Rivista Italiana di Paleontologia e Stratigrafia	volume 119	no. 3	3 pls.	pp. 387-399	November 2013

THE SHELL ULTRASTRUCTURE OF THE GENUS *GLYCYMERIS* DA COSTA, 1778: A COMPARISON BETWEEN FOSSIL AND RECENT SPECIMENS

GAIA CRIPPA

Received: March 5, 2013; accepted: August 26, 2013

Key words: Bivalve biomineralization, Crossed lamellar fabric, Tubules, Arda River, Lower Pleistocene.

Abstract. New data about the shell ultrastructure of species of the genus *Glycymeris* are obtained through a comparison between the fabric of recent specimens from Brittany (France) and fossil specimens collected from the Lower Pleistocene Castell'Arquato Formation cropping out along the Arda River in Western Emilia (Italy). This comparison, made using Scanning Electron Microscope (SEM), results in a strong similarity between the two fabrics, highlighting the good preservation of fossil ones. Both fossil and recent specimens show a well preserved outer simple crossed lamellar layer and an inner irregular and cone complex crossed lamellar layer. The inner and outer layers are separated by an irregular simple prismatic pallial myostracum. These mineralized layers are penetrated by parallel, not ramified and not bifurcated cylindrical tubules, which represent a peculiar character of the Arcoida shells. This analysis provides a more complete picture of *Glycymeris* shell ultrastructure. It shows that *Glycymeris* shell fabric has not changed for the last 2 million years and that the fossil specimens are pristine. Furthermore new data on the pattern and origin of tubules are reported, allowing to conclude that it is unlikely that they have a deterrence function for boring organisms. They may instead function to increase the volume of the organic content of the shell at lower metabolic cost without increasing the shell surface.

Riassunto. In questo lavoro vengono forniti nuovi dati sull'ultrastruttura di alcune specie appartenenti al genere *Glycymeris*, paragonando il fabric della conchiglia di specie fossili provenienti dalla Formazione di Castell'Arquato del Pleistocene inferiore affiorante lungo il Torrente Arda in Emilia Occidentale (Italia) con quello di una specie recente proveniente dalla Bretagna (Francia); questo confronto, effettuato utilizzando il Microscopio Elettronico a Scansione (SEM), risulta in una forte somiglianza tra le due ultrastrutture e mette in evidenza l'eccellente preservazione del fabric degli esemplari fossili.

Sia gli esemplari fossili che quelli recenti presentano un livello esterno a lamelle incrociate semplici e un livello interno a lamelle incrociate complesse coniche o irregolari, separati da un miostraco palleale composto da prismi semplici irregolari. I livelli mineralizzati sono attraversati da tubuli cilindrici paralleli, che non si ramificano e non si biforcano e che rappresentano un carattere distintivo dell'ordine Arcoida. Questa analisi fornisce un quadro più completo dell'ultrastruttura del guscio di specie appartenenti al genere *Glycymeris* e mette in evidenza che il fabric della conchiglia non è cambiato negli ultimi 2 milioni di anni e che il guscio degli esemplari fossili è quello originale. Inoltre vengono mostrati nuovi dati sul fabric e sull'origine dei tubuli: la loro funzione come deterrente contro gli organismi perforatori risulta improbabile; potrebbero invece avere la funzione di incrementare il volume della materia organica nel guscio ad un basso costo metabolico senza dover aumentare la superficie dello stesso.

Introduction

Species belonging to the genus *Glycymeris* Da Costa, 1778, make their first appearance in the Lower Cretaceous and have a rich fossil record (Marwick 1923; Squires 2010). They are widespread and common in fossil faunas and are also abundant in present seas and oceans, being absent from polar, deep-sea regions and brackish water (Thomas 1975). They are free-living inhabitants, often found lying on the surface of the seabed or slightly buried in sandy and gravel substrates with strong bottom currents (up to 100 m of depth) (Stanley 1970; Thomas 1975).

Species belonging to the genus *Glycymeris* Da Costa, 1778 are potential tools in several contexts: palaeoecology, palaeoclimatology, sclerochronology, biology and archaeomalacology are only some of the possible applications of these thick shelled bivalves.

In recent years bivalve shells have been successfully used in palaeoenvironmental reconstruction of

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Dipartimento di Scienze della Terra "Ardito Desio", Università degli Studi di Milano, Via Mangiagalli 34, 20133 Milano, Italy.
E-mail: gaia.crippa@unimi.it

seawater temperature records (Schöne et al. 2011), phytoplankton dynamics (Thébault et al. 2009) and climatic oscillations (Schöne et al. 2003; Carré et al. 2005). In fact bivalves, as brachiopods and planktonic foraminifera, are known to record in their calcium carbonate shells the primary seawater isotope composition with little or no vital effect in their shell layers (Epstein et al. 1953; Erez & Luz 1983; Weiner & Dove 2003; Lecuyer et al. 2004).

Furthermore, bivalves grow episodically, forming incremental growth lines and providing sequential growth increments that can be interpreted as a chronological record, which can be analyzed and used as a geochemical proxy for environmental conditions operating during the growth of mollusk shells (e.g. Hickson et al. 1999; Goodwin et al. 2001; Schöne et al. 2005a; Hallmann et al. 2009; Johnson et al. 2009; Ivany & Runnegar 2010; Royer et al. 2013; Schöne & Gillikin 2013). Molluscs are very useful for these kinds of analyses because they have a widespread distribution across a wide range of climatic zones (polar, temperate and tropical) and environments (estuaries, coastal bays, continental shelves, hydrothermal vents, etc.). The long-lived, cold climate *Arctica islandica* (Linnaeus, 1767) is the most used bivalve for these studies (e.g. Schöne et al. 2005b; Scourse et al. 2006; Butler et al. 2009; Dunca et al. 2009; Schöne & Fiebig 2009); fewer investigations have been carried out with *Glycymeris* shells (Lecuyer et al. 2012; Brocas et al. 2013; Royer et al. 2013). As *Arctica*, *Glycymeris* has a very long life-span of as much as 21-100 years and could reach up to ~200 years (Brocas et al. 2013). Furthermore it has very thick shells and distinct growth lines making this genus a very good tool for geochemical and isotopic analyses. According to Brocas et al. (2013) *Glycymeris glycymeris* (Linnaeus, 1758) is less dependent than *Arctica islandica* on burrowing into the substrate, and it is therefore more directly exposed to the properties of the surrounding water column; thus resulting more climatically sensitive to the seawater variations. Despite this, both *Arctica* and *Glycymeris* are useful tools in palaeoclimatic reconstructions; the habitat preference of *G. glycymeris* complements that of *A. islandica*, and therefore "dog cockles" could be considered a potential temperate equivalent to cold climate *Arctica islandica* shells (Scourse et al. 2006; Brocas et al. 2013).

