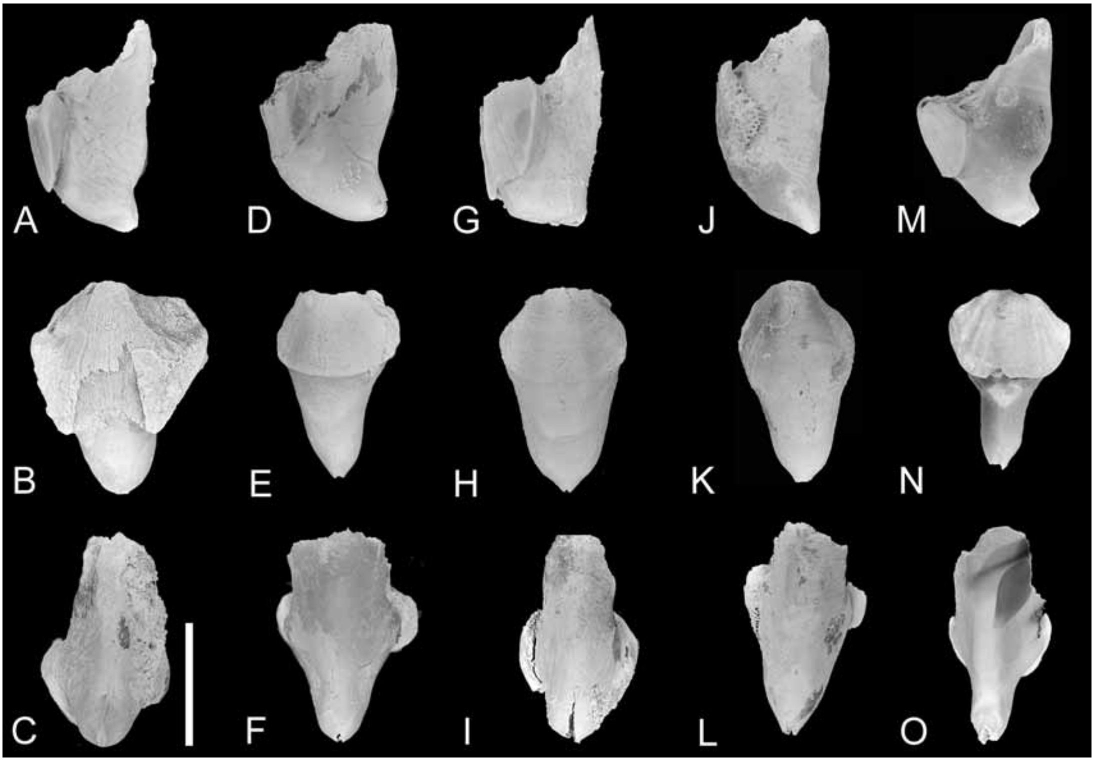

Fig. 6: The species of *Anomalosaepia* from the Eocene Castle Hayne and Cook Mountain Formations of North Carolina and Louisiana. Figure resized from the original Weaver & Ciampaglio (2003: Fig.1). A) *Anomalosaepia alleni* Weaver & Ciampaglio, 2003: Castle Hayne Formation, North Carolina, NCSM 5041 lateral view. B) *A. alleni;* NCSM 7161, ventral view. C) *A. alleni,* NCSM 5041, dorsal view. D) *A. mariettani* Weaver & Ciampaglio, 2003: Castle Hayne Formation, North Carolina, NCSM 4843, lateral view. E) *A. mariettani;* NCSM 7162, ventral view. F) *A. mariettani,* NCSM 4843; dorsal view. G) *A. vernei* Weaver & Ciampaglio, 2003; Castle Hayne Formation, North Carolina, NCSM 7163, lateral view. H) *A. vernei;* NCSM 4788M ventral view. I) *A. vernei;* NCSM 7163, dorsal view. J) *A. andreanae* Weaver & Ciampaglio, 2003; Castle Hayne Formation, North Carolina, NCSM 7164, lateral view. K) *A. andreanae;* NCSM 7165, ventral view. L) *A. andreanae;* NCSM 7164, dorsal view. M-O) *A. jeletzkyi* (Allen, 1968); Cook Mountain Formation, Louisiana, PRI 27553, lateral, ventral and dorsal views; scale bar = 10 mm.

