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SHELL MICROSTRUCTURES IN LOPINGIAN BRACHIOPODS: IMPLICATIONS FOR FABRIC EVOLUTION AND CALCIFICATION

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*Abstract***.** The study of the shell microstructure of brachiopods is fundamental to understand their evolutionary history and their biomineralization process. Here, species of forty Lopingian brachiopods genera, representative of twenty-seven different families, are investigated using the Scanning Electron Microscope. The investigated specimens come from different paleogeographic localities in the Palaeotethys/Neotethys oceans. The studied brachiopods show a large variability of the shell fabric, which is mainly related to the organization of its structural units: laminae, fibers and columns, possibly crossed by pseudopunctae or punctae. For the Strophomenata, the laminar fabric of Productida is crossed by pseudopunctae with taleolae and the laminae are often organized in packages, with the blades oriented about perpendicular to each other; this feature is less evident in the laminar Orthotetida, which bear pseudopunctae without taleoae. For the Rhynchonellata, fibrous fabrics are either impuctate in the Spiriferida, most Athyridida and Rhynchonellida, or with punctae, as observed in the Orthida, Terebratulida and in the Neoretziidae (Athyridida). The fibers show a range of sizes and shapes also in the same specimens and the transition to the columnar layer is different than in Strophomenata.

The arrangement of the structural units revealed that the disposition of the organic membranes, on which biomineralization took place, was highly variable among the taxa. On the other hand, two distinctive features are analogous among distantly related groups, i.e. the Strophomenata and the Rhynchonellata: the presence of a columnar tertiary layer underlying the secondary fabric and the alternations between fibers/laminae of the secondary layer and columns of the tertiary layer. This suggests that there are common factors controlling the development and evolution of the shell fabric in all rhynchonelliformean brachiopods that can be linked to their taxonomical position, to their environmental requirements and to constraints imposed by their low-energy life-style. This should be taken into account to understand how these calcifying organisms responded and will respond to environmental and climate change in past and future times.

INTRODUCTION

The shell microstructure of calcifying organisms plays a critical role since it can affect parameters such as exoskeleton mechanical proprieties, functionality and metabolic cost (Palmer 1983; Palmer 1992; Schmahl et al. 2012). Due to the growing concern about the effects of anthropogenic CO_2, modern calcifying organisms have been studied to assess how they modify the shell microstructure, in response to global warming and acidification of seawater (e.g. Roger et al. 2012; Cross et al. 2016; Milano et al. 2016). This knowledge is a key step to understand how these organisms can survive in a changing environment. Unfortunately, comparative and experimental studies on modern organisms cannot fully address the evolutionary response on the long-term timescale (Cross et al. 2015). From this perspective, the fossil record of brachiopods provides the best archive to study the evolution of shell microstructure through geological time (Williams & Cusack 2007).

Modern rhynchonelliformean brachiopods display a small number of taxa, which bear multilayered calcite shells with different hierarchical microstructures and mechanical proprieties (Pérez-Huerta et al. 200; Griesshasber et al. 2007; Schmahl et al. 2008; Göetz et al. 2009). However, during the Paleozoic, they have been one of the major calcifying phyla. Two classes of rhynchonelliformean brachiopods dominated the Paleozoic benthic communities (e.g. Curry & Brunton 2007): the Strophomenata,

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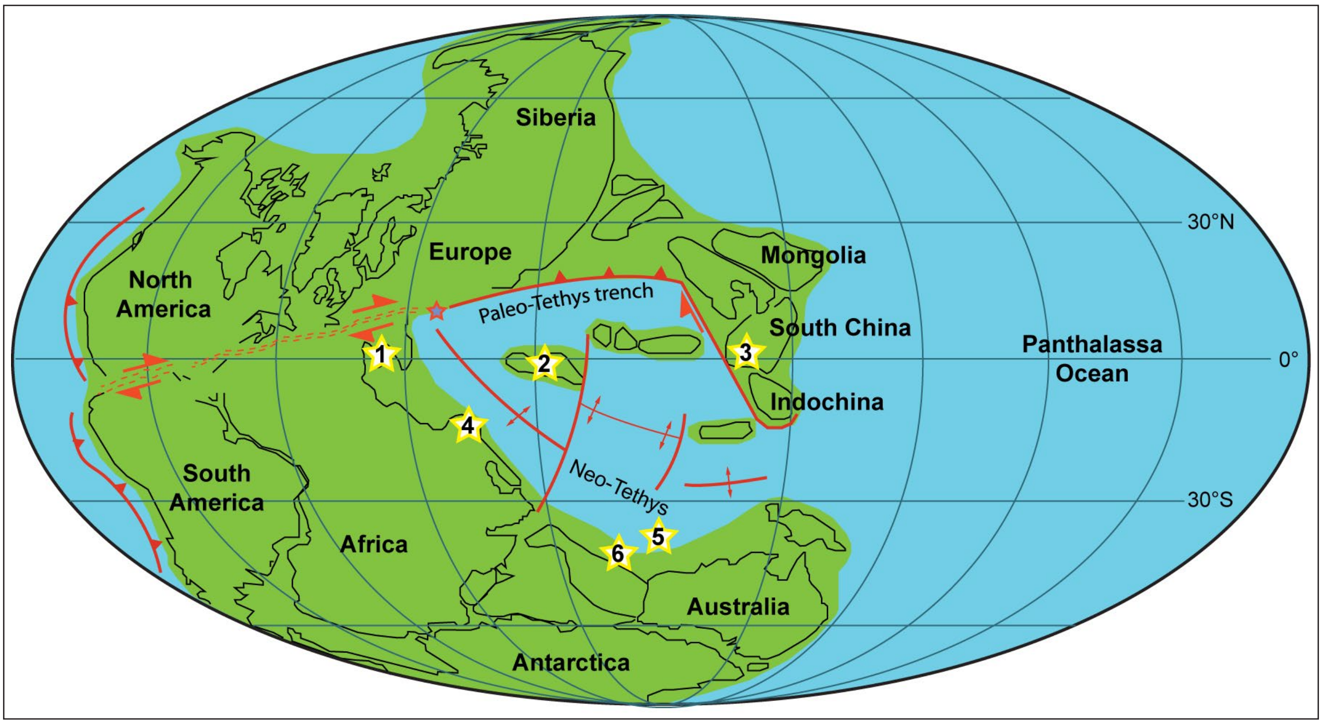


Fig. 1 - Lopingian paleogeographic reconstruction showing the setting of the formations from which the brachiopod shell structure was investigated. The yellow stars refer to the paleogeographic positions of the different studied localities: 1 - Southern Alps (Dolomites), 2 - Northern Iran, 3 - South China, 4 - Turkey, 5 - Southwestern Tibet, 6 - Salt Range (Pakistan) (modified after Muttoni et al. 2009; Angiolini et al. 2015).

which became extinct at the end of the Permian, and the Rhynchonellata, still extant. These two taxa show general differences in their body plan organization and shell microstructure (Williams 1968; Williams 1997). In the two classes, the multilayered shell biocomposite is different, as the secondary layer is laminar in the Strophomenata, while it is fibrous in the Rhynchonellata (Williams 1997). The tertiary layer is morphologically similar in the two classes, being columnar in both (Göetz et al. 2009; Garbelli et al. 2014a). Cambrian and Ordovician brachiopods had already evolved different types of organocarbonate fabric (Williams 1970; Brunton 1972), but only certain fabric types survived the end-Permian extinction event (e.g. Erwin 2006; Shen et al. 2011; Garbelli et al. 2017).

Brachiopods low-Mg calcite shells are highly resistant to diagenesis; thus, they have a high potential to preserve the original shell microstructure and fabric during the fossilization process (i.e. Angiolini 1993; Garbelli et al. 2012). The study of various shell microstructures of fossils can return information about physiological changes of biomineralization in these organisms. The purposes of this paper are: 1) to investigate the microstructure of shell fabric in several Lopingian brachiopod taxa, with a focus on describing its variability, and 2) to discuss its implication on the evolution of Rhynchonelliformea brachiopods fabric. This can help us in a better understanding of macroevolutionary patterns of biomineralization and to assess the possible key factors, which promote fabric change during a period of environmental and climatic perturbations at a global scale, such as during the Lopingian.

MATERIALS AND METHODS

Materials. The shell microstructure of several species belonging to 39 genera of Lopingian brachiopods (Tabs 1, 2) was studied using a Scanning Electron Microscope (SEM).

A total of 450 brachiopods shells have been analyzed. The observed specimens were collected from different paleogeographic localities in the Palaeotethys/Neotethys oceans (Fig. 1). The material comes from the Nesen Formation, Alborz Mountains, northern Iran (Angiolini & Carabelli 2010; MPUM 9907-10050); Julfa Formation, Ali Bashi Formation and Boundary Clay, Ali Bashi Mountains, northwestern Iran (Ghaderi et al. 2014; Garbelli et al. 2014b; MPUM 11616-11657, MPUM 11723-11724); Selong Group, southern Tibet (MPUM 11703-11707); Gyanyima Formation, southwestern Tibet (Shen et al. 2006, 2010; MPUM 11682-11702); Bulla Member, Dolomites, Northern Italy (Posenato 2001, 2009; Brand et al. 2012; MPUM 11720-11722); Gomaniibrik Formation, Hazro, Turkey (unpublished data collected by A. Baud: MPUM 11708-11719); Changhsing Limestone and Dalong Formation, South China (unpublished data collected in 2014 by L. Angiolini, G. Crippa, C. Garbelli, S.Z.

