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ULTRASTRUCTURAL AND CHEMICAL COMPARISON BETWEEN GLADII IN LIVING COLEOIDS AND APTIAN COLEOIDS FROM CENTRAL RUSSIA

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Abstract: The paper reports ultrastructural and chemical studies on gladii of living and fossil coleoids in order to elucidate the original composition of the fossil gladii. Gladii of the Aptian *Nesisoteuthis simbirskensis* Doguzhaeva and of the living coleoids *Berryteuthis magister* Berry and *Loligo* sp. were studied with scanning electron microscopy (SEM) and energy dispersive spectrometry (Link). In all three taxa the gladius is laminated, being composed of alterating solid and less solid laminae. In living coleoids each lamina consists of fibrous sheaths. In the fossil *N. simbirskensis* the laminae are composed of globular aggregates of tiny granules. The globules are ca. 0.1–0.4 µm in diameter and arranged in chains.

Link analyses on the gladii of living coleoids show the elements: S, P, Si, Fe, Cl, Al, K and Ca. The first two elements, S and P, are dominant, whereas Ca has the lowest concentration. The fossil gladius and ink were analysed with the same instrument in the holotype of *N. simbirskensis* and in an imperfectly preserved, unnamed Aptian coleoid (Hecker & Hecker, 1955, p. 37, note 2). In the two specimens the gladius and ink both show Ca as the dominant element followed by P and S. This indicates that they are composed of calcium phosphate. In *N. simbirskensis* the split surfaces between the part and counterpart of the gladius are covered by a thin layers of barite.

The fossil ink in the two Aptian specimens, like the dried ink in the living *B. magister* and *Loligo* sp, has a globular ultrastructure. Originally organic, but post-mortally phosphatized, fish scales, which co-occur with *N. simbirskensis*, also have a globular ultrastructure but the globules are smaller than those in the gladius. Also the "horny" siphonal tube in ammonites found in the same beds has a globular ultrastructure, whereas the ammonite shell wall and septa show a well-preserved nacreous structure.

The role of bacteria in the post-mortem phosphatization which gives rise to the globular ultrastructure of the fossil gladius, ink and organic debris in *N. simbirskensis* is discussed. The presence of morphologically similar globules is shown in the soft tissues of Recent *Loligo* after a year of drying, and in the muscle tissue of Recent *Nautilus* after 20 years in alcohol. In *Loligo* the globular aggregates represent colonies of bacteria; in *Nautilus* they seem to be formed by coagulation of proteins in the muscle fibres.

The laminated gladius in *N. simbirskensis* is here interpreted as originally composed of an organic, probably chitinous, material that became phosphatised during fossilization. This agrees with the results previously obtained in Jurassic "fossil squids" (Doguzhaeva & Mutvei, 2003). It is still unclear whether the gladius in extinct squid-like coleoids lost its mineral composition in pre-Jurassic times or whether it was composed of organic material from the beginning. In belemnoids the mainly organic pro-ostracum seems to have developed as an innovative structure (Doguzhaeva & al., 2002, 2005a, b).

Key words: Early Cretaceous coleoid, shell morphology and ultrastructure, gladius, ink sac, evolutionary morphology, Russia

INTRODUCTION

The available interpretations of the original composition of the gladii in "fossil squids" are contradictory. All gladii of "fossil squids" which have been analysed, found either in the Solnhofen Formation in Bavaria, in the Kimmeridge Clay in southern England, and in Upper Jurassic and Aptian beds in Central Russia, consist of calcium phosphate. Gladii were therefore considered to have been originally mineralized (Naef, 1922; Jeletzky, 1966; Hewitt, Lazer, Moorhouse, 1983; Donovan & Toll, 1988; Hewitt & Whyte, 1990; Engeser & Keupp, 2002). Hecker & Hecker (1955) assumed that the Upper Jurassic and Aptian gladii from Central Russia were mainly organic in life, as in the case of modern squids. Bandel & Leich (1986) were of the opinion that the original mineral of the gladius in *Trachyteuthis* was aragonite. Hewitt and Jagt (1999) shared this interpretation of the primary aragonitic mineralization of the gladii in *Trachyteuthis* and *Loligosepia,* and accepted the idea of their post-mortem replacement by francolite. However, Hewitt & Wignall (1988, p. 153) thought that "the shell of *Trachyteuthis* was largely composed of a laminar fabric of chitin material, with a crystalline material concentrated in folded layers over the median dorsal surface". They assumed that the chitin in this fossil might have been secondarily replaced by brushite. According to Doguzhaeva & Mutvei (2003), the gladii of the Jurassic squids *Loligosepia, Trachyteuthis* and *Teudopsis* were originally of organic, apparently chitinous composition.

The present paper describes scanning electron microscopic (SEM) and energy dispersive spectroscopic (Link) studies on the gladii of the Aptian *Nesisoteuthis simbirskensis* Doguzhaeva from Central Russia and the living squids *Berryteuthis magister* Berry and *Loligo* sp. The object of the study is to elucidate the original composition of the gladius of *N. simbirskensis.*

MATERIAL STUDIED AND METHODS

The material studied includes: (1) gladius of the holotype of *N. simbirskensis* Doguzhaeva (no. PIN 3871/391) from the Lower Aptian, vicinity of the village Shilovka, Uljanovsk region, Middle Volga, Central Russia (Doguzhaeva, 2005; Doguzhaeva & Mutvei, 2005); (2) gladius and ink from an unnamed "fossil squid" (Hecker & Hecker, 1955, p. 37, footnote 2), together with scales of pelagic fish and aptychi from the same beds, and (5) five gladii each of the living squids *Berryteuthis magister* Berry and *Loligo* sp.