mirrored the descriptions given by Naef (1922) for *Beloptera*. As no guard-like sheaths of *Beloptera* have yet been recovered from North Carolina, Weaver, Ciampaglio & Chandler (2007) found it prudent to describe these steinkerns as *Beloptera*? sp.

Oligocene coleoids

There have only been two genera of coleoid cephalopods recovered from Oligocene aged sediments of North America; *Oligorostra alabami* Ciampaglio & Weaver, 2008 and *Oligosella longi* Ciampaglio & Weaver, 2008. Guard-like sheaths of these two diminutive genera were recovered from the Chickasawhay Limestone Washington Co., Alabama through sieving. *Oligorostra alabami* (Fig. 8) is thought to be a spirulid because of it lacks the ventral plate of belosaepiids and the straight phragmocone of belemnosellids. *Oligosella longi* (Fig. 9) was determined to be a coleoid cephalopod by Ciampaglio & Weaver (2008) based on over all morphology and mineralogy of the sheath, but was so diminutive and unlike any other coleoid that it's higher taxonomy is uncertain. Ciampaglio & Weaver (2008) remark that their specimens may be embryonic.

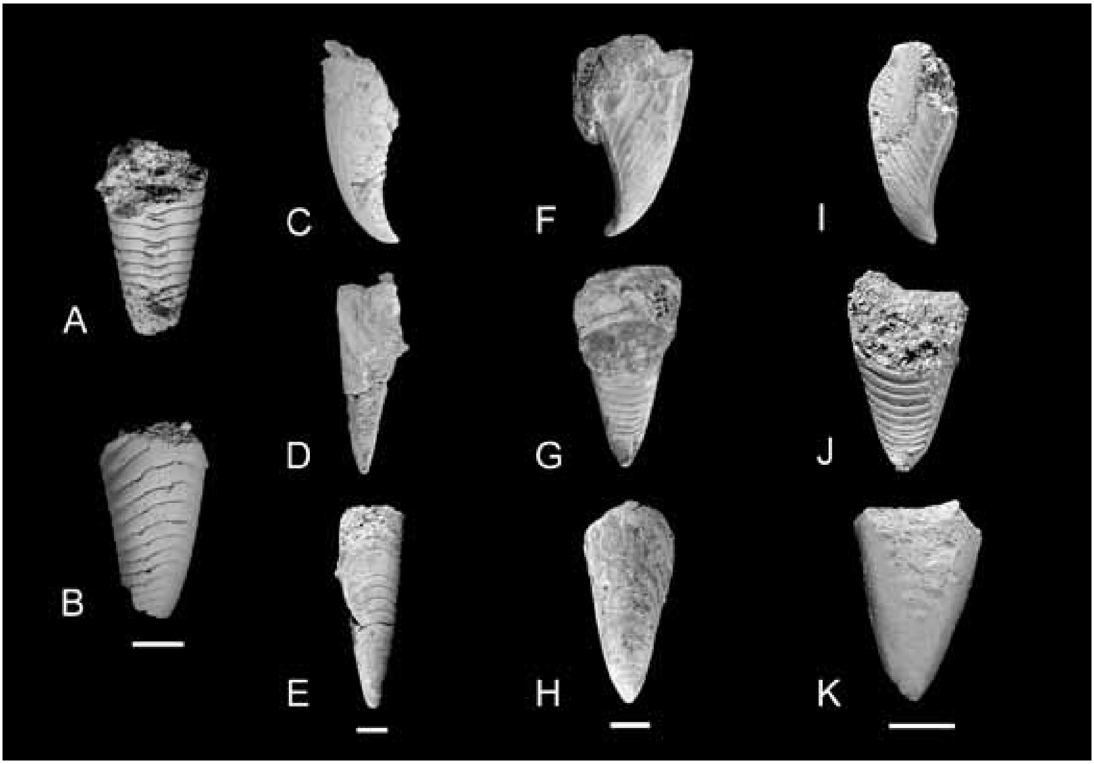

Fig. 7: Coleoid phragmocone steinkerns from the Eocene Castle Hayne Formation, North Carolina. Figure resized from the original Weaver, Ciampaglio & Chandler (2008: Pl. 1). A-B) *Beloptera*? sp.; UNC 1416, ventral and dorsal views. Images provided by Dr. Joseph Carter UNC. The specimen appears to be lost. C-E) *Beloptera*? sp.; NCSM 7714, lateral, ventral and dorsal views. F-H) *Anomalosaepia* sp.; NCSM 7713, lateral, ventral and dorsal views. I-K) *Anomalosaepia* sp.; NCSM 9262, lateral, ventral and dorsal views; scale bar = 10 mm.