Class	Order	Family	Genus	Layers observed	Structures observed	Comments
Strophomenata	Orthotetida Wagen, 1884	Meekellidae Stehli, 1954	*Orthotetina* Schellwien, 1900	L, C?	P, no T, S	massive shell composed mainly of laminae, which seem very compact
			Alatorthotetina He Xi-Lin & Zhu Mei-Li, 1985	L	P, no T, S	
			Meekella White & St. John, 1867	L	P, S	the specimen does not show good preservation
	Productida Sarytcheva & Sokolskaya, 1959	Strophalosidae Schuchert, 1913	*Marginalosia* Waterhouse, 1978	L		rather thick laminar layer, >0.5 mm, poor preservation
		Aulostegidae Muir-Wood & Cooper, 1960	*Edriosteges* Muir-Wood & Cooper, 1960	Pr?, L	P	
		Richthofeniidae Waagen, 1885	*Richthofenia* Kayser, 1881	L	P, T	shell wall extremely rich in pseudopunctae, some of which are strongly persistent through it.
		Permianellidae He & Zu, 1979	*Permianella* He & Zu, 1979	L	P, T	
		Monticuliferidae Muir-Wood & Cooper, 1960	*Costatumulus* Waterhouse in Waterhouse & Briggs, 1986	L		the specimen does not show good preservation
		Linoproductidae Stehli, 1954	*Linoproductus* Chao, 1927b	L	P, T, E	cross bladed lamination evident
		Productidae Gray, 1840	*Tyloplecta* Muir-Wood & Cooper, 1960	L, C	P, T, S, E	massive shells, both laminar and columnar are thick, frequent alternation of the two
			Araxilevis Sarytcheva in Sarytcheva & Sokoloskaya, 1965	L, C	P, T, S	
		Productellidae Schuchert, 1929	*Cathaysia* Ching in Wang, Ching & Fang, 1966	L	P, T	
			Paryphella Liao in Zhao & others, 1981	L	P, T	
			Haydenella Reed, 1944	L	P, T	
			Costiferina Muir-Wood & Cooper, 1960	L	P, T, E, S	
			Retimarginifera Waterhouse, 1970	L	P, T, E, S	thick laminar layer, >2mm
			Transennatia Waterhouse, 1975	L, C?	P, T	
			Spinomarginifera Huang, 1932	L, C	P, T, E, S	alternation of fibrous and columnar layers; high variability for the presence of columnar layer
		Lyttoniidae Wagen, 1883	*Leptodus* Kayser, 1883	L	P, T, E	cross bladed lamination

Tab. 1 - List of studied taxa and synthetic results of characters detected in the shell fabric of Strophomenata; Pr - primary layer; L - laminar layer; C - columnar layer; P - pseudopunctae; S - spines; E - endospines; T- taleolae.

Class	Order	Family	Genus	Layers observed	Structures observed	Comments
Rhynchonellata	Orthida Schuchert & Cooper, 1932	Schizophoriidae Schuchert & LeVene, 1929	*Acosarina* Cooper & Grant, 1969	Pr, F	Pu	
		Enteletidae Waagen, 1884	*Enteletes* Fischer de Waldheim, 1825	Pr, F	Pu	keel and saddle
			Peltichia Jin & Liao in Jin & Sun, 1981	F	Pu	thick fibrous layer, >1 mm, small fibers
	Spiriferida	Martiniidae Waagen, 1883	*Martinia* M'Coy, 1844	F, C		
		Ambocoeliidae George, 1931	*Paracrurithyris* Liao, 1981	F		
		Choristitidae Waterhouse, 1968	*Alphaneospirifer* Gatinaud, 1949	F, C?		
		Spiriferellidae Waterhouse, 1968	*Spiriferella* Chernyshev, 1902	F, C?		
		Trigonotretidae Schuchert, 1893	*Neospirifer* Frederiks, 1924	F, C		thick columnar layer
		Reticulariidae Waagen, 1883	*Squamularia* Gemmellaro, 1899	F		
		Elythidae Frederiks, 1924	*Bullarina* Jin & Sun, 1981	F		
		Elythidae Frederiks, 1924	*Permophricodothyris* Pavlova, 1965	F, C		alternation of fibrous and columnar layers
	Spiriferinida Ivanova, 1972	Paraspiriferinidae Cooper & Grant, 1976	*Paraspiriferina* Reed, 1944	Pr, F	Pu	
	Athyridida Boucot, Jhonson & Staton, 1964	Athyrididae Davidson, 1881	*Araxathyris* Grunt, 1965	F, C		
			Transcaucasathyris Shen et al., 2004	F, C		
			Comelicania Frech, 1901	F, C		
		Neoretziidae Dagys, 1972	*Hustedia* Hall & Clarke, 1893	Pr, F	Pu	very small fibers, ~4 µm in width
	Rhynchonellida Khun, 1949	Stenoscismatidae Oehlert, 1887	*Stenoscisma* Conrad, 1839	F, C		massive columnar layer
		Wellerellidae Licharew, 1956	*Uncinunellina* Grabau, 1932a	F		
	Terebratulida Waagen, 1883	Notothyrididae Licharew, 1960	*Notothyris* Waagen, 1882	F, C	Pu	
		Gillediidae Campbell, 1965	*Hemiptychina* Waagen, 1882	F, C	Pu	
		Dielasmatidae Schuchert, 1913	*Dielasma* King, 1859	F, C		not well preserved

Tab. 2 - List of studied taxa and synthetic results of characters detected in the shell fabric of Rhynchonellata; Pr - primary layer; F - fibrous layer; C - columnar layer; Pu - punctae.

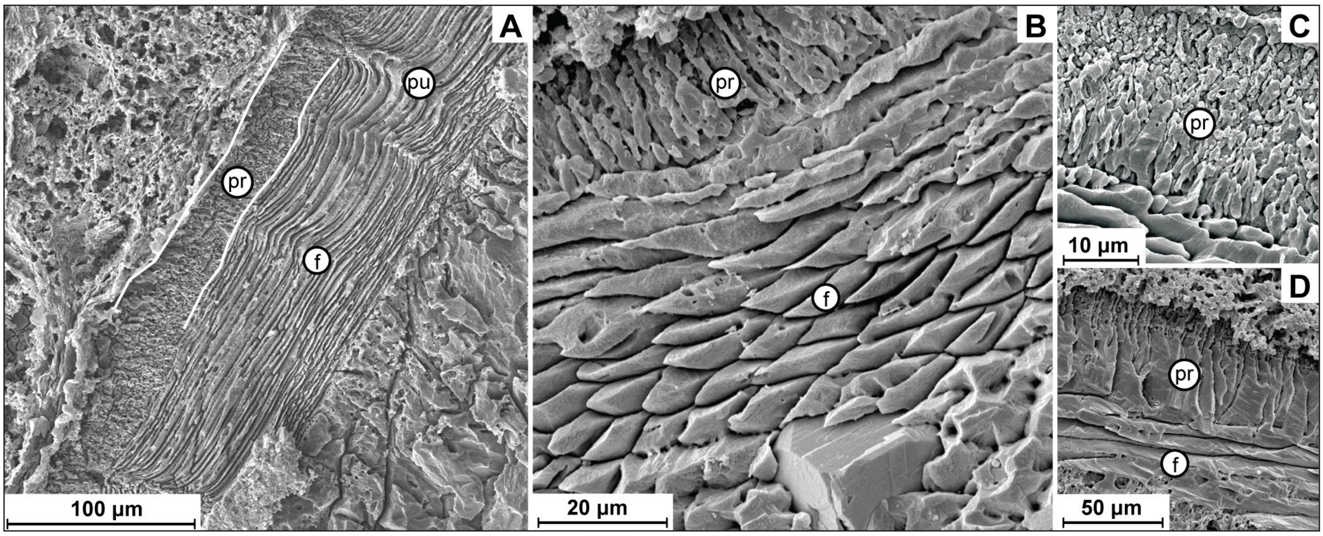


Fig. 2 - A) Shell sequences showing the outermost primary layer (pr) and the inner fibrous layer (f) crossed by punctae (pu), *Hustedia* sp. MPUM 11659 (CH60-15); B) transition from the outermost primary layer (pr) and the fibers (f), which preserve their original shape and are oriented subparallel to the outer shell surface, *Paraspiriferina alpha* MPUM 11676 (CH12-3); C/D) details of outermost primary layer (pr) showing different morphologies, possibly related to the degree of alteration; the first one is coarser, the second one more compact, *Enteletes lateroplicatus* MPUM10000 (IR 332-1) and *Transcaucasathyris* sp. MPUM 11658 (CH30-4) respectively.

Shen, D.X. Yuan; MPUM 11658-11861). The material is housed at Dipartimento di Scienze della Terra "A. Desio", Università di Milano (MPUM catalogue numbers).