The well preserved gladius of *N. simbirskensis* was extracted from a small, ca 50 mm long, dense, dark-grey, sideritic concretion that was split into two halves. The fractured surfaces of the concretion expose a longitudinally split gladius. The larger, thicker portion is 26 mm long and 6 mm wide and the thinner counterpart is 23 mm long and 4 mm in maximum width. The specimen has an ink sac distinguished by its flask-like shape and its content of black ink. The ink sac could be observed through the thin gladius that became transparent in alcohol. At the location of the ink sac the gladius is deformed and broken. The black ink fills the cracks.

Both split surfaces of the gladius, and a cross section through its anterior edge, were studied with the SEM without etching. The structure of pelagic fish scales, which consisted originally of organic substance, and which occur abundantly in the same sideritic concretion, was compared with that of the gladius. The fossil gladius, ink and fish scales were also analysed for their elemental composition with Link. The samples were coated with gold for ultrastructural examination, and either with carbon or nickel for Link analysis.

The study was carried out at the Palaeontological Institute of the Russian Academy of Sciences, Moscow, and at the Department of Palaeozoology at Swedish Museum of Natural History, Stockholm.

The specimens are stored at the Palaeontological Institute of the Russian Academy of Sciences, Moscow.

OBSERVATIONS

1. The ultrastructure of the gladius, ink and organic debris in *N. simbirskensis*

1. 1. Gladius (Pl. 1, A–B; 2, A–F)

The gladius consists of ca 1–2 μm thick laminae, each composed of a set of thinner lamellae (Pl. 2, A–C). The lamellae consist of globules that are more or less regularly shaped and 0.05–0.2 μm in diameter (Pl. 2, C–E). In some laminae a fibrous arrangement of granules is present (Pl. 2, F). Each globule is formed of an aggregate of still smaller particles (Pl. 2, E). In some laminae the globules are loosely packed (right side of Pl. 2, D), whereas in others they are compactly packed (left side of Pl. 2, D). In the central keel region of the gladius the globules form aggregates that are larger and more compactly packed than those outside the keel. The laminae are traversed by vertical micro-pores with diameters of ca 0.05–0.2 μm, being close to the size of the globules (Pl. 2, E). About 100 pores were counted on a square with 5μm sides in the central keel region. However, in some other places the pores are less numerous or not visible. Numerous cracks in the gladius, caused by compaction, are filled by heterogeneous debris.

A longitudinal scar occurs on the right side of the median keel on the dorsal surface of the gladius (sc, Pl. 1, B). Along the scar the growth lines are irregular and form a V-shaped lobe indicating the the gladius was damaged during life and that the injury was repaired by the animal. Similar scars have been described in gladii of living sepiida (Bello & Paparella, 2003).

1. 2. Fossil ink (Pl. 3, A–D)

Because of compaction of the gladius the ink sac is partly destroyed and the ink is exposed in cracks in the gladius (Pl. 3, A). In addition to the ink, the ink sac probably contains pieces of gladius and remnants of soft tissues (Pl. 3, B) that penetrated into the ink sac during the early post-mortem period. Under low magnification the ink looks like a glassy, structureless substance (Pl. 3, C). Under higher magnification aggregates of globules, ca 0.3 μm in diameter, can be distinguished. They are embedded in a mass of small particles that do not form globules (Pl. 3, D). Each globule consists of smaller particles (Pl. 3, D). The globular ultrastructure is similar to that seen in the ink of living and extinct coleoids of different geological ages (see Doguzhaeva & al., 2004).

1. 3. Soft tissue debris (Pl. 3, B, E–F)

Debris of soft tissues is observed within the ink sac (Pl. 3, B), in cracks of the fractured laminae of the gladius, and also around the gladius. In all these places the debris has an angular or globular shape and seems to be mixed with sediment from the concretion. Besides, the ink sac contains indeterminate micro-fragments of unknown origin in the shape of distinct bands of criss cross fibres (Pl. 3, F). The organic debris also contains numerous micro-organisms that are often organized in colonies (Pl. 3, E).

2. Aptian fish scales (Pl. 5, A) and aptychi

The Aptian fish scales are laminated, consisting of several (five or six) laminae of globular ultrastructure (Pl. 5, A). The globules are ca 0.03 μm in diameter and, thus, about ten times smaller than the globules in the gladius and ink substance. Each globule is composed of smaller particles.

The Aptian aptychi studied also have a globular ultrastrucutre. The globules are ca. 0.2 μm. in diameter and arranged in fibre-like chains.

3. Gladius and ink in the living squids *Berryteuthis magister* (Pl. 4, A–G) and *Loligo sp.* (Pl. 5, B, F)

The gladius is laminated (Pl. 4, G; 5, B) and flexible when wet, but fragile, longitudinally folded (Pl. 4, A), deformed (Pl. 4, B) or crushed into elongated fragments, when dried.

It seems to absorb and retain water between the laminae. This possibly explains why the gladius is flexible in wet condition and fragile in dried condition.

The dorsal surface of the gladius bears a mid-dorsal rib, known as the rib of rigidity (e.g. by Bizikov, 1996), that encloses a hollow, ventrally open, tunnel (Pl. 4, A, C). The dorsal surface of the rib bears growth lines whereas the rest of the dorsal surface is smooth (Pl. 4, A). The gladius is thickened along the crest of the rib and along a narrow, longitudinal thickened zone on each side of the rib (Pl. 4, A–C). The laminae are separated by empty interspaces in the central part of the thickened zones but they become tightly packed close to the dorsal and ventral surfaces of the gladius (Pl. 4, E). Under high magnification the lamination is not homogenuous but consists of alternating compact and porous laminae (Pl. 5, F). The latter are perforated by numerous micropores, 0.3–0.6 µm in diameter. Each lamina has a fibrous ultrastructure, the diameter of the fibres ca 0.06 µm.