Miocene coleoids

There has been only one report of Miocene aged coleoids recovered from southern North America, *Amerirostra americana* (Berry, 1922) from the Isthmus of Tehuantepec, Mexico (Fig. 10). This genus, a spirulid, was originally described by Berry (1922) as *Spirulirostra americana*. However, Jeletzky (1966, 1969) re-examined these specimens and determined, due to differences in phragmocone, the guard-like sheath, and other differences from the European and Australian *Spirulirostra*, that these are separate from *Spirulirostra* and named the new genus *Amerirostra* for them. Since 1922 no other Miocene aged coleoid cephalopods have been reported.

Discussion

In total, guard-like sheaths of three species of *Belemnosella*, ten species of *Belosaepia* and five species of *Anomalosaepia* have been described from Eocene sediments of the Atlantic and Gulf Coastal regions of southern North America (Gabb 1860; Meyer & Aldrich 1886; Palmer 1937, 1940; Stenzel 1941, Jeletzky 1966, 1969; Allen 1968; Garvie 1996; Weaver & Ciampaglio 2003). Phragmocone steinkerns of *Beloptera*? and *Anomalosaepia* have been described from the Eocene of North Carolina (Weaver, Ciampaglio & Chandler 2007). From the Oligocene of Alabama, *Oligorostra alabami* and *Oligosella longi* have been described by Ciampaglio & Weaver (2008) and one species of *Amerirostra* (Berry 1922; Jeletzky 1969) has been described from the Miocene of Mexico.