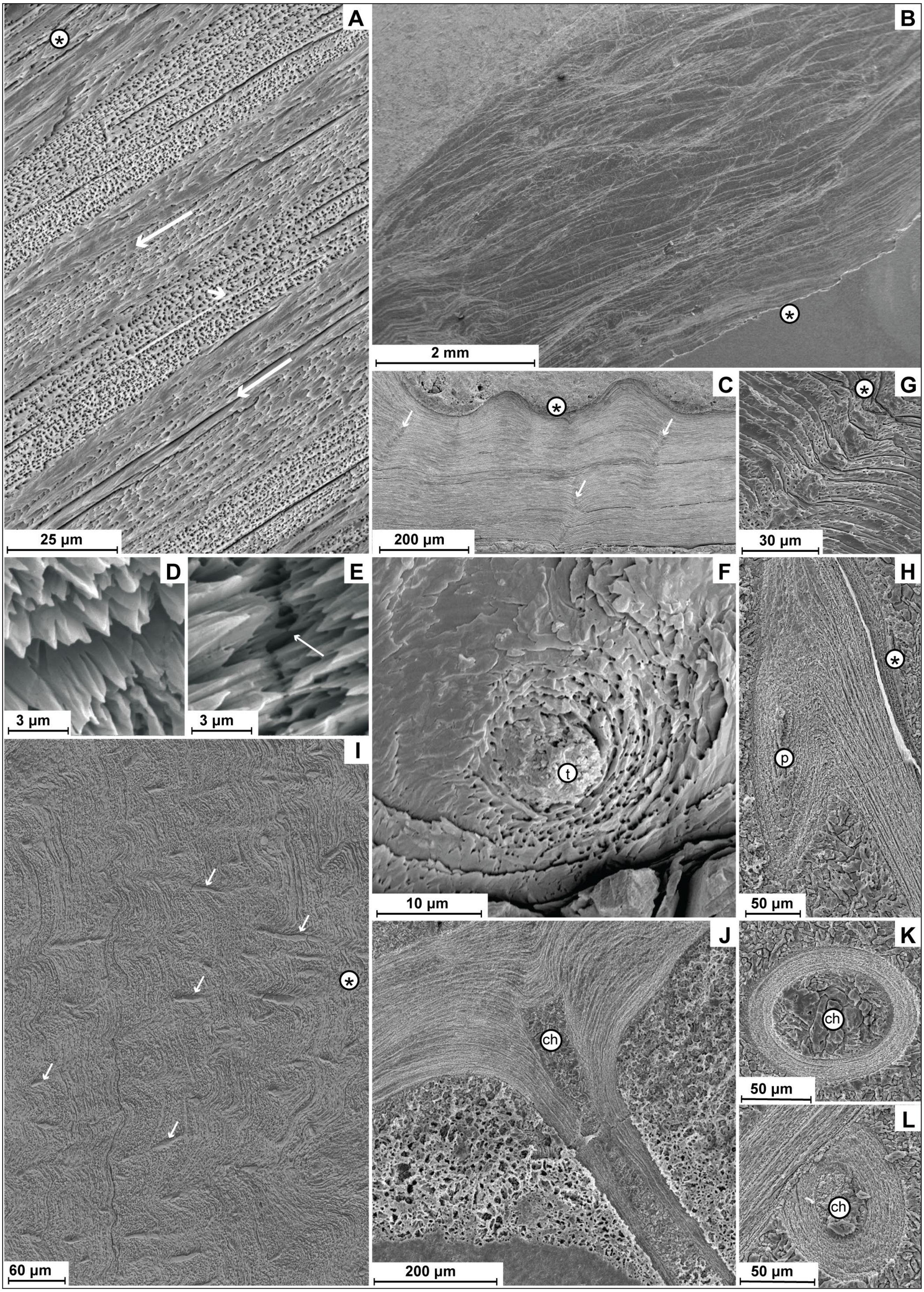
Methods. The studied brachiopod specimens were cut along their longitudinal and transverse axes, embedded in resin, polished and then etched with 5% HCl for 15 seconds. In addition, acetate peels of some specimens were prepared with a cellulose acetate film and acetone (CH$_3$)$_2$CO. The exposed surfaces were metal coated with Au by the sputtering process and then inspected with two scanning electron microscopes (SEMs): 1) a Cambridge S-360 featuring a LaB6 source and an acceleration voltage of 20kV, located at the Dipartimento di Scienze della Terra A. Desio, Milan, Italy (DPT); 2) a LEO-1530VP with an acceleration voltage between 10 and 15kV, located at the Nanjing Institute of Palaeontology and Geology, Nanjing, China.

Thin sections of specimens were also analyzed by cathodoluminescence with a cold cathode luminoscope (Nuclide ELM2) operating at 10 kV with a beam current of 5-7 mA. The instrument is located at DPT. Exposure to the electronic beam (before taking photomicrographs) was on the order of 1530 seconds, not to force shell material to luminescence, and it was consistent for all specimens. In addition, light exposure for photographs was uniform and set to 1.2 seconds for consistency with a Nikon Coolpix 4500, operating at 400 ISO. Cathodoluminescence (CL) microscopy is a powerful technique to study biominerals, and is particularly important in paleontology to assess the preservation of carbonate shells (Machel 2000; Barbin 2013; Angiolini et al. 2012). Results are presented in the supplementary materials.

Results: ultrastructure, micromorphology and shell fabric of Lopingian brachiopods

The investigated taxa show the typical brachiopod shell successions described by Williams (1997), composed of an outermost primary layer, a secondary fibrous or laminar layer and, when present, an innermost columnar tertiary layer. The primary layer is not always present because it is only a few micrometers thick, and it easily undergoes corrasion. This outermost layer has been observed

Fig. 3 - A) cross-bladed laminar layer showing packages of laminae orthogonally oriented (arrows), *Paryphella* sp. MPUM 11671 (CH136-2); B) shell thicker than 4 millimeters, but composed entirely of a laminar fabric, *Costiferina indica* MPUM 11684 (GY79); C) laminar layer crossed by pseudocpunctae (arrows); the laminae are folded to produce the external ornamentation of costellae, *Alatorthotetina* sp. MPUM 11711 (EBHZ80-16); D/E) details of the laminae composed of aligned blades/laths; in longitudinal section (E) discontinuities are evident between the structural units (arrow), *Cathaysia* sp. MPUM 11664 (CH71-8); F) pseudopunctae formed by cone in cone inwardly deflected laminae with an inner core of calcite, the taleola(t), planar view, *Spinomarginifera* sp. MPUM 11616 (JU117-1); G) details of C showing a pseudopuncta, without taleola, crossing the secondary shell and deflecting the laminae inwardly, *Alatorthotetina* sp. MPUM 11709 (EBHZ65-12); H) pseudopunctae formed by inwardly deflected laminae protruding in the inner shell to form an endospine, *Haydenella* sp. MPUM 11677 (CH4-5); I) laminar layer in a coral-like Richtofenioid, crossed by numerous pseudopunctae (arrows), *Richthofenia lawrenciana* MPUM 11682 (GY52); J) longitudinal section of the distal part of a spine composed of laminar secondary layer with a channel (ch) filled by diagenetic calcite, *Spinomarginifera helica* MPUM 11710 (EBHZ71-10); K/L) cross sections of an isolate spine and of the proximal portion arranged sub-parallel to the outer shell surface respectively, *Spinomarginifera helica* MPUM 11710 (EBHZ71-10); in all the pictures the asterisks (*) indicate the outermost part of the shells.


A
*
25 µm
B
*
2 mm
C
*
200 µm
G
*
30 µm
D
3 µm
E
3 µm
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10 µm
H
*
p
50 µm
I
*
60 µm
J
ch
200 µm
K
ch
50 µm
L
ch
50 µm

Genera	Max thickness of the laminar fabric (µm)	Thickness of laminae (µm)		Number of measures	Number of specimens	Number of species
		Mean	SD			
Orthothetina	240	0.36	0.09	6	1	1
Alatorthothetina	710	0.44	0.04	273	9	1
Richtofenia	1700	0.52	0.07	16	2	1
Linoproductus	520	0.41	0.05	7	1	1
Tyloplecta	1600	0.34	0.02	108	6	2
Araxilevis	790	0.33	0.06	4	1	1
Cathaysia	190	0.27	0.06	20	2	1
Paryphella	230	0.36	0.00	31	3	1
Costiferina	190	0.59	0.01	93	7	2
Retimarginifera	2100	-	-	-	-	-
Transennatia	220	-	-	-	-	-
Spinomarginifera	510	0.31	0.04	783	46	8
Leptodus	250	-	-	-	-	-

Tab. 3 - Summary of the data measured in the shells with laminar secondary fabric.