Valves of species of *Glycymeris* have also been found in different Prehistoric sites (Parks-Barrett 2001; Dimitrijevic & Tripkovic 2006); they were shaped to create shell artefacts, such as bracelets, pendants, rings or other ornaments (Bayman 2002), but were also designed to other purposes, such as the substrata foundations of pavements and house floors in littoral settlements, or as eye applications on the famous statuette of Ur (Ronen 1980; Bar-Yosef 1991, 2002; Sivan et al. 2006). Another interesting application of *Glycymeris* shells is to use them to test the shell damage linked to fishing gears (e.g. trawls or dredges) in order to differentiate between severely impacted and lightly fished areas of the sea bed (Ramsay et al. 2000).

The study of the shell ultrastructure under scanning electron microscope (SEM), besides providing important information about the shell fabric, represents a fundamental step before doing geochemical and isotopic analyses. It is very important to test the preservation of fossil shell ultrastructure in order to exclude phenommena of diagenetic alteration which could modify the results of the geochemical and isotopic analyses, thus yielding erroneous data.

Although they are so useful, the shell ultrastructure of the genus *Glycymeris* has been poorly studied and described. Waller (1980) in his work on the shell and mantle ultrastructure of the order Arcoida described some of the characters of *Glycymeris* shells. Carter (1990) briefly analyzed the ultrastucture of some *Glycymeris* species, and so did Rogalla & Amler (2007) with *Glycymeris glycymeris*. Shibata (1976, 1979), Waller (1980) and Reindl & Haszprunar (1996) examined the function and the structure of the characteristic tubules that penetrate all the shell layers of Arcoida, but little is said about *Glycymeris* shell ultrastructure.

The aim of this paper is thus to describe and figure the shell ultrastructure of four fossil species of *Glycymeris*, collected in the Lower Pleistocene Castell'Arquato Formation, Northern Italy, and to compare it with the shell ultrastructure of one recent species collected in Brittany, France, in order to give a more complete picture of their shell ultrastructure and thus to complement data from previous works.

Material and methods

The fossil specimens here analyzed belong to the species *Glycymeris glycymeris* [MPUM 11202 (ACG14-4), MPUM 11203 (ACG14-11), MPUM 11205 (ACG27bis-8)], *Glycymeris insubrica* (Brocchi, 1814) [MPUM 11202 (ACG9-2), MPUM 11205 (ACG31-1), MPUM 11206 (ACG49-1), MPUM 11207 (ACG53-16), MPUM 11206 (ACG59-1)], *Glycymeris inflata* (Brocchi, 1814) [MPUM 11204 (ACG29bis-1)] and *Glycymeris* sp. [MPUM 11202 (ACG24-4), MPUM 11208 (ACG76-25)]. They were collected from the Lower Pleistocene Castell'Arquato Formation cropping out along the Arda River, in the town of Castell'Arquato, Western Emilia, Northern Italy (Fig. 1A). The Arda River marine succession is about 240 m-thick and it is composed mainly of sandstone, siltstone and claystone with rich fossiliferous levels. The fossil species of *Glycymeris* sampled in the Arda River mainly comprise disarticulated valves although occasionally there are some articulated specimens. They were collected in sandstones and siltstones with a variable amount of clay in the first basal 100 metres. They show an excellent preservation, sometimes preserving the original color pattern and the organic external ligament.

The ultrastructure of these fossil specimens was compared with that of two recent specimens of *Glycymeris glycymeris* [MPUM 11202 (AG1), MPUM 11202 (AG2)] collected in a shore shell accumulation of

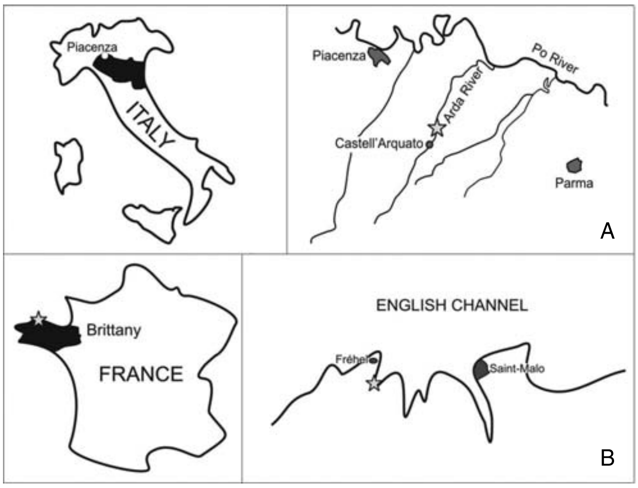


Fig. 1 - A) Sketch map with the position of the fossil samples collected along the Lower Pleistocene Castell'Arquato Formation cropping out along the Arda River, Western Emilia, Italy. B) Sketch map with the location of the recent samples collected along the Northern Coast of Brittany, France.