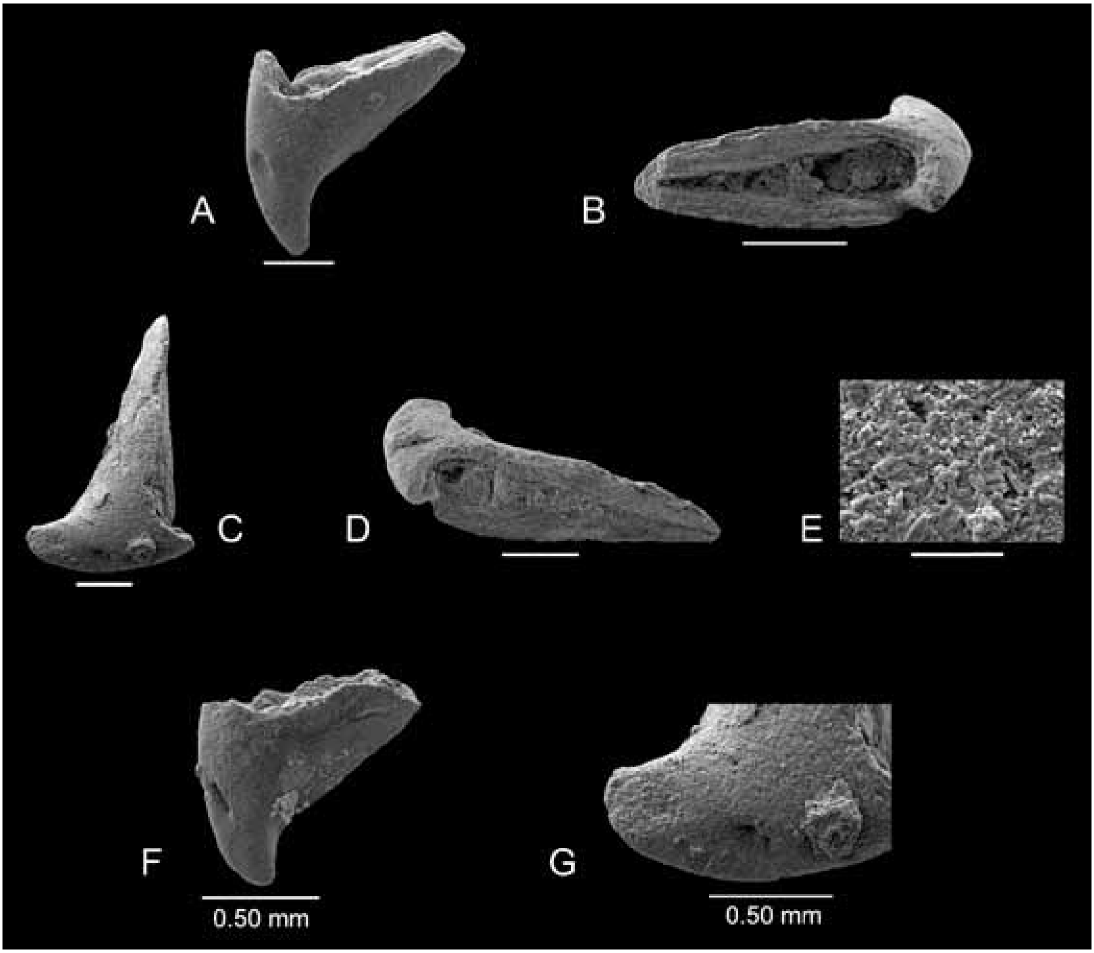

Fig. 8: Oligocene coleoid *Oligorostra alabami* Ciampaglio & Weaver, 2008; Chickasawhay Limestone, Alabama. Figure resized from the original Ciampaglio & Weaver (2008: Fig. 5); scale bar = 0.5 mm. A) *O. alabami;* NCSM 10980, left lateral view; scale bar = 0.5 mm. B) NCSM 10980, internal view; scale bar = 0.5 mm. C) NCSM 10978, right lateral view; scale bar = 0.5 mm. D) NCSM 10978, internal view; scale bar = 0.5 mm. E) NCSM 10978, close-up of mineralization of guard-like sheath; scale bar = 0.02 mm. F) NCSM 10985, left lateral view; scale bar = 0.5 mm. G) 10978, close-up view of apical spine showing pit; scale bar = 0.5 mm.

Though published research on Paleogene and Neogene aged coleoid cephalopods from southern North America has been at best sporadic, considerable taxonomic work has been done, particularly on Eocene aged specimens. Due to gaps in geographic and stratigraphic coverage an overall picture of Paleogene and Neogene coleoid cephalopods through space and time from North America has yet to emerge. Field collection of Eocene aged specimens is desperately needed in South Carolina, Georgia and Florida and from Oligocene and younger sediments of North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Texas to give a more complete picture these coleoids throughout the Cenozoic. Future research is needed on microstructures of all of species mentioned through out this paper, as well as, on any new specimens recovered from field sampling. Once these studies are complete perhaps more correlations could be made with Paleogene and Neogene faunas of Europe and some of the phylogenetic problems of certain taxa such as *Anomalosaepia*, *Oligorostra* and *Oligosella* can be resolved.