The dried ink has a globular ultrastructure. Each globule has a diameter of 0.2–0.5 µm and consists of tiny particles. As experimentally demonstrated, regular or less regular shape of globules depends on the mode of drying. If the ink is dried slowly within the opened ink sac the globules are regularly shaped. If the ink is placed between cover glasses and heated the globules do not form but the ink consists of flattened, irregularly shaped, structural units.

4. Results of chemical analysis

4. 1. The gladius of the Aptian *Nesisoteuthis* and an unnamed coleoid (Pl. 6, A–B), fossil ink (Pl. B–D), aptychi and fish scales (Pl. 5A)

The dominant elements in the gladius of *Nesisoteuthis simbirskensis* and an Aptian unnamed coleoid (Hecker & Hecker, 1955) are Ca, P, S, Fe, followed by the minor elements, Si, Ti, Al, Zn, Md, K. In strongly pyritized spots the peak of S is almost as high as for Ca and the peak of Fe higher than that of P (Pl. 6, A). In other places the peaks of Ca and P are the highest (Pl. 6, B). In *N. simbirskensis* the split surfaces between the part and counterpart of the gladius are covered by a thin layers of barite.

The dominant elements in the fossil ink of *Nesisoteuthis simbirskensis* and the Aptian unnamed coleoid are Ca, P, S and Fe, and the minor elements Al, Mg, U.

Also in the Aptian aptychi and fish scales the dominant elements are Ca, P and S, and the minor elements Fe, Si, Mg.

The results clearly demonstrate that the fossil gladius, ink, aptychi and fish scales, co-occuring in Aptian beds, consist of calcium phosphate.

4. 2. The gladii in the living squid *Berryteuthis magister* (Pl. 6, C–D)

The dominant elements are S, P, Si, and the minor elements Cl, K, Ca, Fe. The elements show quantitative variations, although S always has the highest peak. In some places the peak of Si is higher that that of P (Pl. 6, C) but lower in others (Pl. 6, D).

5. Discussion

5.1. Ultrastructural and chemical comparisons with gladii previously studied

The ultrastructure of the gladius has previously been described in the Jurassic *Loligosepia, Trachyteuthis* and *Teudopsis* (Doguzhaeva & Mutvei, 2003). All these gladii are multi-laminated, each lamina being composed of tablet-like or chain-like aggregates of tiny globules with a diameter of 0.3–0.4 µm. The globular chains in the laminae represent fossilized fibres, which reveal the organic composition of the Jurassic gladii. The gladius of the Aptian *N. simbirskensis,* examined herein, shows a similar ultrastructure: it is laminated and each lamina is composed of globules that are arranged in indistinct fibres (Pl. 2, F).

In the living coleoids *Berryteuthis* and *Loligo* the gladii are laminated and consist of fibrous sheaths. It seems reasonable to assume that also the laminae in the fossil gladii originally consisted of organic fibres that have been transformed diagenetically into chains of globules.

According to previous elemental analysis (Doguzhaeva & Mikhailova, 2002) the mandibles in the Aptian heteromorph ammonite *Australiceras* are also composed of calcium phosphate like the Lower Aptian fossils studied herein, whereas the ammonite shells are unaltered aragonitic.

5.2. Potential role of microorganisms in preservation of the Aptian gladii from Volga

The spectra of the gladius in *Nesisoteuthis* exhibit the barite on the split surfaces of the main part and counter-part. The marine microorganisms can precipitate this mineral. The recently obtained data on the chemistry of the marine water column demonstrate the presence of suspended marine barite. The particles of barite are a universal component of water in the Atlantic and Pacific oceans. Barite has been previousely reported in benthic protozoans (see Church, 1986). It has been found in the siphuncle of the Carboniferous orthoconic cephalopods from the Buckhorn asphalts (unpublished data by Doguzhaeva & Mapes). These data indicate the potential role of the microorganisms participeinting in barite precipitation and in the exceptional preservation of the gladii examined.

5.3. The role of Recent marine bacteria in decomposing chitin, and its bearing on the interpretation of mineral replacement of chitin in ancient environments

Million of tons of chitin are deposited in the exoskeletons of marine crustaceans every year. Although chitin is a water-insoluble and chemically resistant substance there are no large accumulations of chitin on the sea bottom. Marine bacteria play a dominant role in the decomposition of chitin. These bacteria were studied in the Black Sea where accumulations of chitin are produced by gammarids and other amphipods (Crustacea) by shedding their exoskeletons 40–50 times per year (Imsheneckiy, 1933; Kopp & Markianovich, 1950; Markianovich, 1959). The chitin-decomposing bacteria are widelspread all over the Black Sea where they live in various environments at the depths from 100 m to 2000 m. They occur most abundantly in zones with a high content of hydrogen sulphide (Kopp & Markianovich, 1950). This led first to the conclusion that the decomposition of chitin by bacteria mainly occurred in anaerobic conditions. Later it was shown that bacteria also decompose chitin in oxygen-rich zones (Markianovich, 1959). Long-term experiments with the chitin-decomposing bacteria from the Black Sea show that the chitin became soft in three months and total decomposition was usually achieved after 3–7 months. The experiments carried out in aerobic conditions showed that the appearance of ammonium hydrate marks the end of the total decomposition, resulting in the formation of mineral components. The rate of destruction of the chitin by different chitin-decomposing bacteria depends on environmental conditions, being higher in water that is rich in sulphuric acid. The chitin-decomposing bacteria in the Black Sea include several species, most having a stick-like shape of length ca. 0.2–1 µm.