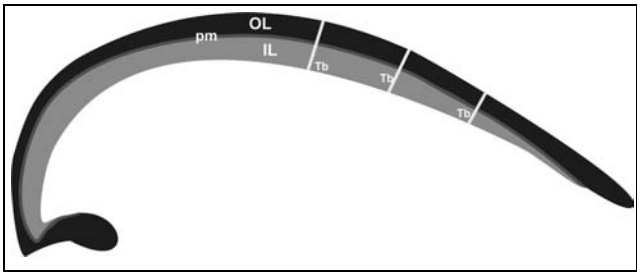


Fig. 2 - Longitudinal section of the shell of the genus *Glycymeris* showing the mineralized layers. OL: outer layer; IL: inner layer; pm: pallial myostracum; Tb: tubules.

articulated and disarticulated specimens piled up by tide currents at Baie de la Fresnaye, near the towns of Fréhel and Pléboulle in Brittany, France (Fig. 1B).

The fossil specimens were prepared and cleaned from the sediment using a small air drill, in case of hard sediment, or a scalpel, in case of soft sediment. After that, the length and the width of both recent and fossil specimens were measured using a caliper (to the nearest 0.1 mm) and at each of them has been assigned a unique ID to identify it.

To prepare specimens for the Scanning Electron Microscope (SEM) analyses shells were cut longitudinally along the axis of maximum growth (the axis perpendicular to the growth lines) using a diamond saw; the sections obtained were embedded in an epoxidic resin, forming blocks which contain several sections of different shells; every block was polished, ground smoothed and etched with 5% chloridric acid for 15-20 seconds in order to reveal the details of the ultrastructures; finally the block was coated with gold and observed at SEM Cambridge S-360 with lanthanum hexaboride (LaB6) cathodes.

Shell ultrastructure

Fossil and recent specimens

Species of *Glycymeris* have an aragonitic shell with an outer crossed lamellar layer, an inner irregular and cone complex crossed lamellar layer and an irregular simple prismatic pallial myostracum. All the mineralized shell layers are penetrated by cylindrical tubules, which are an important character of the ultrastructure of the order Arcoida.

The outer layer is composed by simple crossed lamellae and occupies the whole outer part of the shell, under the organic periostracum, from the marginal band to the hinge plate, defining a sort of "zebra pattern" (Pl. 1, figs B, C, G). In particular the hinge plate and the marginal band, which is the growth surface between the pallial line and the outer shell margin (Waller 1980), are composed entirely by simple crossed lamellae. The thickness of the outer layer shows an identical trend among different specimens: the maximum thickness is in the marginal band, then it begins to decrease starting from the pallial line, reaches its minimum dorsally in correspondence with the umbonal curvature and returns to be prevalent in the hinge plate (Fig. 2). The hierarchical organization of the lamellae is here easily discernible. First order lamellae are extremely variable in thickness (5-30 μm) and appear as a series of linear to branching oriented lenses, which could also curved (Pl. 1, fig. C); they are clearly observable at SEM thanks to the alternated brightness – linked to differences in electron scattering (Tschudin 2001) – of the adjacent first order lamellae, which are inclined in two opposite directions (Pl. 1, figs C-H). The first order lenses show a double pattern in the outer layer (Pl. 1, figs B, C, G): 1) in the internal part they are linear and parallel to each other, 2) in the external part they are irregular, almost parallel to the outer surface and with a better contrast compared to the adjacent lamellae in the inner part and, linear and parallel to each other in the outer part. The third order lamellae are usually 0.3-1 μm thick and sometimes the fourth order granules that form the third order elements are observable in well preserved fossil specimens and in the recent ones (Pl. 1, fig. H). They are very similar to the fourth order elements figured by Dauphin & Denis (2000, fig. 2J); in contrast the second order lamellae are not always well defined.

The inner layer is present only internally with respect to the pallial line. It is delimited by the pallial myostracum and it does not extend inside the hinge plate (Fig. 2). As for the outer layer, also for the inner shell layer a typical thickness trend is recognized among the different specimens: it starts dorsally to the pallial line and increases its thickness towards the umbonal region, where it disappears abruptly (Fig. 2). The inner layer is composed by complex crossed lamellae, both irregular and cone ones (Pl. 2, figs A-E). The cone complex crossed lamellae consist of an aggregation of elongate rectangular rods of aragonite which radiate from the central part of the cone (Kobayashi & Samata 2006) (Pl. 2, figs B, D), as it has been observed also by Tschudin (2001, fig. 3) in SEM sections. The distinct lamellar hierarchy of the outer layer is not so clear. The inner layer is less organized and more irregular in relation to the outer layer. Only the third order elements are evident, showing a similar thickness to those of the outer layer (Pl. 2, figs A-C, E).

The outer and inner layers are separated by a pallial myostracum which is formed by a stockade of parallel and elongated irregular simple prisms. It starts in correspondence with the pallial line and finishes abruptly in the internal margin of the hinge plate (Fig. 2; Pl. 2, figs F-H). The thickness of the pallial myostracum varies among the specimens. It is clearly distinguishable and it cannot be confused with inner layer growth lines, which are usually thinner. Furthermore the pallial myostracum identifies the boundary between the inner and outer layers, which are easily recognizable at SEM because usually the inner layer shows a darker coloration than the outer layer (Pl. 1, figs A, B; Pl. 2, fig. F).

Growth lines cross both the inner and outer layers with a different orientation and a different grade of discrimination among the lamellae and the two layers. In fact in the inner layer they are more distinct than in the outer layer; they are parallel to the inner and outer shell surfaces (Pl. 1, fig. A), following the curvature of the shell and consist of parallel and elongated irregular simple prisms (Pl. 2, figs C, E). In the outer shell layer the growth lines are evident mainly in the marginal band and in the hinge plate, whereas in other parts of the shell they are not so easy to distinguish. In the marginal band they are slightly inclined to the outer surface, then they curve and become nearly parallel to the outer surface (Pl. 1, fig. G; Pl. 3, fig. C). In the hinge plate they are concentric and parallel to the direction of growth of the hinge plate itself (Pl. 3, fig. B). They seem to be composed by an elongation of the third order rods (Pl. 1, fig. D). Growth lines on the outer surface of the shell appear as concentric striations, which correspond internally to the outer layer growth lines (Pl. 1, figs D, G; Pl. 3, fig. C).