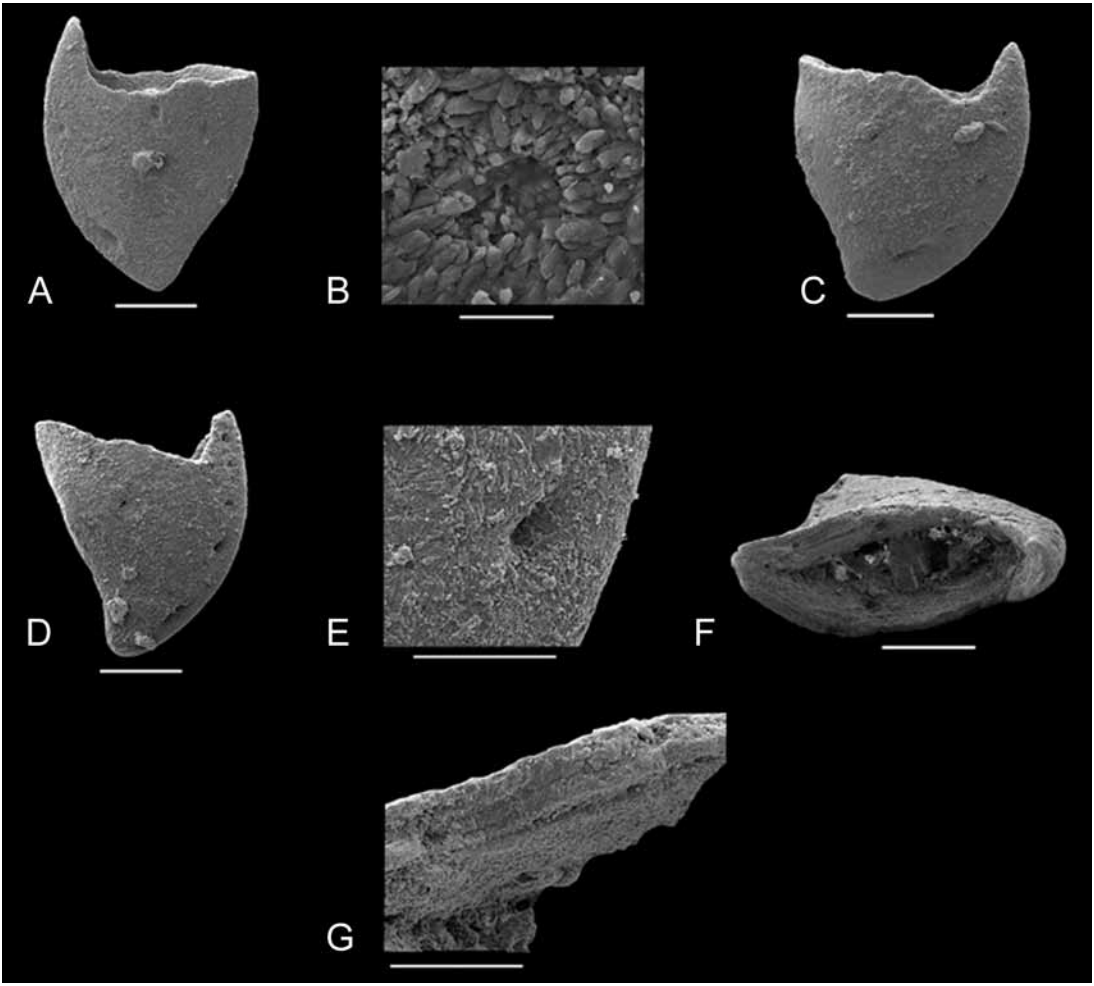

Fig. 9: Oligocene coleoid *Oligosella longi* Ciampaglio & Weaver, 2008; Chickasawhay Limestone, Alabama.. Figure resized from the original Ciampaglio & Weaver (2008, Fig. 6). A) *O. longi;* NCSM 10984, left lateral view; scale bar = 0.5 mm. B) NCSM 10984, close-up view of mineralization in and around one of the small pits; scale bar = 0.02 mm. C) NCSM 10987, right lateral view; scale bar = 0.5 mm. D) NCSM 10982, right lateral view; scale bar = 0.5 mm. E) NCSM 10982, close-up view of apical spine showing pit, scale bar = 0.2 