The recent findings suggest that in the Mesozoic also marine bacteria played a significant role in decomposition of chitin in skeletons. As shown above, the globular ultrastructure of the fossil gladii is hitherto known in three Jurassic and one Lower Cretaceous genera of coleoid cephalopods. Besides, the Aptian fish scales and aptychi have a globular ultrastructure. All the globules consist of tiny particles. In the gladii the globules are 0.3–0.5 µm in diameter but in fish scales about ten times smaller. This shows that the size of the globules does not exceed the size of the modern chitin-decomposing bacteria that have a diameter of about 1µm or less. The bacteria seem to rework the chitinous material of the gladius and accumulate P. The possible step-by-step scenario of the replacement of chitin by calcium phosphate cannot so far be reconstructed. The size of the granules suggests that they are at least partly a product of bacterial metabolism. The chitin-decomposing bacteria could create a microenvironment favouring the precipitation of phosphates. The latter occurred in low oxygen condition, such as in the Lower Aptian of the Uljanovsk region. Here the Lower Aptian black claystones and shales contain abundant bituminous organic material, numerous druses of gypsum crystals, dispersed pyrite, marcasite, siderite and ankerite-siderite concretions. The lamination in the calcareous concretions and claystones, interlayered with beds of fine-grained glauconitic sandstones, laminated aleurolite limestones and marls, indicate a generally shallow water environment with variable depth regime. Such a lithology indicates an unstable, deoxygenated milieu favouring the precipitation of phosphorus (Doguzhaeva, 2002).

After drying for one year the gladius of living *Loligo* shows laminae that are disintegrated into small, plate-like elements (Pl. 5, C–D). Colonies of globular bacteria were found in the soft tissue surrounding the gladius (Pl. 5, E). The ink in living *Loligo* sp., *Berryteuthis magister* and other coleoids studied has also a globular ultrastructure even after being dried and heated for ca. 30 min. Fossil coleoid ink has a similar globular ultrastructure. In the latter case the aggregates of globules were apparently formed as a result of coagulation of melanin into small particles (the pigment melanin is the main component of ink in living coleoids) and thereafter transformed into bigger globules. In the compressed ink sac of the Aptian *N. simbirskensis* the fossil ink has in places a globular ultrastructure, in other places it is structureless, apparently because the particles were not coagulated into globules. In Recent *Nautilus* after 20 years in alcohol the muscular mantle shows a globular ultrastructure. This is probably caused by a coagulation of proteins in the muscle fibres into globules.

Recent studies show that the exposed surfaces of the bacteria include reactive chemical compounds, e.g. phosphates and carboxylates, which are responsible for ionic interaction with solutes. Once an ion is added to such a chemical group, it becomes a nucleation site for further precipitation. The bacteria have the highest surface area-to-volume ratio that makes them highly interactive with the surrounding elements. This explains their great productivity in the precipitation of ions from the environment and development of fine-grained minerals (Fortin et al., 1997).

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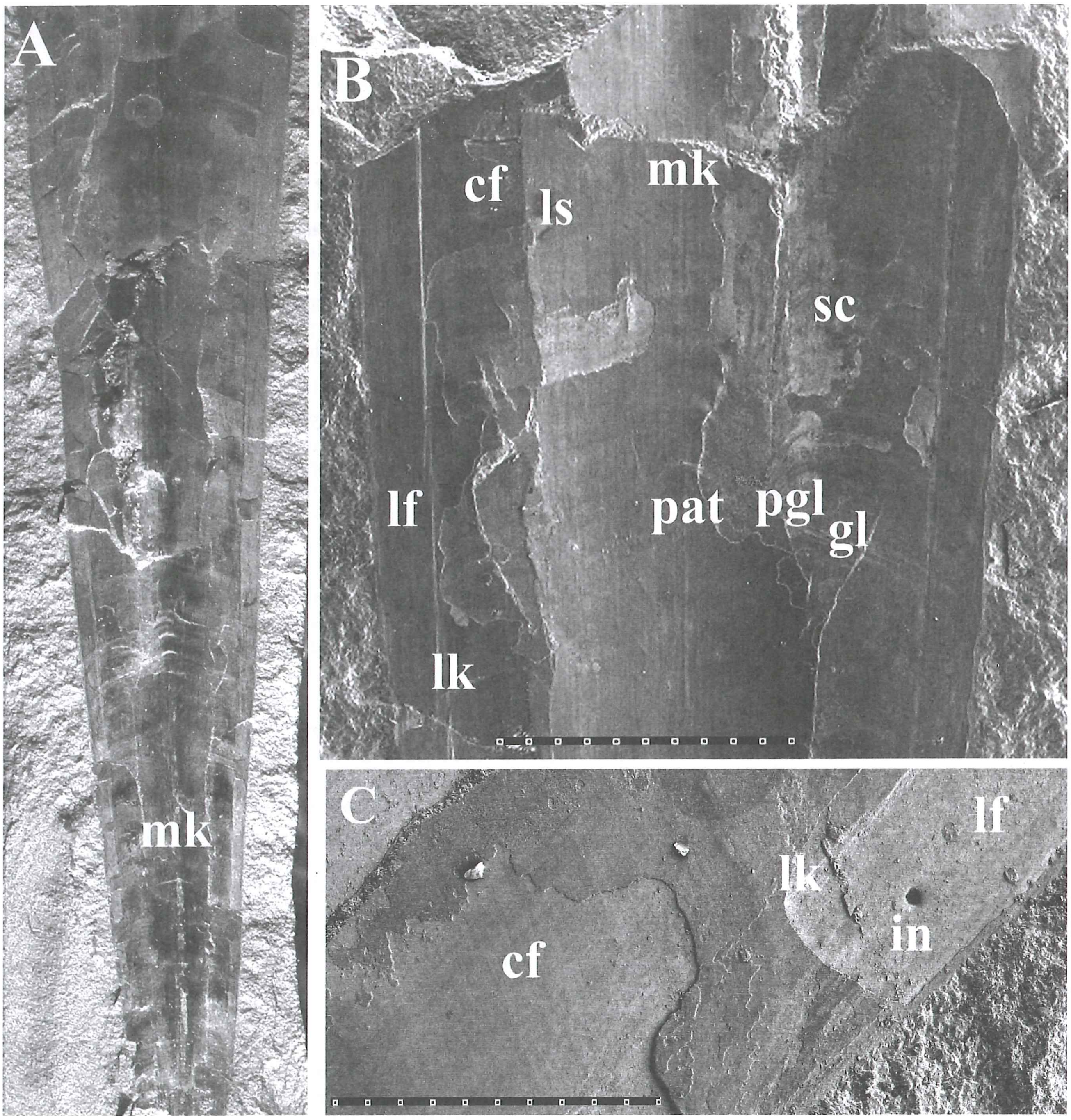