Genera	Max thickness of the secondary fibrous fabric (µm)	Width of the fibers (µm)				Number of measures	Number of specimens	Number of species
		Mean	SD	min	MAX			
Acosarina	470	-	-	-	-	-	-	-
Enteletes	500	11.00	2.36	8.31	16.01	10	2	2
Peltichia	2120	12.21	2.32	7.97	14.88	6	1	1
Martinia	205	-	-	-	-	-	-	-
Paracruruthyris	310							
Alphaneospirifer	80*	10.43	0.27	9.32	11.38	9	2	1
Spiriferella	200	13.19	1.78	9.97	16.71	18	1	1
Neospirifer	830	9.86	2.28	5.07	12.84	15	2	1
Squamularia	200	-	-	-	-	-	-	-
Permophricodothyris	240	9.15	3.46	3.58	13.96	21	4	2
Paraspiriferina	80*	-	-	-	-	-	-	-
Araxathyris	770	26.13	7.09	13.40	41.88	20	1	1
Trnscaucasathyris	200	27.51	13.44	11.18	56.69	20	3	1
Comelicania	260	14.52	1.82	9.06	18.16	19	3	2
Hustedia	90*	6.41	0.90	5.14	7.17	4	1	1
Stenoscisma	290	-	-	-	-	-	-	-
Uncinunellina	50*	-	-	-	-	-	-	-
Nothothyris	150	24.54	6.61	13.74	32.34	7	1	1
Hemiptychina	210	10.14	2.64	7.01	13.47	11	2	1
Dielasma	90*	-	-	-	-	-	-	-
Prelissorhynchia	100*	-	-	-	-	-	-	-

Tab. 4 - Summary of the data measured in the shells with fibrous secondary fabric.

in the Rhynchonellata *Enteletes*, *Acosarina*, *Transcaucasathyris*, *Paraspiriferina*, *Hustedia* and traces of the outermost layer were observed in *Paracrurithyris*; the layer usually appears to be recrystallized (Figs 2A, B, C, D). Its thickness is rather constant along the shell and slightly varies between taxa. The thinnest primary layer observed belongs to *Paraspiriferina* (~ 15 µm), and the thickest one belongs to *Transcaucasathyris* (~ 50 µm), indicating a range of thickness similar to the one observed in modern Rhynchonellata (Williams & Cusack 2007).

The outermost primary layer was not observed in the analyzed Strophomenata specimens.

The other two inner layers are thicker; thus, they are more easily preserved compared to the outermost layer.

Laminar fabric

In adult specimens, the laminar secondary layer is usually between 50 and 400 µm thick, but it exceeds 1 mm in some genera, such as *Alatorthotetina* (Orthotetida), and *Tyloplecta* and *Araxilevis* (both Productida) (Figs 3A, B, C). In some genera of Productida, such as *Retimarginifera* and *Costiferina*, laminar fabrics thicker than 2 and 3 mm respectively have been observed. Table 3 summarizes the maximum thickness of the laminar fabric observed for several genera. The laminae are composed of single blades/laths, which are 0.6-2 µm wide (Figs 3D, E; Fig. 4). The thickness of laminae is between 0.2 and 0.6 µm, and a summary of the obtained measurements is presented in Tab. 3. In the species represented by a high number of specimens, i.e. the species of *Spinomarginifera*, there are significant differences in the mean thickness between individuals of the same species. The thinnest laminae have been measured in *Spinomarginifera iranica*, and can be thinner than 0.2 µm. The thickest laminae have been observed in *Costiferina*, and can be thicker than 0.7 µm. The blades length is not measurable in transverse or longitudinal sections, but observations along the fracture surfaces suggest that they can be longer than 50 µm.

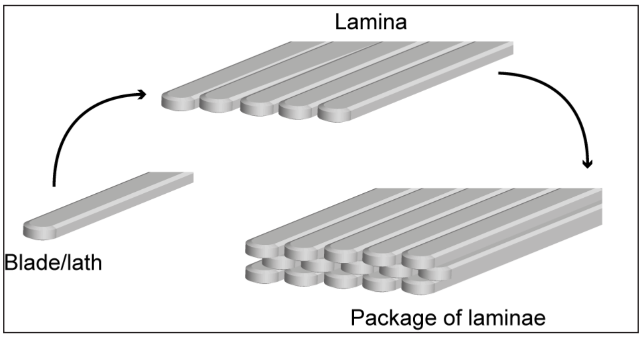


Fig. 4 - Schematic drawing illustrating the structural organization of the laminar fabric. Single long shaped crystals of calcite, the blades, are aligned with the major axis parallel to each others, composing a single lamina; several laminae are packed together to form a layer.

In several Productida, i.e. *Spinomarginifera*, *Haydenella*, *Cathaysia*, *Tyloplecta*, *Fusiproductus*, *Leptodus*, *Araxilevis*, *Transennatia*, *Perygeyerella*, and *Costiferina*, the laminae are organized in packages with the axis of blades oriented about perpendicular to each other (Fig. 3B). This feature is less evident in the investigated Orthotetida. The orthotetid *Alatorthotetina* and *Orthotetina* have pseudopunctae composed only of a slightly arcuate, anteriorly inclined trail of cone in cone laminae that are inwardly deflected, without any evidence of internal core forming a taleola (Fig. 3G). In the studied Productida, the laminar layers are crossed by pseudopunctae with taleolae (Fig. 3F) and a certain variability in the size and density of pseudopuncation between taxa has been observed (compare Figs 3A, B with 3I). Pseudopunctae can produce endospines when they protrude inwardly in the shell wall (Fig. 3H). Spines are hollow and have and internal tubular structure (Figs 3L, M, N).

Fibrous fabric

The fibrous layer is composed of stacked fibers, but a certain amount of variability was observed in the thickness and size of the fibers, their shape, and their reciprocal arrangement. The maximum thickness of this fabric ranges from a few tens of micrometers in *Hustedia* (Fig. 2A) up to 2 mm in *Peltichia* (Fig. 5A). The mean width of the fibers in cross section varies from approximately 6 μm to 27 μm (Tab. 4; Figs 5B, G, H, I, L). Even the cross-section outline varies, from a "keel and saddle" profile to a more sub-diamond outline. In some taxa, such as *Transcaucasathyris*, *Araxathyris*, and *Notothyris*, the size and shape of the fibers are not consistent in all the secondary layer, but, gradual, significant differences were observed (Figs 5D, E, G, H, O). In these taxa, the width of fibers show a relative higher standard deviation, due to the huge differences in the minimum and maximum width of fibers. The maximum width can be three times wider than the minimum one (see Tab. 4). In other genera, such as *Peltichia*, *Paracrurithyris*, *Permophricodothyris*, and *Comelicania*, the shape and size of fibers are more homogeneous in different regions of the shell (Figs 5C, L) and standard deviation is smaller. Also in these taxa, differences in fibers size and shape are observed in the umbonal region, where convolute fibers are present. *Hustedia* has the smallest fibers, with a maximum width of 10 μm, confirming the observation of MacKinnon (1974) for the Retziidina. About the orientation of the fibers through the shell substance, in *Peltichia* (Orthida) and *Paracrurithyris* (Spiriferida) there are abrupt changes in the orientation of the main longitudinal axis of the fibers (Figs 5A, C). On the other hand, in other groups, the directions of fibers are more consistent, as in *Permophricodothyris*. In some genera the change in orientation of the longitudinal axis is gradual, and it is coupled with an increase of fiber size and a change of shape (Figs 5D, M). For example, in the cross section of *Transcaucasathyris*, *Araxathyris*, and *Notothyris*, the outermost fibers are smaller and flatter, with a keel and saddle outline, whereas inwardly they become larger, with a diamond shape outline.

In the order Orthida, Spiriferidina and the family Neoretziidae (Order Athyridida), these fabrics are perforated by punctae (Figs 2A, 5K, 6A-F). The diameter has the same order of magnitude in different genera. These perforations deflect the fibers outwardly (Figs 6E, F). In cross section, the infilling of the channels, which perforate the secondary layer, shows regularly disposed hole, which could be traces of internal structures of the mantle extensions (Figs 6A, C). In the Orthida *Acosarina*, in the Spiriferinida *Paraspiriferina* and in the the Neoretziidae *Hustedia*, the infill of the punctae was detected through the recrystallized primary layer (Figs 6B, F), but no canopy has been observed.

Columnar fabrics

Different taxa of both classes, Strophomenata and Rhynchonellata, bear a well-developed columnar tertiary layer. Its thickness easily exceeds 1 mm (Figs 7B, F). The genera, which produce a well-developed columnar layer, are *Tyloplecta*, *Araxilevis*, and *Spinomarginifera* in the Productida; *Permophricodothyris*, *Martinia*, *Neospirifer*, and *Spiriferella* in the Spiriferida; *Araxathyris*, *Transcaucasathyris*, and *Comelicania* in the Athyridida; *Notothyris* and *Hemiptychina* in the Terebratulida. In some genera, only traces of columnar layer have been observed because of the poor preservation: *Alphaneospirifer*, *Stenoscisma*, and *Dielasma*. In a few species, an intraspecific variability for the development of the columnar layer was observed: some species of *Spinomarginifera* from the Julfa (Iran) and Gomaniibrik (Turkey) formations show a well-developed columnar tertiary layer. On the other hand, none of the numerous conspecific specimens investigated from the Nesen Formation (Iran) has shown any well-developed tertiary layer.

The morphology of the columnar layer is very similar among classes, but the Rhynchonellata show polished surfaces with well-defined accretion of micrometric bands (Fig. 7H) more frequently than Strophomenata, in which the columns tend to have a coarser appearance (Fig. 7C). At sub-micrometric scale the structure is different (Figs 7D, I): the columns of Strophomenata appear with smaller and coarser micro-granular units than the columns of Rhynchonellata.