mm. F) NCSM 10979, internal view; scale bar = 0.5 mm. G) NCSM 10979, edge of guard-like sheath showing mineral layering; scale bar = 0.2 mm.

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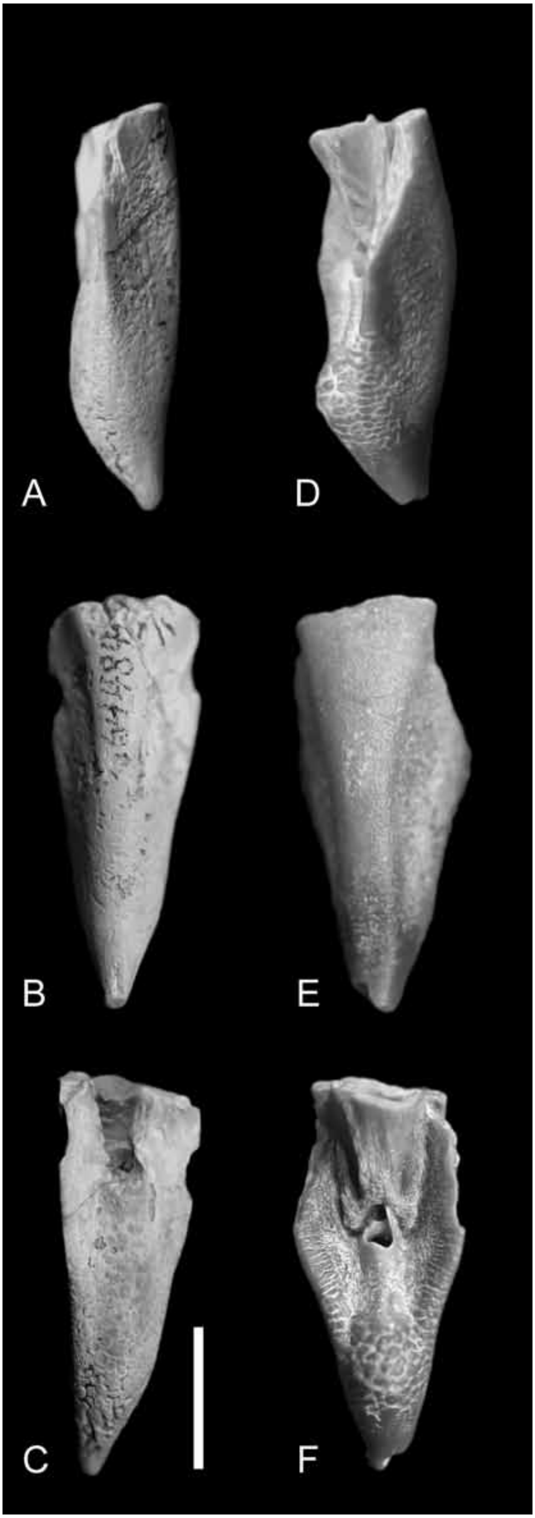

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Fig. 10: Miocene coleoid *Amerirostra americana* (Berry, 1922) from an unnamed formation, Mexico. A-C) *A. americana;* USNM 644842a, lateral, dorsal, ventral views. D-F) USNM 644841a, lateral, dorsal and ventral views; scale bar = 10 mm.

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