Plate 1. A–C. ***Nesisoteuthis simbirskensis*** **(no. PIN 3871/391). A. General view of the gladius exposed along the split surface; × 2. B. Anterior portion of the counterpart of the gladius in Fig. 1A to show the central (cf) and lateral (lf) fields, the median (mk) and lateral (lk) keels, growth lines (gl), longitudinal striation (ls), crest of the median keel (pat), healed longitudinal scar (sc); scale bar is 1.5 mm.**

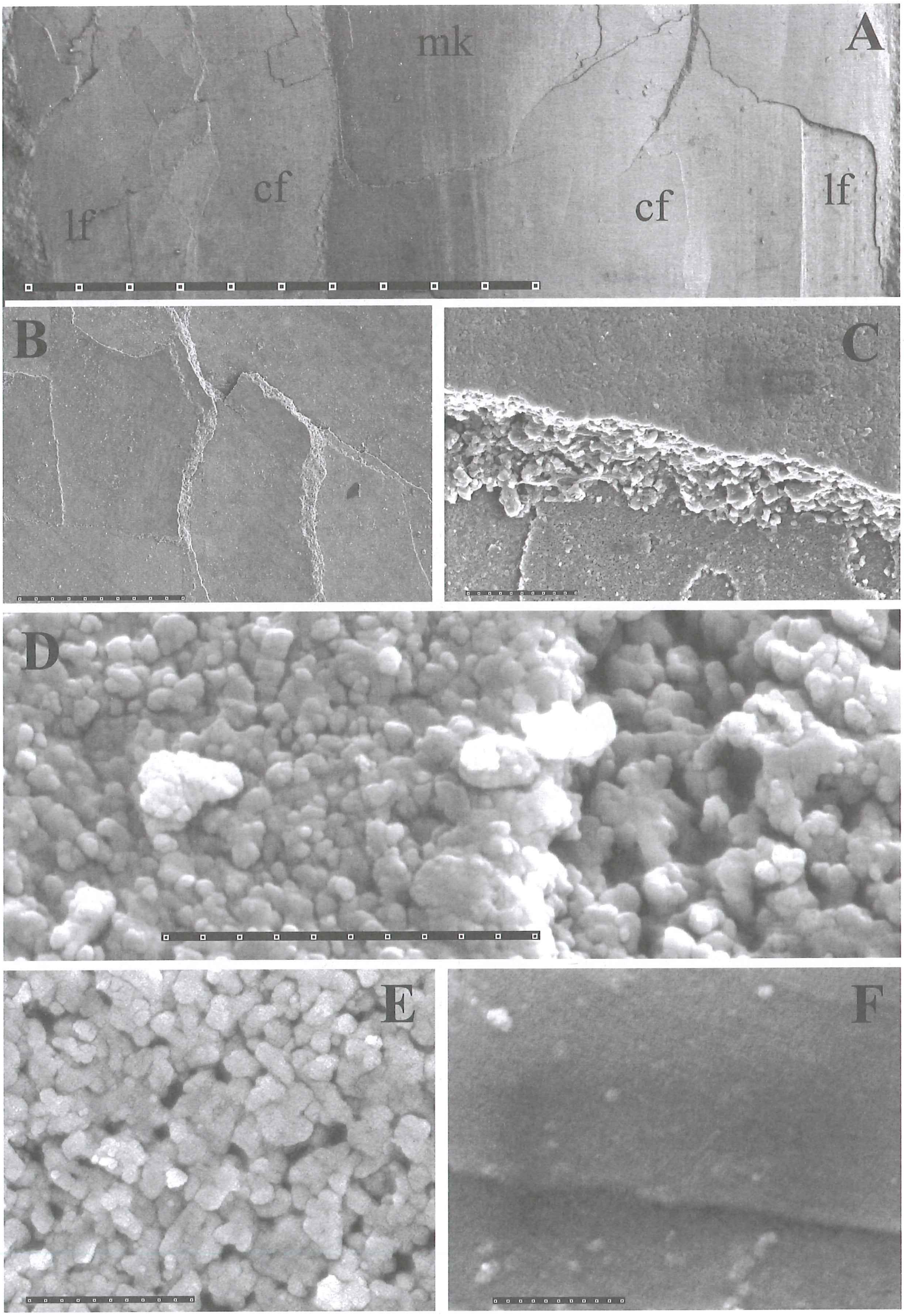


Plate 2. A–F. *Nesisoteuthis simbirskensis* **(no. PIN 3871/391). A. Portion of the dorsal surface of the gladius in higher magnification; scale bar is 1 mm. B. Detail of Fig. 1 to show sublayers in the fractured gladius; scale bar is 3 mm. C. Close-up of 2B to show the globular structure in the fractured gladius; scale bar is 300 µm. D. Close-up of 2C to show the globular ultrastructure of the laminae; the globules are compactly packed on the left hand side but loosely packed on the right hand side; scale bar is 3 µm. E. Globular ultrastructure of the gladius; each globule consists of numerous tiny particles; note the micro-pores between the globules; scale bar is 1.2 µm. F. Sufaces of two laminae of the gladius to show fibrous ultrastructure; each fibre consists of chains of small globules; scale bar is 0.6 µm.**