In several genera, intercalations of laminar/fibrous layers inside the columnar layer have been observed (Figs 7A, E). This feature was observed in *Tyloplecta*, *Araxilevis*, and *Spinomarginifera* in the Productida, in *Permophricodothyris* in the Spiriferida (Fig. 7E). The transition from the laminar/fibrous to the columnar layer shows a certain pattern in variability. In the Strophomenata, the transition is restricted to a few micrometers with a rather abrupt change of fabric (Fig. 7J); also in the Rhynchonellata, some taxa show this feature (Fig. 7E), but others show a gradual shift from fibers to columns (Fig. 7K) in the growth direction.

Discussion

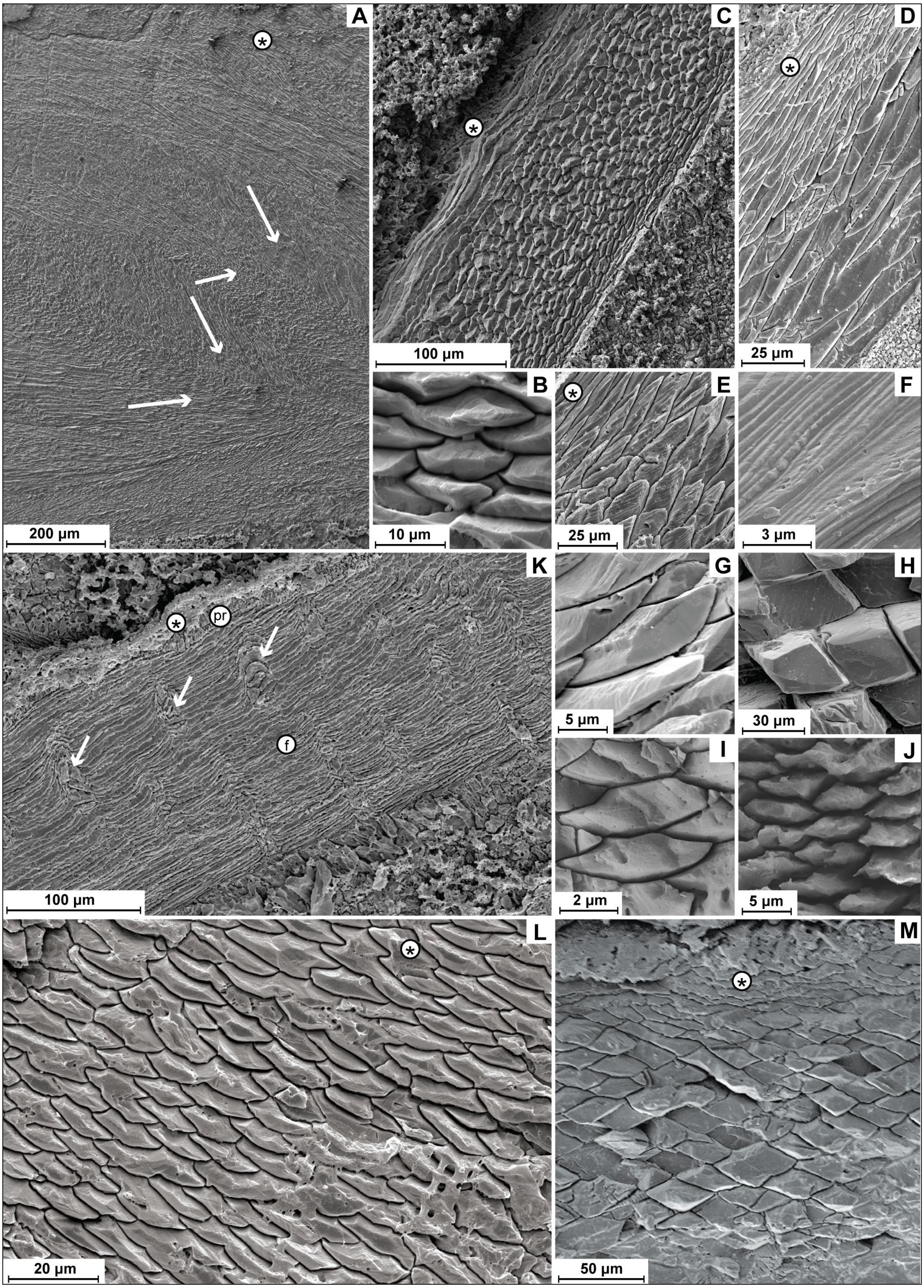
Variability in Lopingian brachiopod shell microstructure and its implications

The observed variability in microstructure is in agreement with the already known differences between the laminar fabric in the Strophomenata and the fibrous one in the Rhynchonellata (Williams 1997; Williams & Cusack 2007), but it also reveals some important aspects of the ontogenetic development of the tertiary columnar layer.

Laminar fabrics. Despite the homogeneous basic structure of the laminae, which are composed of aligned structural units (Fig. 4), and even though these fabrics are virtually indistinguishable between the Orthotetida and Productida, some differences are present. For example, laminae organized in packages with the axis of blades oriented about perpendicular to each other are more frequently observed in the Productida than in the Orthotetida (compare Figs 3A, 3C). These two orders have also differences related to the pseudopunctae, the structures crossing the shell, which are slightly arcuate, anteriorly inclined trail of inwardly deflections of the laminae (Williams 1997). In *Orthotetina* and *Alatorthotetina* (Orthotetida), the pseudopunctae are composed of deflected laminae, layered around a core of amalgamated laminae. On the other hand, in all the Productida, the pseudopunctae possess an inner rod-core of calcite called taleola. These two types of pseudopunctae correspond to those described by Williams (1997).

The reason why Orthotetida and Productida show these differences could be explained by the independent origin and evolution of the two groups (Dewing 2004). In fact, Orthotetida probably evolved from a group of the ancestral Billingselloidea, in which the fabric is stratiform laminar (Williams 1970; Williams & Cusack 2007);

Fig. 5 - A) fibrous layer in which several changes in the direction of fiber growth are evident (arrows), *Peltichia* sp. MPUM 11660 (CH60-8); B) details of A showing the fibers in cross section with a keel and saddle outline; C) fibrous layer in which the structural elements are consistently oriented parallel to the outer shell surface, *Paracrurithyris pygmaea* MPUM MPUM 11661 (CH30-11); D/E) fibrous layer in which the fibers change their orientation, progressively modifying the orientation of the growth axis from the outer to the inner shell, *Transcauscasathyris* sp. MPUM 11620 (JU136-1); F) accretionary bands on the fibers, *Transcaucasathyris* sp. MPUM 11621 (JU140-2); G) cross section of the fibers in the outermost layer of the same taxon of D, *Transcaucasathyris* sp. MPUM 11618 (JU121-1; H) cross section of the fibers in the innermost fibrous layer of the same taxon of D, *Transcaucasathyris* sp. MPUM 11622 (JU89 -1); I) cross section of the fibers in, Terebratulida fam. gen. sp. ind. MPUM 11683 (GY6-12); J) cross section of the fibers in *Hustedia* sp. MPUM 11662 (CH30-15); K) shell composed of an outermost primary layer (pr) and a fibrous secondary layer (f) crossed by punctae (arrows), *Acosarina minuta* MPUM 11665 (CH72-11); L) fibrous secondary layer of *Comelicania* sp. MPUM 11720 (VB9B-1); M) fibrous secondary layer of *Transcaucasathyris* sp. MPUM 11723 (JU1-1); in all the pictures the asterisks (*) indicate the outermost part of the shells.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instead, the primitive Productida evolved from the Plectambonitoidea, which bear a fibrous fabric (Brunton 1972). It is possible to infer that Orthotetida and Productida had different abilities to biomineralize their shell due to the following reasons;

(1) the two orders have a distinct origin of the laminar fabric;

(2) they bear two different types of pseudopunctae;

(3) in the Productida, the pseudopunctae with taleola greatly vary in diameter and length in the same shell and they can be differently spaced depending on the shell position and taxon; instead, the pseudopunctae of Orthotetida seem more homogeneous in the same shell.

These differences may be related to the mobility of the mantle, the ability of membrane secretion, and the capacity of changing secretion rate of calcium carbonate (Williams 1968). For example, the accelerated apical growth of pseudopunctae can produce tubercles or endospines in the interior surface of valves in Productida, as observed in genera such as *Spinomarginifera*. In certain species of Richthofenioids, the endospines of ventral valve are branched and amalgamated to form a net (i.e. the coscinidium), which was covered by the mantle epithelium, exposing more surface to the environment (Williams 1997). These features are not present in Orthotetida, revealing differences in the biomineralization process between the two groups, despite the similarity in the fabric and shell structure. In addition, the production of spines indicates the differences in growing abilities of the two groups. The growth mechanisms of spines are disparate, as illustrated by Alvarez & Brunton (2001) and Pérez-Huerta (2013). Alvarez & Brunton (2001) showed that the Productida grew spines through a peculiar process; they developed hollow spines filled by mantle tissue where the tip is composed of cells, thus the spines can potentially grow endlessly. These spines are common in all Permian Productida, while the Orthotetida did not develop such spines. This supports the occurrence of significant differences between these groups in terms of shell biomineralization. All these observations suggest that the Productida had a more plastic shell fabric and a greater flexibility to modify the shell growth. This is consistent with the evolutionary history of the two groups, with the Productida reaching the highest level of morphological shell variation (Brunton et al. 2000).