An irregular prismatic layer is present in the inner part of the hinge plate of the best preserved specimens. It is composed by elongated and parallel simple prisms perpendicular to the inner surface of the shell with prism widths of 0.5-3 μm and it appears as a prolongation of the irregular prismatic pallial myostracum in the hinge plate. It represents the attachment site for the pedal retractor muscles (Pl. 3, figs. A, B). As pointed out by Taylor et al. (1969), the irregular prismatic shell fabric commonly associated with muscle-attachment areas is found in a variety of bivalve shells.

The comparative analysis of fossil (Pl. 1, figs A-C, E, F; Pl. 2, figs A, B, D, F-H; Pl. 3, figs A-E, G) and recent (Pl. 1, figs D, G, H; Pl. 2, figs C, E; Pl. 3, figs F, H) shell ultrastructures of several species of *Glycymeris* shows a strong similarity between the two fabrics. No important differences have been noticed, except the obvious fact that the recent specimens show a better defined and clear fabric.

Tubules

Both fossil and recent species of *Glycymeris* here analyzed show a densely perforated structure (Pl. 1, fig. A; Pl. 2, figs B, F; Pl. 3, figs C-H). Tubules penetrate all the shell layers: no data is available on their relationship to the periostracum as the latter is not preserved in the studied specimens. In the examined material, tubules do not appear as empty perforations but they are filled seemingly by the resin used for the specimens preparation (Pl. 2, fig. B; Pl. 3, figs C-H).

The distribution of tubules is restricted to the part of the shell characterized by the occurrence of the inner layer, that is from the pallial line to the umbonal region

PLATE 1

Scanning electron microscope images showing the ultrastructure of shells of species of *Glycymeris*; IL: inner layer; GL: growth lines; OL: outer layer; OS: outer shell surface; Pm: pallial myostracum; Tb: tubules.

A) Shell section showing the outer layer, the pallial myostracum and the inner layer with its growth lines; note the darker coloration of the inner layer with respect to the outer layer. Fossil specimen of *Glycymeris glycymeris* [MPUM 11205 (ACG27bis-8)].

B) Shell section of the ventral part of the valve showing the outer layer with the external "zebra pattern", the pallial myostracum and the inner layer, which is gradually disappearing near the pallial line; also note that here some tubules are not perpendicular to the outer surface but they are inclined and protrude in the marginal band gradually decreasing their length and disappearing without reaching the outer surface. Fossil specimen of *Glycymeris inflata* [MPUM 11204 (ACG29bis-1)].

C) Double pattern of the first order lamellae of the outer layer: irregular, branching and with a better contrast compared to the adjacent lamellae in the external part and linear and parallel to each other in the internal part. Fossil specimen of *Glycymeris glycymeris* [MPUM 11205 (ACG27bis-8)].

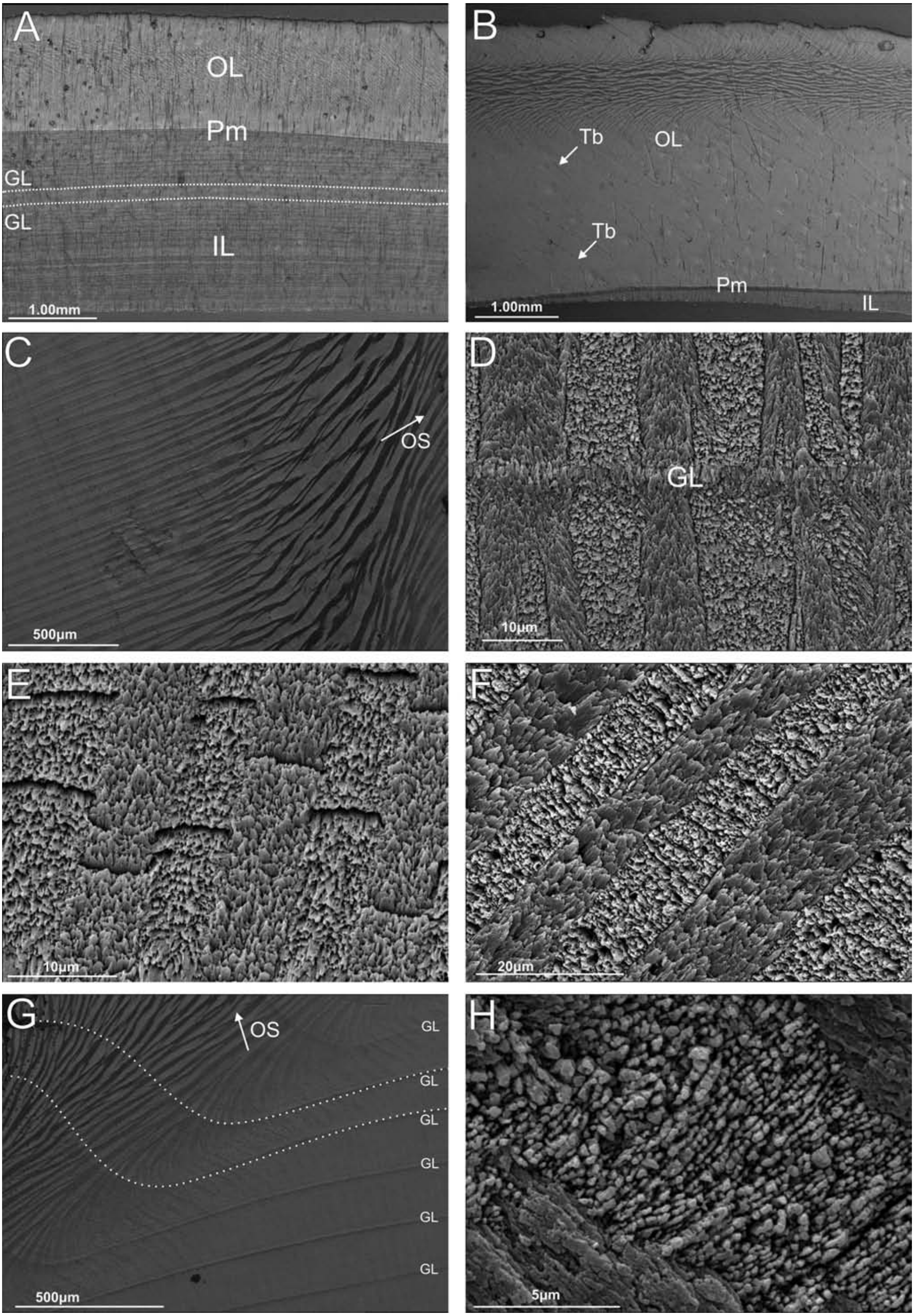
D) First order lamellae of the outer layer crossed by growth lines, evident because of the different brightness. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG1)].