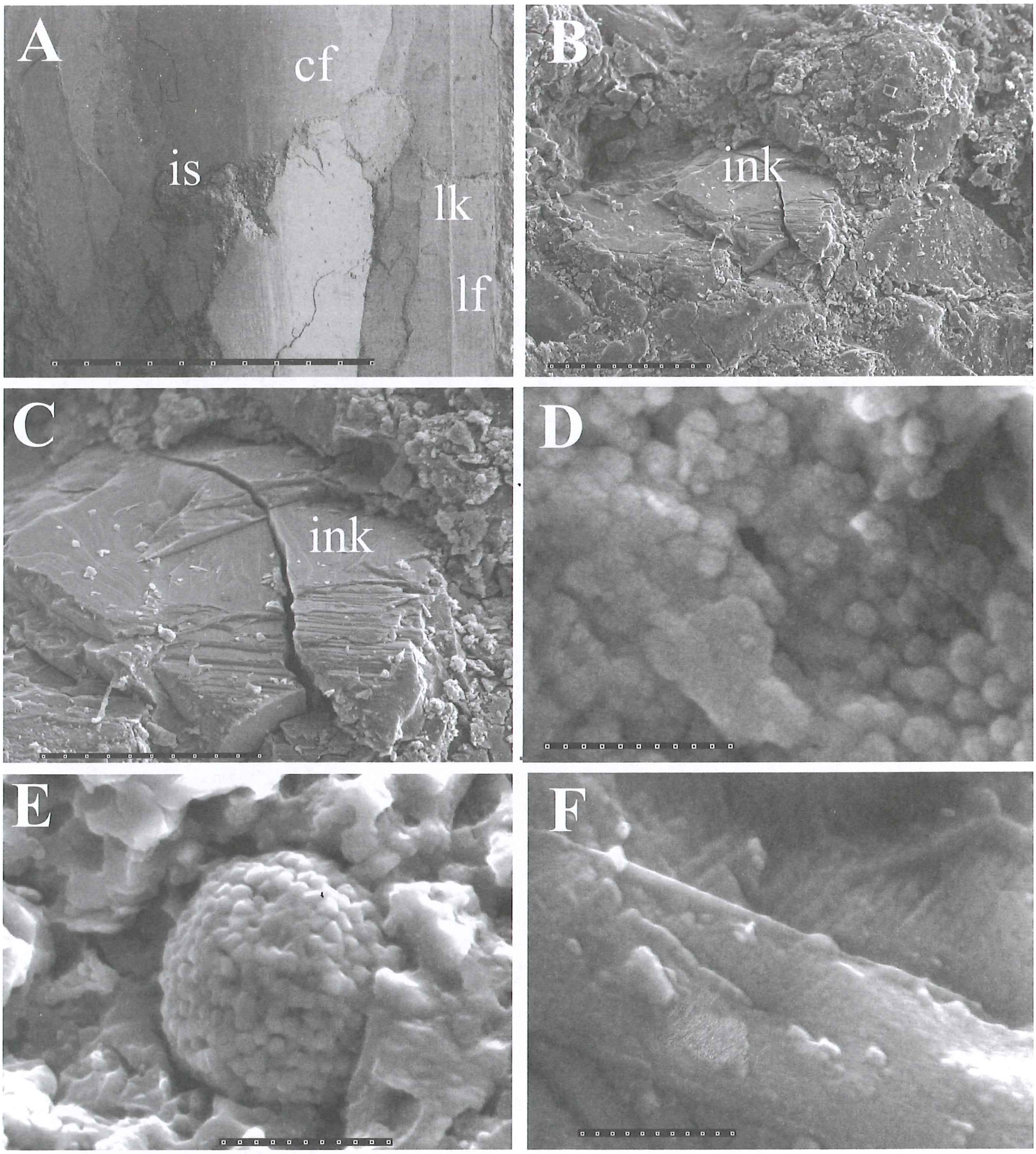


Plate 3. A–F. ***Nesisoteuthis simbirskensis*** **(no. PIN 3871/391). A. Fractured gladius above the ink sac (is); scale bar is 3 mm. B. Organic debris within the ink sac; scale bar is 60 µm. C. Fossil ink in low magnification; scale bar is 30 µm. D. Globular structure of the fossil ink in higher magnification; scale bar is 0.6 µm. E. Colony of micro-organisms in ink sac; scale bar is 3 µm. F. Undetermined fragments of distinct bands with fibreous structure in ink sac; scale bar is 1.2 µm.**