Fibrous fabric. Three factors affect the variability of this fabric: the size, the shape of the fibers, and the orientation of their longitudinal axis through the shell substance. Basing on previous observations on Paleozoic brachiopods (e.g. MacKinnon 1974), the range in size of the fibers is a taxonomic feature. For example, the athyridid genus *Hustedia* has rather small fibers, reaching up to 10 μm in width, with rounded keel and saddle, as also described by MacKinnon (1974). From the same suborder Athyrididina, but under a different family, *Transcaucasathyris* shows fibers with a smoothed keel and saddle outline in the outer part of the shell (Fig. 5G); however, the fibers become diamond shaped inwardly (Fig. 5H). In species of this genus, the outer fibers have a small size, up to 10 μm in width, but mature inner fibers can be as wide as 40 μm. Diamond shaped fibers with large size have been observed in other taxa of this order (MacKinnon 1974), but they belong to another suborder than the studied one, the Koninckinidina. This reveals that the shape and size of fibers could be a diagnostic feature for lower taxonomic ranks, probably at the generic or specific level. However, in *Araxathyris* and *Transcaucasathyris*, which are close relatives (Angiolini & Carabelli 2010; Garbelli et al. 2014b), the change of fiber shape and size is very similar, and this indicates that the overall organization of shell fabric is more significant to draw phylogenetic relationships between different genera, than the observation of single structural elements. In the same family Athyrididae, *Comelicania* has a different shell fabric organization, with fibers showing a consistent keel and saddle shape through the all secondary layer, and the width of the fibers comprised between 10 and 20 μm (Fig. 5L). It is worthy of note that *Comelicania* is also assigned to a different subfamily, the Comelicaninae (Posenato 2001; Alvarez & Rong Jia-yu 2002), whereas both *Araxathyris* and *Trancaucasarthyris* are in the subfamily Spirigerellinae (Shen et al. 2004). This observation suggests that the overall shell fabric organization could be indicative of phylogenetic relationship at low taxonomic rank, such as family, subfamily, and genera.

The taxa possessing a secondary fibrous shell show a different ability of producing accessory structures perforating the shell layers: the punctae. Six different genera, belonging to four different orders, have been studied here: *Acosarina*, *Enteletes*, and *Peltichia* in the Orthida; *Paraspiriferina* in the Spiriferi-

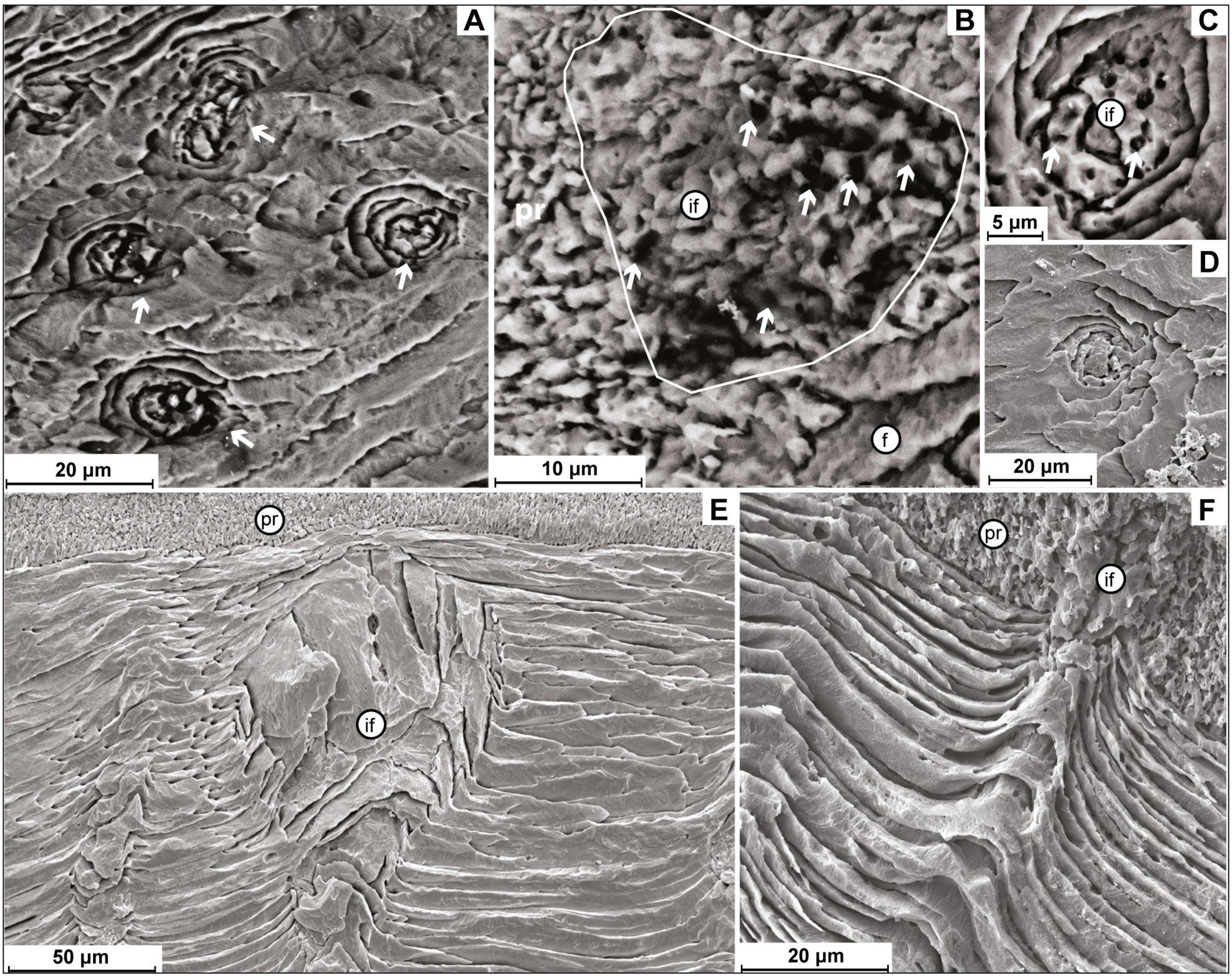


Fig. 6 - A/C) Cross section of the punctae and detail of the infilling (if) showing numerous voids (arrows), *Acosarina* sp. MPUM 11665 (CH72-11); B) infilling (if) of a puncta in the primary layer; the infill shows voids (arrows), *Acosarina* sp. MPUM 11665 (CH72-11); D) cross section of a puncta in *Paraspiriferina alpha* MPUM 11676 (CH12-3); E) longitudinal section of a puncta in Orthida with the infilling (if) which does not cross the primary layer (pr), *Enteletes* sp. MPUM 10000 (IR332-1); F) puncta crossing the outer secondary layer, deflecting the fibers outwardly and penetrating into the inner part of the primary layer (pr), infilling (if), *Hustedia* sp. MPUM 11659 (CH60-15).

nida; *Hustedia* in the Athyridida; *Notothyris*, *Hemyptychina*, and *Dielasma* in the Terebratulida. The punctae show the same organization when crossing the secondary shell. Here, their diameter is between 10 and 20 µm, but it becomes wider close to the primary layer (Fig. 6E). In the Schizophoridae *Acosarina*, the infill of the puncta penetrates the thin layer covering the secondary layer, which is assumed to be a recrystallized primary layer, as proposed by Williams & Harper (2000). In addition, this infill retains some voids, both in the primary and secondary layer (Figs 6B, C). These voids are regularly disposed, with a diameter of approximately 1-2 µm, and they could represent the trace of the microvillous branches of the punctae, which also perforate the primary shell. In analogy to the modern craniformis, which bear branched punctae perforating all the shell layers (Williams 1997), this observation seems to support the hypothesis that the punctae were in direct contact with periostracum without any evidence of a canopy, as in the primitive enteletoid *Schizoporia* (Williams & Harper 2000). On the other hand, it is difficult to exclude the possibility of presence/absence of a canopy in the shell, because this thin structure, located in the uppermost part of the primary layer, is easily lost during the diagenesis. This infill was not observed in the recrystallized primary layer of *Enteletes* or *Peltichia*. In the former, the fibers suture upward the punctae, adjacent to the primary layer (Fig. 6E). In the second one, the primary layer was absent in the studied specimens. Note that the punctae of *Peltichia* were smaller, less dense and scarcely persistent through the shell. These observations suggest that the family Enteletidae, which evolved from the Schizophoriidae (Williams & Harper 2000), could have evolved different types of punctae that did not perforate the primary layer, but a preservation bias cannot be excluded.

In the Neoretziidae *Hustedia*, the infill of punctae permeated the primary recrystallized layer, highlighting that the puncta was connected to the outer periostracum (Fig. 6F), but there are not evidences of a canopy.