E) Well preserved first order lamellae of the outer layer; note the empty spaces between the lamellae, probably occupied in alive specimen by the organic matrix. Fossil specimen of *Glycymeris insubrica* [MPUM 11202 (ACG9-2)].

F) Well preserved crossed lamellae of the outer layer: first, second and third order elements are clearly observable. Fossil specimen of *Glycymeris glycymeris* [MPUM 11202 (ACG14-4)].

G) Double pattern of the outer layer with branching to subparallel first order lamellae, crossed by growth lines. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG1)].

H) Detail of a first order lamella showing the fourth order granules that compose the third order rods. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG1)].


A
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Pm
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1.00mm
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up to the point where the inner layer suddenly disappears. No tubule has been found in the marginal band or in the hinge plate. In both the inner and outer layers they cut the crossed lamellar fabric pointing out that they are secondary formed (Pl. 2, figs B, F; Pl. 3, figs C-H). Tubules diameter varies among the different specimens but within the same specimen they have more or less the same size, ranging between 2 and 10 µm in agreement with the ranges reported by Waller (1980).

Tubules are mostly parallel to each other and perpendicular to the outer and inner shell surfaces with no intersection between them and no bifurcation (Pl. 1, fig. A; Pl. 2, fig. F; Pl. 3, fig. C). They perforate all the mineralized layers in a straight line without changing their direction at the passage between the inner and outer layers (Pl. 2, fig. F). The end of tubule, penetrating the outermost shell layer, flares at its distal opening (Pl. 3, fig. F). In a few instances there is a change of their direction but only in correspondence with the pallial line or in the umbonal region where the inner layer disappears. Here tubules are inclined and protrude in the marginal band and in the hinge plate with an angle of 60-65 degrees; in these regions (end of the inner layer in the pallial line and in the hinge plate) both inclined and straight tubules gradually decrease their length disappearing without reaching the outer surface (Pl. 1, fig. B; Pl. 3, fig. C).

Discussion

Fossil and recent specimens at SEM share the same ultrastructure, with an outer crossed lamellar layer, an inner irregular and cone complex crossed lamellar layer and an irregular simple prismatic pallial myostracum (Fig. 2; Pl. 1, figs. A, B), as described by Carter (1990) in species of the genus *Glycymeris*.

In contrast, Popov (1992) and Rogalla & Amler (2007) subdivided the outer layer of Carter (1990) into a middle and an outer layer, thus describing three layers in addition to the pallial myostracum. However, their subdivision mainly results from a change in the pattern of the first order elements in the simple crossed lamellae rather than from the occurrence of two real distinct layers. In fact the middle and outer layers of Popov (1992) and Rogalla & Amler (2007) correspond respectively to pattern 1 and pattern 2 of the outer layer described in the paragraph on shell ultrastructure (Pl. 1, figs B, C, G). For these reasons, the subdivision of Carter (1990) in two layers in addition to the pallial myostracum is preferred and is followed in this paper.

Growth lines, in both recent and fossil specimens are evident mainly in the inner layer and in the outer layer of the hinge plate and of the marginal band, whereas in other parts of the shell they are not clearly observable at SEM. They are important characters of *Glycymeris* shells that need to be identified also with other methods (see next paragraph). A study of oxygen isotope composition of aragonitic shells of *Glycymeris glycymeris* by Berthou et al. (1986) and Brocas et al. (2013) demonstrated that these growth lines are formed annually, with their boundaries corresponding to winter shell growth cessation.

An important consequence of the strong similarity between fossil and recent fabrics is that the ultrastructure of the shells of the species of the genus *Glycymeris* has not changed for over 2 million years. These species are particularly conservative in their morphological diversity and this conservative character is seen not only in their morphology but also in their shell ultrastructure.

The analysis of *Glycymeris* shell ultrastructure have interesting implications also on the debated position of the family Glycymerididae Dall, 1908, being part of the superfamily Arcoidea Lamarck, 1809 or of the superfamily Limopsoidea Dall, 1895. Newell (1969), includes the Glycymerididae in the Limopsoidea near the family Limopsidae Dall, 1895, based on the rounded shell form and on the weak ornamentation. However, the two families differ by several morphological char-

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PLATE 2

Scanning electron microscope images showing the ultrastructure of shells of species of *Glycymeris*; IL: inner layer; IS: inner shell surface; GL: growth lines; OL: outer layer; Pm: pallial myostracum; Tb: tubules.

A) Irregular complex crossed lamellar inner layer with third order rods inclined in different direction. Fossil specimen of *Glycymeris inflata* [MPUM 11204 (ACG29bis-1)].

B) Cone complex crossed lamellar inner layer cut by a tubule. Fossil specimen of *Glycymeris insubrica* [MPUM 11206 (ACG49-1)].