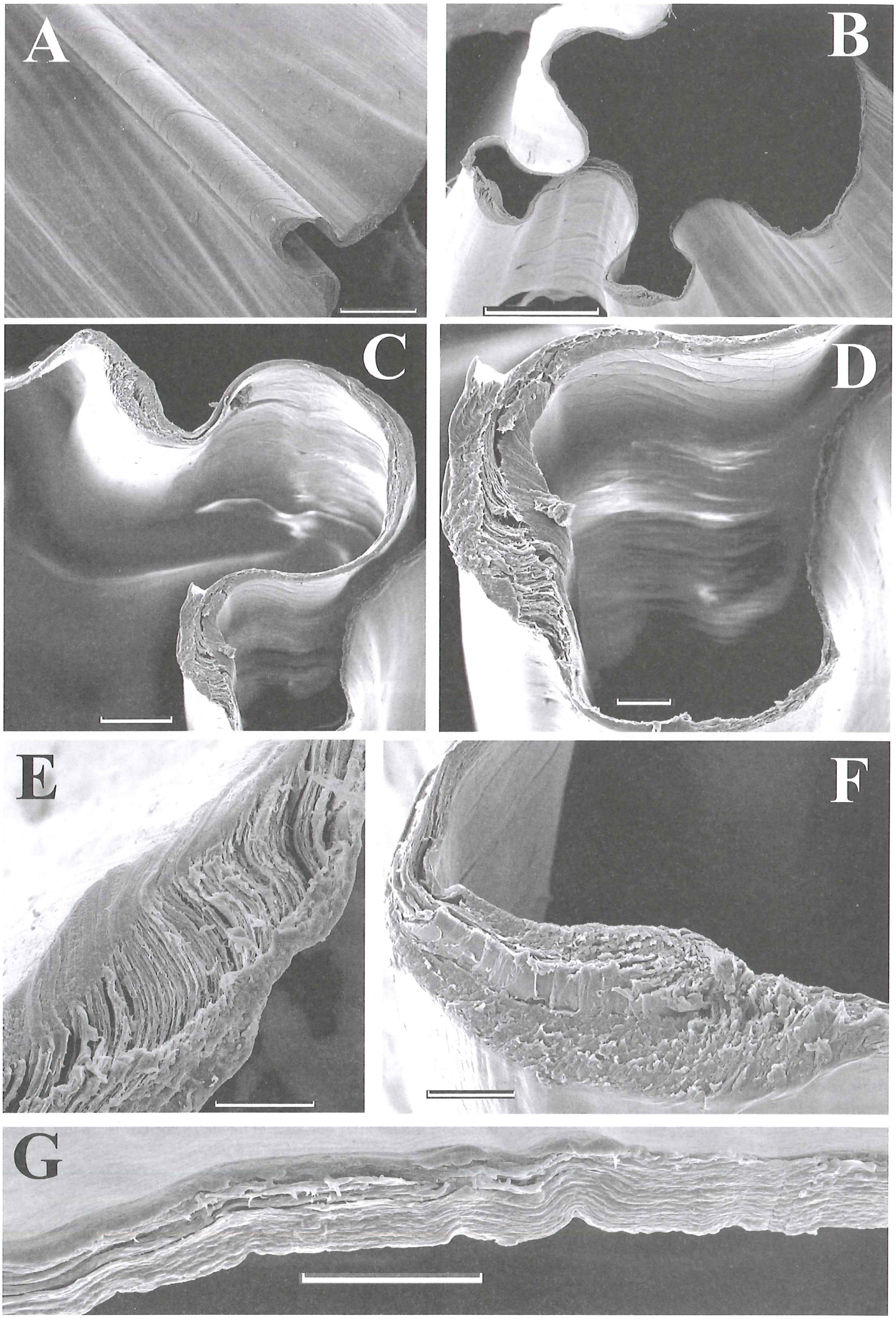
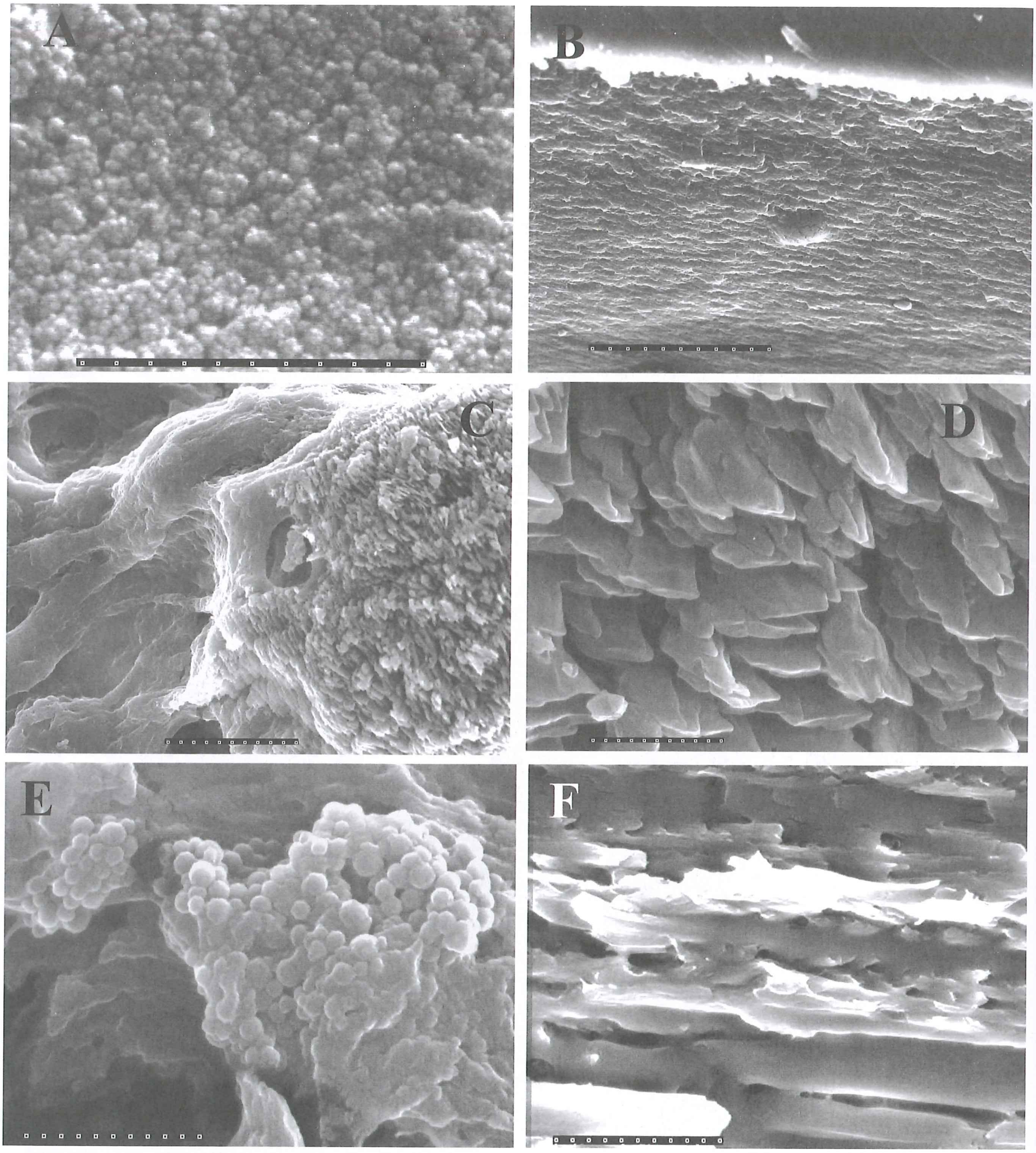