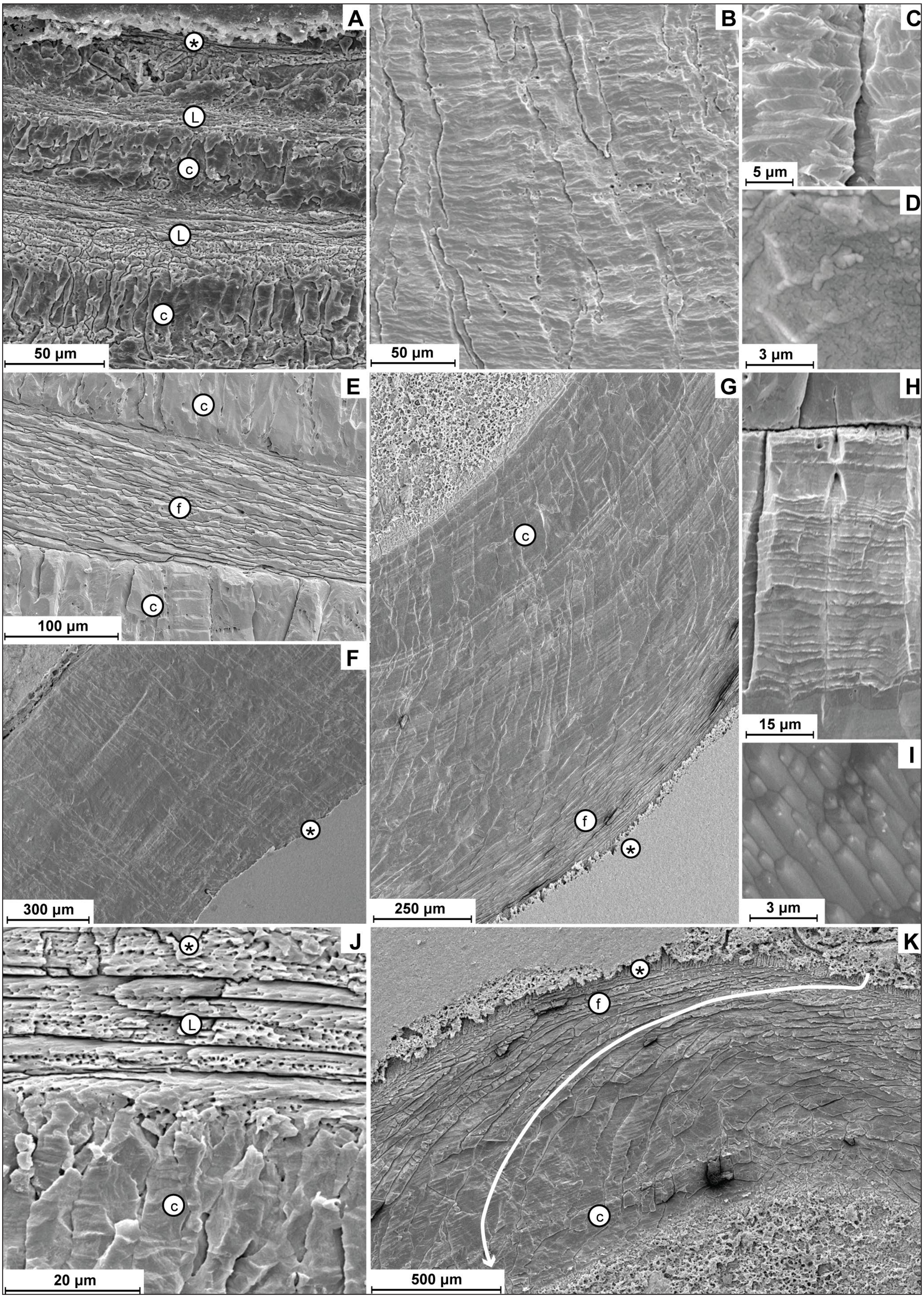
For the Terebratulida, no specimen showed the primary layer being preserved, hampering comparison with modern taxa.

Despite the possible differences in punctae among Permian brachiopods, the presence of these perforations imposes developmental constrains to the outer epithelium of the mantle. In addition, they bear specific biological functions, such as storage compartments, sensorial activity, and protection against boring organisms (Owen & Williams 1969; Thayer 1977; Curry 1983; Pérez-Huerta et al. 2009). To be noted that several authors found a positive relationship between density of punctae and seawater temperature (Campbell 1965; Peck et al. 1987; Ackerly et al. 1993), suggesting that the variability in the distribution and density of these structures discloses disparate thriving abilities among taxa. The major variation is in *Peltichia*, where the punctae appear less dense, when compared with those of *Acosarina*, another species of Orthida. These two species occur together in the Lopingian-Lower Triassic successions of South China, but only *Acosarina* has been recovered in the Extinction Interval (Shen & He 1991). On the other hand, *Peltichia* disappears below the extinction horizon (Shen & Shi 2007), suggesting that density of punctae may have played some role in the differential survivorship during the Lopingian.

Columnar fabrics. The columnar layer is present in both classes, but not in all the studied taxa, confirming the homoplastic origin of this feature at different taxonomic levels (Smirnova & Popiel-Barcyz 1991). The development of the columnar layer during ontogenesis is similar to the one described in Williams (1968), but the studied material reveals a few distinctive features in some taxonomic groups. In the Strophomenata, the transition to the columnar layer is principally controlled by a specific activity of the mantle, which ceases secretion of the organic strand that delimits the morphology of single blades and laminae and starts to produce a continuous columnar layer. However, in the Rhynchonellata, the transition may vary from gradual to abrupt. For example, when *Transcaucasathyris* and *Comelicania* are compared, it is evident that the transition is gradual in the former and abrupt in the latter (see Figs 8A, B). This probably corresponds to a gradual demise of organic sheet production in species of the former genus, and an abrupt demise in the latter.

Another interesting feature is that this shift between the two secretory regimes can be reversible in several taxa of both classes. The shell fabric of species of *Permophricodothyris* (Rhynchonellata) shows intercalations of fibrous and columnar layers (Fig. 7E, see fig. 4F in Garbelli et al. 2012). On the other hand, species of *Transcaucasathyris* – in the same class – do not show these alternations, highlighting a difference in the ability to modulate the production of the organic matter sheets. Analogous examples are also present in the Strophomenata, such as *Tyloplecta*, *Araxilevis*, and *Spinomarginifera*, where there are intercalations of laminae and columns (Fig. 7A, see fig. 4C in Garbelli et al. 2012). The function and cause of the reversion in the secretory mechanism remain unclear. To figure

Fig. 7 - A) Shell composed of an alternating sequence of laminar (l) and columnar layers (c), *Spinomarginifera helica* MPUM 11708 (EBHZ15-15); B) massive columnar-like layer, *Araxilevis intermedius* MPUM 9959 (IR 311-6b); C) bands of accretion and discontinuity between two adjacent prisms, *Araxilevis intermedius* MPUM 9959 (IR 311-4); D) prisms surface showing a microgranular-like texture, *Tyloplecta persica* MPUM 9949 (IR317-4); E) fibers (f) intercalated between two columnar layers (c), *Permophricodothyris iranica* MPUM 10044 (IR332-5); F) thick columnar layer, *Comelicania* sp. MPUM 11721 (VB9B-2); G) shell composed of an outer fibrous (f) and an inner columnar (c) layer, *Transcaucasathyris araxensis* MPUM 11723 (JU10-4); H) details of F showing bands of accretion on the prisms surface, *Comelicania* sp. MPUM 11721 (VB9B-2); I) details of columnar layer showing the boundaries between microgranules, *Transcaucasathyris araxensis* MPUM 11620 (JU136-1); J) transition between laminar and columnar layer, *Spinomarginifera iranica* MPUM 11617 (JU1-2); K) transition from fibrous to columnar layer in the Athyridida *Transcaucasathyris* sp., the arrow shows the change in orientation of the fiber into a prism, *Transcaucasathyris* sp. MPUM 11619 (JU131-4); in all the pictures the asterisks (*) indicate the outermost part of the shells.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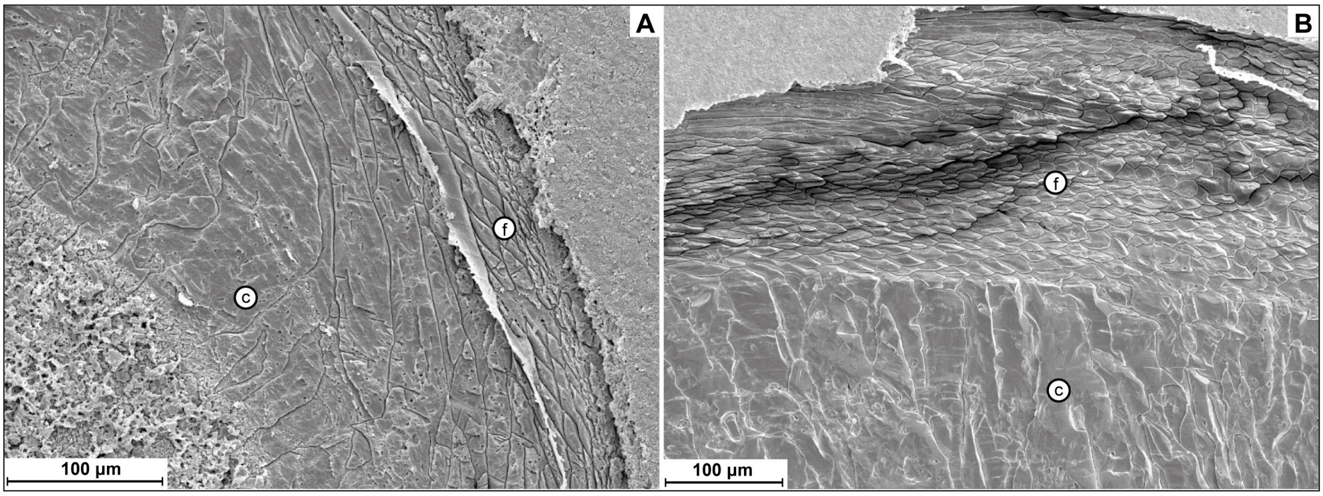