C) Irregular complex crossed lamellar inner layer crossed by growth lines made of irregular simple prisms. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG2)].

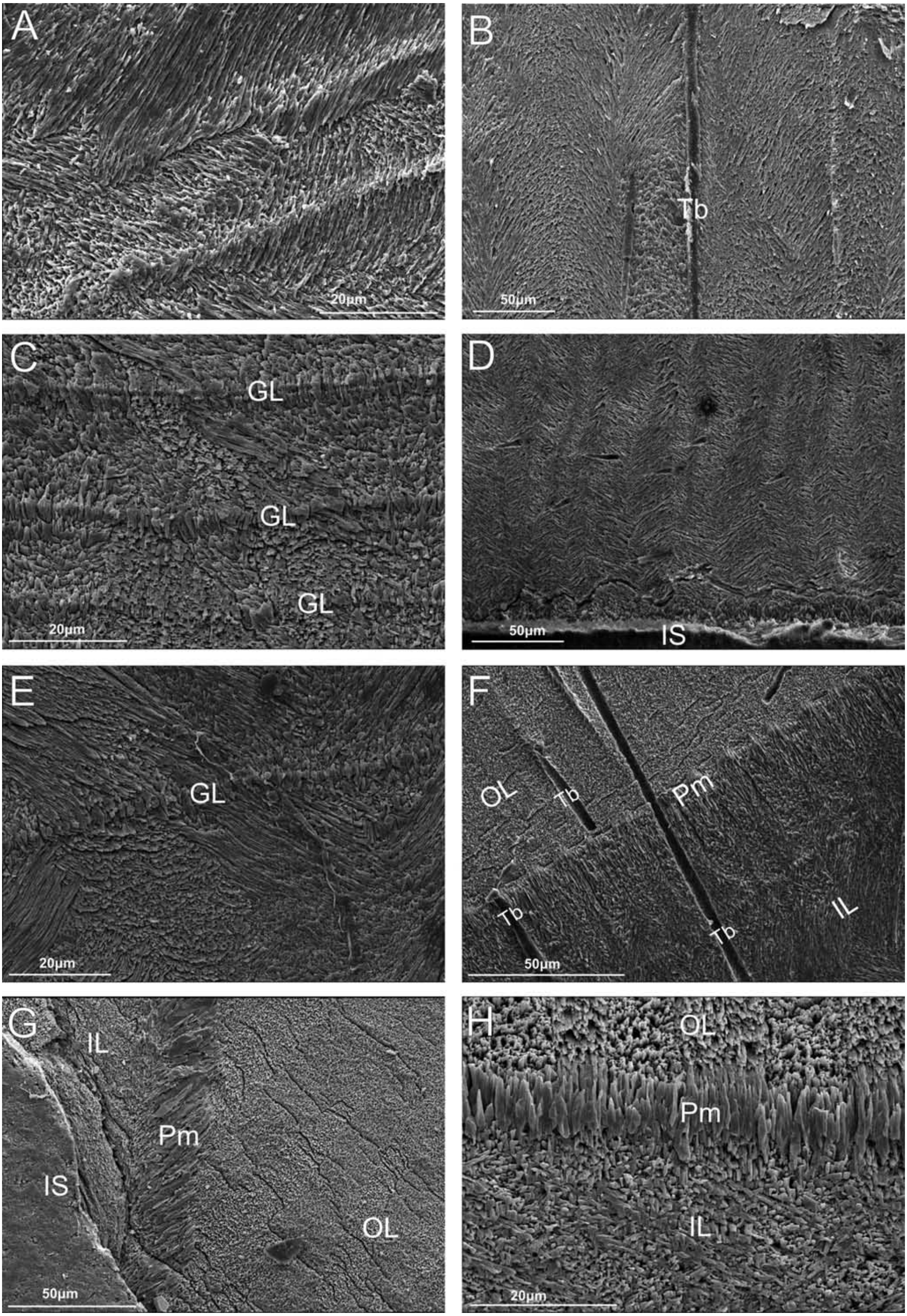
D) Cone complex crossed lamellar inner layer; third order elements radiate from the cone apices. Fossil specimen of *Glycymeris glycymeris* [MPUM 11203 (ACG14-11)].

E) Irregular complex crossed lamellar inner layer crossed by irregular prismatic growth lines; prisms of growth lines are made by third rods prolongation. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG2)].

F) Sharp contact between outer and inner layers represented by the prismatic pallial myostracum; note that tubules cross linearly all the mineralized layers cutting the fabric. Fossil specimen of *Glycymeris glycymeris* [MPUM 11205 (ACG27bis-8)].

G) Prismatic pallial myostracum disappearing in the inner part of the hinge plate. Fossil specimen of *Glycymeris glycymeris* [MPUM 11205 (ACG27bis-8)].

H) Prismatic pallial myostracum; note that the pallial myostracum is usually thicker than the inner layer growth lines and cannot be confused with them. Fossil specimen of *Glycymeris insubrica* [MPUM 11206 (ACG59-1)].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acters: the Glycymerididae have a massive form with a duplivincular ligament, lack of a functional byssus and a slightly larger anterior adductor muscle, whereas the Limopsidae are generally small with an alivincular-multivincular ligament, a functional byssus and a larger posterior adductor muscle (Oliver & Holmes 2006). Subsequently, Carter (1990) and Oliver & Holmes (2006) placed the family Glycymerididae in the superfamily Arcoidea near the family Cucullaeidae Stewart, 1930 because of their similar hinge dentition, ligament structure and shell ultrastructure. Both the families are characterized by the lack (or rarity) of crossed-matted/lineated fabric and of an outer prismatic layer in their shell ultrastructure. In the specimens here examined no crossed matted/lineated fabric and no outer prismatic layer have been observed confirming the affinity of the genus *Glycymeris* with the family Cucullaeidae and hence its closer relationship with the superfamily Arcoidea, rather than with the superfamily Limopsoidea.