Plate 4. A–G. Gladius ultrastructure in the modern squid *Berryteuthis magister*. A. Surface view of the gladius; scale bar is 1 mm. B. Cross section of the gladius; scale bar is 1 mm. C. Thickened portions of the gladius along the crest of the mid dorsal rib and lateral ribs; scale bar is 300 µm. D. Thickening of the gladius in higher magnification; scale bar is 100 µm. E, F. Close ups of lateral thickenings in Fig. C; scale bar is 100 µm. G. Cross section of the gladius; scale bar is 100 µm.



Palte 5. A–F. Globular ultrastructure of Lower Aptian pelagic fish scale; scale bar is 0.6 µm. B. *Loligo sp.*, fractured gladius showing micro-laminated ultrastructure; scale bar is 30 µm. C. *Loligo sp.*, soft tissues on the left side and gladius on the right side after one year of drying; scale bar is 15 µm. D. detail of 5C to show multi-plate structure of the chitinous laminae of the gladius; scale bar is 1.5 µm. E. *Loligo sp.*, globular bacteria within the soft tissues after one year of drying; scale bar is 1.5 µm. F. *Loligo sp.*, ultrastructure of the gladius showing alternating solid and porous laminae; scale bar is 6 µm.

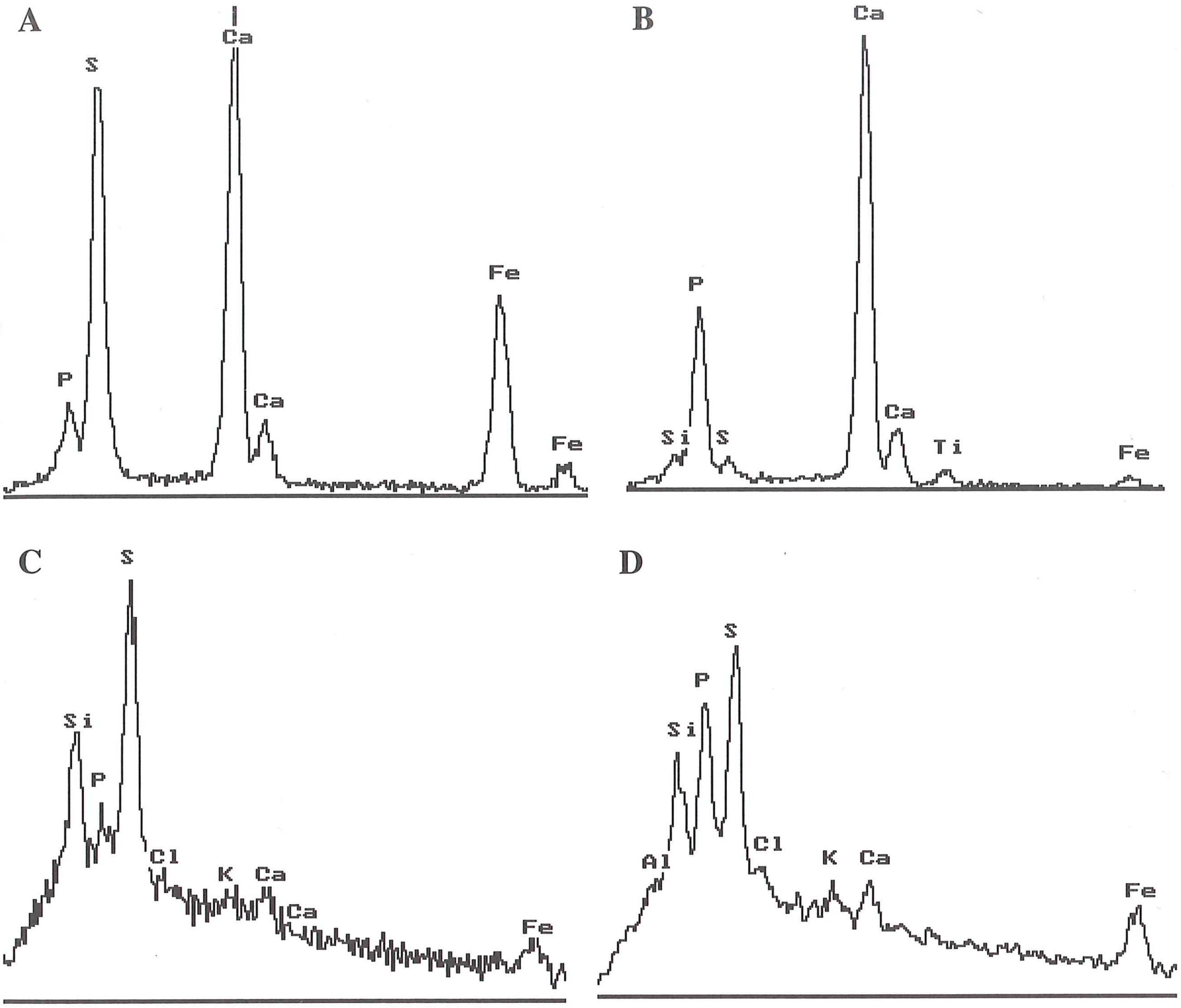


Plate 6. A–D. Elemental composition of an unnamed Lower Aptian gladius (Hecker & Hecker, 1955), Shilovka village, Middle Volga, Central Russia. C–D. Elemental composition of gladius in living squid *Berryteuthis magister.*