Tubular structures are known from unmineralized and mineralized shells of molluscs, polyplacophorans, fissurellid gastropods, brachiopods, bryozoans, ostracods and cirripedian crustaceans (e.g. Owen & Williams 1969; Klepal & Barnes 1975; Keyser 1980) and they have also been discovered in Early Cambrian mollusk-like organisms (Kouchinsky 2000). They are a fundamental character in the Arcoida shells (Shibata 1979) and thus of *Glycymeris* ultrastructure. They are present in both fossil and recent specimens but their function is far from being clarified. They do not result from bioerosion: tubules are usually straight and regular, whereas burrows of bioerosion are usually larger in diameter (even if bacteria can produce smaller erosions) and form complex and irregular networks (Shibata 1979); furthermore, according to Reindl & Haszprunar (1996), no boring algae, fungi or bacteria have been found to penetrate the shell.

Tubules are occupied in alive specimen by caeca which are extensions of the outer mantle epithelium composed each by a single, specialized epithelial cell (Reindl & Haszprunar 1996), which is chemically active at its tip, apparently capable of dissolving calcium carbonate in order to perforate the shell (Waller 1980). It thus seems that although the mantle projections that form tubules are able to dissolve calcium carbonate, they cannot completely penetrate the organic material forming the periostracum (Waller 1980).When the shell-dissolving tip of a cellular projection reaches the undersurface of the periostracum, its etching effect continues, as shown by the flared distal extremity of the tubule (Pl. 3, fig. F).

Waller (1980) noticed that the complex fabric of the inner shell layer is secreted around the mantle projection forming the tubule and is not crossed, indicating that in this area the tubules are primary rather than secondary. In the examined specimens tubules cut the fabric also in the inner shell layer suggesting that also in this case they are formed secondarily, after the secretion of the inner layer (Pl. 2, figs B, F; Pl. 3, figs B-F).

The function of the tubules and caeca is still debated, and several are the hypotheses proposed (Lutz & Rhoads 1977; Waller 1980; Reindl & Haszprunar 1996).

The most supported one is that they represent a deterrence for boring organisms and this functional interpretation is strengthened by the taxonomic distribution of tubules. They are common in epifaunal bivalves in many unrelated groups (Oberling 1964) but are uncommon in infaunal taxa (Waller 1980). La Perna (2006) found tubules in the epibyssate juveniles of *Ambrogia mytiloides* (Brocchi, 1814) but they are absent in the endobyssate adults of the same species and this may represent another evidence that shell tubules are actually involved in deterring microborers, as the caeca could remain chemically active during the bivalve life. In the examined specimens some of the tubules seem to extend to the marginal band or to the hinge plate in order to protect through chemical secretion also these regions that cannot otherwise be reached by tubules as

PLATE 3

Scanning electron microscope images showing the ultrastructure of shells of species of *Glycymeris*; ISP: irregular simple prisms; IS: inner shell surface; GL: growth lines; Mb: marginal band; OS: outer shell surface; Tb: tubules.

A) Irregular simple prismatic layer in the inner part of the hinge plate representing the attachment site for pedal retractor muscles. Fossil specimen of *Glycymeris insubrica* [MPUM 11205 (ACG31-1)].

B) Irregular simple prismatic layer in the inner part of the hinge plate; note also the concentric growth lines. Fossil specimen of *Glycymeris* sp. [MPUM 11208 (ACG76-25)].

C) Tubules gradually decrease their length and do not reach the outer surface in the marginal band; note the curve growth lines that are traceable also on the outer surface of the shell. Fossil specimen of *Glycymeris insubrica* [MPUM 11207 (ACG53-16)].

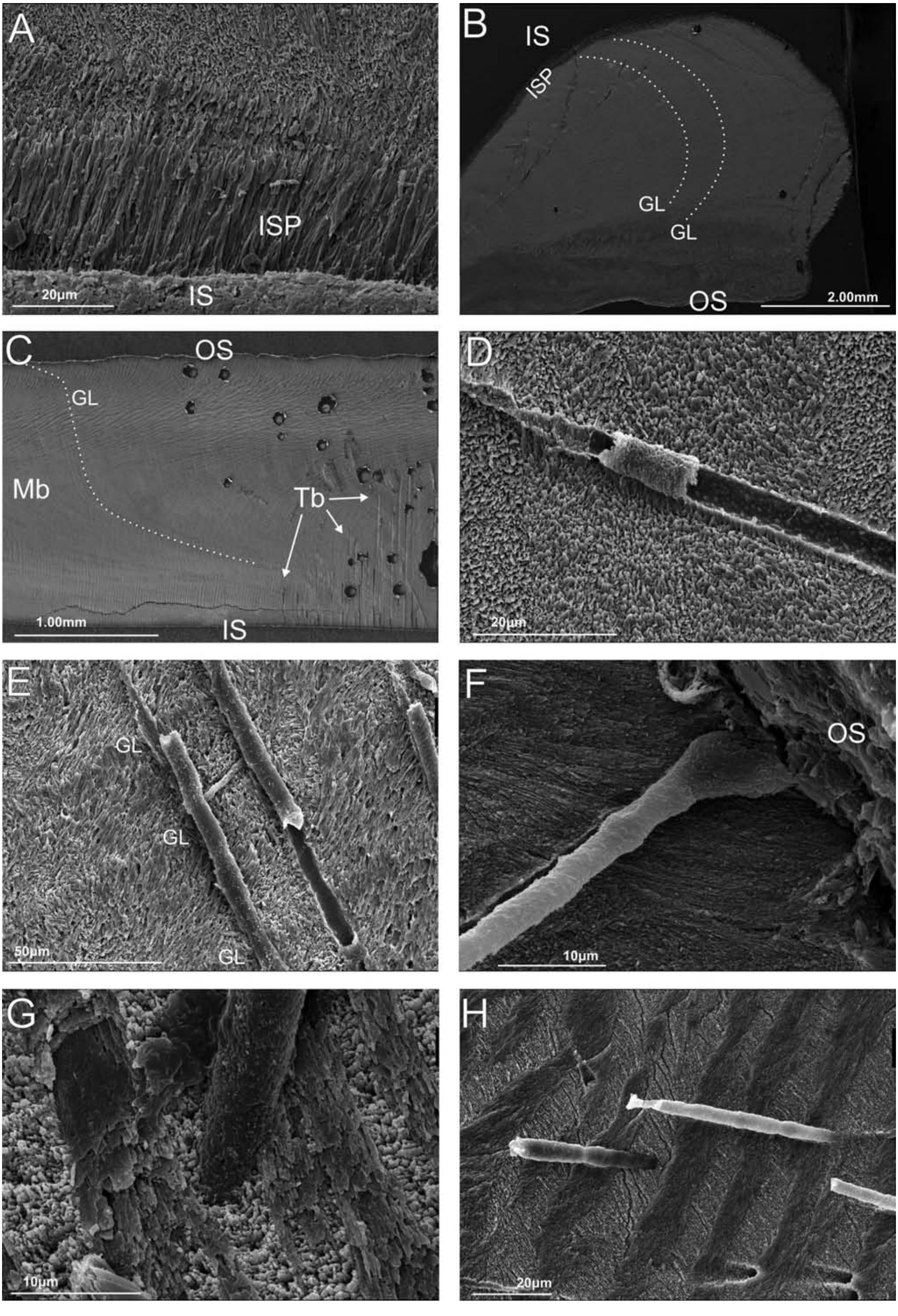
D) Tubule in the outer layer; note that the fabric is cut by the tubule, pointing out that it is secondary formed. Fossil specimen of *Glycymeris* sp. [MPUM 11202 (ACG24-4)].

E) Parallel cylindrical tubules cutting the irregular complex crossed lamellae and the growth lines of the inner layer; the tubule is secondary formed because it cuts the fabric. Fossil specimen of *Glycymeris insubrica* [MPUM 11206 (ACG59-1)].

F) Flared distal end of tubule near the outer surface; note that the tubule flares only in its distal end confirming that the chemical secretion occurs only in the tip of the unicellular mantle extension. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG1)].

G) Tubule cutting the well preserved first and second order lamellae of the outer layer. Fossil specimen of *Glycymeris glycymeris* [MPUM 11203 (ACG14-11)].

H) Cylindrical tubules filled by the epoxidic resin cut the simple crossed lamellar fabric of the outer layer. Recent specimen of *Glycymeris glycymeris* [MPUM 11202 (AG2)].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the mantle does not occur here. In fact the mantle edge at the ventral margin is highly mobile and constantly shifting relative to the shell surface, so that it would be impossible for fine epithelial extensions into the shell to form or to be maintained (Waller 1980). The marginal band and the hinge plate are important zones to protect from boring organisms and if tubules have actually a deterrence function one would expect to find more tubules here than in other regions of the shell and especially to find them perforating also the periostracum. However, in these regions tubules, if present, do not reach the outer surface (Pl. 1, fig. B; Pl. 3, fig. C) and they do not perforate the periostracum. Furthermore the hard crossed lamellar ultrastructure and the thick shell already represent an adequate defense mechanism from boring organisms. These new observations together with those deriving from the literature allow to conclude that a deterrence function for tubules is unlikely.

Tubules are not limited to aragonitic shells (e.g. tubules perforating the calcitic layers of Mytilidae as observed by Carter 1990), but most of them are found in the aragonitic crossed lamellar fabric. The latter has a lower organic content with respect to the other bivalve fabrics. Organic material is very expensive to produce and the shell has to be produced quickly and, metabolically speaking, cheaply (Palmer 1983). Tubules may thus have the function to increase the volume of the organic content in the shell at lower metabolic cost without increasing shell surface.

Conclusion

Species of the genus *Glycymeris* have a widespread distribution both in past and in present seas and are very useful tools in several contexts. The SEM analysis of their ultrastructure represents a very important step to test their preservation, understand their evolutive patterns and also to better comprehend their shell fabric and the origin and function of tubules. The analysis of the shells of several species of *Glycymeris* indicates a strong similarity between fossil and recent fabrics; the outer simple crossed lamellar layer, the inner irregular and cone complex crossed lamellar layer and the irregular simple prismatic pallial myostracum are clearly distinct and nearly identical in both the fossil and recent specimens. As for the growth lines, they are not so easily distinguishable at SEM, for these reasons it is better to support sclerochronological analyses with other methods in order to better underline them, as the use of the Mutvei's solution (Schöne et al. 2005c), the fluorescence (Wanamaker et al. 2009), peels or thin sections.

The present study further underlines the importance of species of the genus *Glycymeris* for palaeoclimatical, palaeoecological and sclerochronological analyses. Fossil and recent shell ultrastructures show the same fabric suggesting that: 1) fossil specimens from the Castell'Arquato Formation of the Arda River (Western Emilia, Italy) are pristine and suitable for geochemical and isotopic analyses; 2) *Glycymeris* ultrastructure has not changed for the last 2 million years; 3) the lack of crossed matted/lineated fabric and of a prismatic outer shell layer confirms the affinity of the genus *Glycymeris* with the family Cucullaeidae and hence their closer relationship to the superfamily Arcoidea; 4) the tubules deterrence function for boring organisms is unlikely. They may instead function to increase the volume of the shell organic content at lower metabolic cost without increasing shell surface.

Acknowledgements. Prof. Lucia Angiolini is deeply thanked for her precious and useful suggestions and comments on the paper. Curzio Malinverno and Agostino Rizzi are thanked for their technical support with specimens preparation and SEM analyses. Prof. Rafael La Perna and an anonymous reviewer are thanked for their suggestions and comments on the paper.

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