Fig. 8 - Comparison of shell sequence in two taxa of Athyridida; A) shell sequence in *Transcausathyris* sp., showing a gradual change from fibers to prisms, with fibers growing in size inwardly; B) shell sequence of *Comelicania* sp., showing a clear transition from secondary fibrous to columnar fabric, with fibers rather homogeneous in size and shape through all the shell; in all the pictures the asterisks (*) indicate the outermost part of the shells.

out whether this character is controlled by the ontogenetic program that regulates shell production, by environmental stimuli or by the interplay of both these factors, it is important to understand brachiopod biomineralization. Angiolini et al. (2012) reported that species of *Gigantoproductus* show interruptions in the secretion of the columnar layer in correspondence of growth lines represented by irregular laminae or grains. The authors interpreted it as a feature related to spawning or more probably seasonal growth interruptions. Similar features have also been observed in large productids, such as *Araxilevis* and *Tyloplecta*. It is important to understand that in these taxa, such kind of intercalations of laminar and columnar fabrics are present throughout the shell. In the taxa here studied, these intercalations tend to be thicker in the outermost shell, where they show a clear laminar fabric. The intercalations become thinner inwardly, where they appear as the growth lines described in Angiolini et al. (2012) (Fig. 9). This could highlight that there is a common physiological mechanism for both intercalations of laminar layers and growth lines, despite their different pattern. The growth lines observed in species of *Gigantoproductus* show a frequency of formation caused by regular environmental perturbations (Angiolini et al. 2012). On the other hand, the intercalations recorded in the species here studied show a less organized pattern of distribution and the laminar intercalations have a different thickness.

Laminar and columnar fabrics must have different mechanical proprieties and the higher organic content in the former confers more tolerance to deformation, as observed in the fibrous fabric (Schmahl et al. 2012). On the other hand, the small size of structural units, in this case the blades, confers higher hardness (Goetz et al. 2009). Therefore, we can hypothesize that the intercalations are related to interactions with the substrate during the growth of the organism, which could be more affected by mechanical stress in juvenile stages. This explains also why outwardly the intercalations are thicker, when the shell is thinner, if compared to subsequent life stages. In addition, the mechanical stress due to the substrate is random and this could explain why the intercalations are irregularly disposed, in contrast to the growth lines observed by Angiolini et al. (2012), which are caused by regular seasonal fluctuations.

Permophricodothyris (Spiriferida) and *Comelicania* (Athyridida) bear a columnar prismatic layer and show similar microstructures as those observed in the Productida. In the former, the columnar layer is interrupted by intercalations of fibrous layer, which can vary in thickness; in the latter, there are evident interruptions of columns growth, but without any evidence of fibers. These analogies would disclose that a common genetic mechanism drives the development of this fabric feature in all rhynchonelliformean brachiopods. On the other hand, Productida show a higher frequency of intercalations than taxa of the other orders. The diverse mechanical stress imposed by the semi-infaunal life style typical of most of the Productida

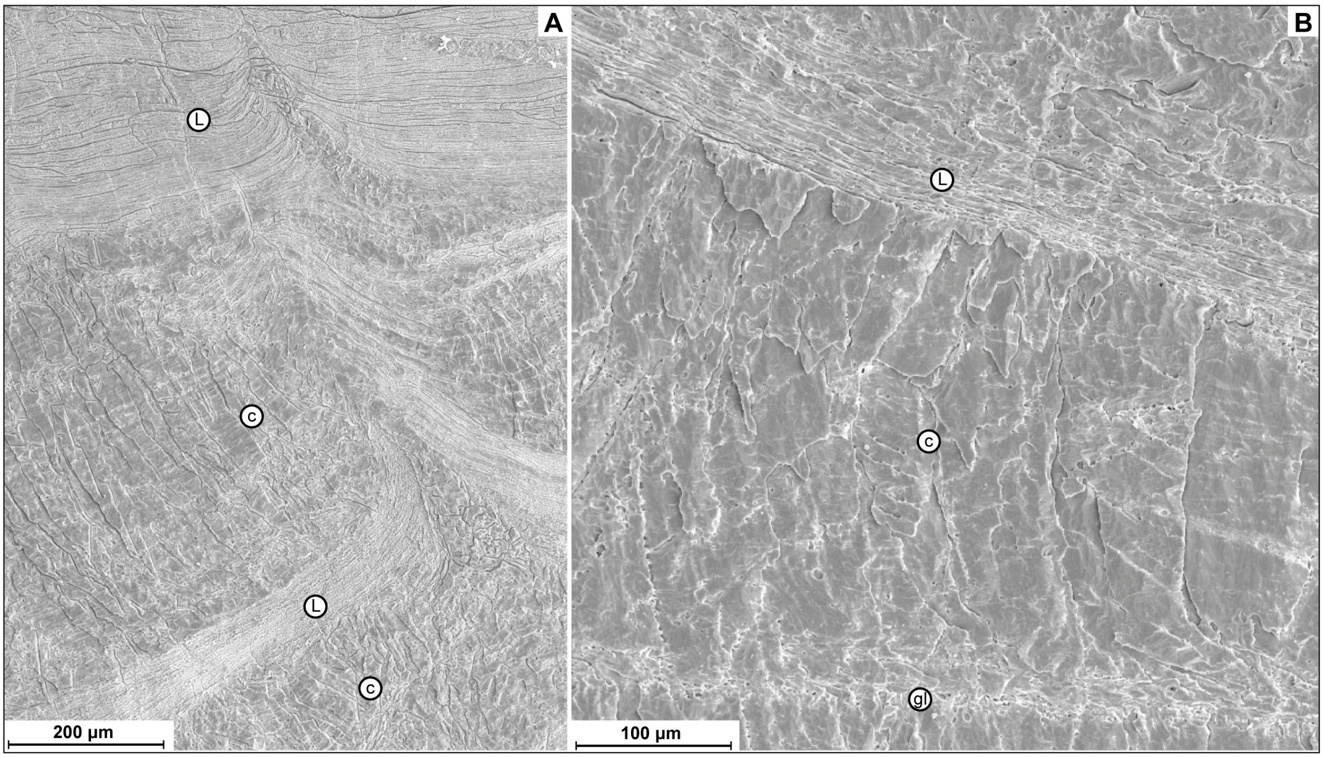

Fig. 9 - Alternation between laminar (l) and columnar (c) fabric in the Productida *Tyloplecta persica*; inwardly the intercalations become thinner, until they appear similar to the growth lines (gl) of Angiolini et al. (2012); A) outermost shell, *Tyloplecta persica* MPUM 9942 (IR877-1); B) inner shell, *Tyloplecta persica* MPUM 9942 (IR879-12); the asterisks (*) indicate the outermost part of the shells.

(Strophomenata), compared to the epifaunal free or attached lifestyles in the taxa of Rhynchonellata, could be one of the factors which acted on evolutionary time scale to promote the acquisition of a laminar shell fabric in the phyletic lineage of this group.

Paleobiological and evolutionary implications

Different life styles and ecologies, coupled with the small genome size of the Phylum Brachiopoda (Adachi et al. 2013), suggest that the analogies observed in the Permian brachiopod shell structure are the result of repetitive adaptations, as it has been proposed for the numerous convergent shell morphologies (Brunton et al. 2000).

The main differences in shell structure of calcifying Rhynchonelliformea, besides the presence/absence of punctae and pseudopunctae, are related to the fabrics they can produce: laminar (composed of blades), fibrous, columnar, or several combinations of these, and to the size and shape of the structural units. However, the observations on the newly studied materials and previous published studies (Williams 1968; Brunton 1972; Williams 1970; Williams & Brunton 1993; Dewing 2004) indicate that:

(1) even if the size of blades composing the laminae can be larger than those observed in the Permian taxa investigated here (also see Dewing et al. 2004), the laminar fabric appeared to have evolved from a fibrous one (see Brunton 1972) by a reduction of size of the structural elements, and a consequent change in their relative spatial organization;

(2) the fibers seem to have a large range of variation in size (Tab. 4) and the fibers can have a wide range of outlines, from diamond to keel and saddle, or very flat in shape (Fig. 5);

(3) the size of the structural elements can be significantly different among specimens of the same species from different stratigraphic positions (Garbelli et al. 2017);

(4) for some species, it has been observed a gradual and continuous change in size and shape from fibers to columns (Fig. 8A), suggesting that an ontogenetic gradual change from one fabric to another is possible.

These observations reveal that simple variations in size, shape and arrangement of structural

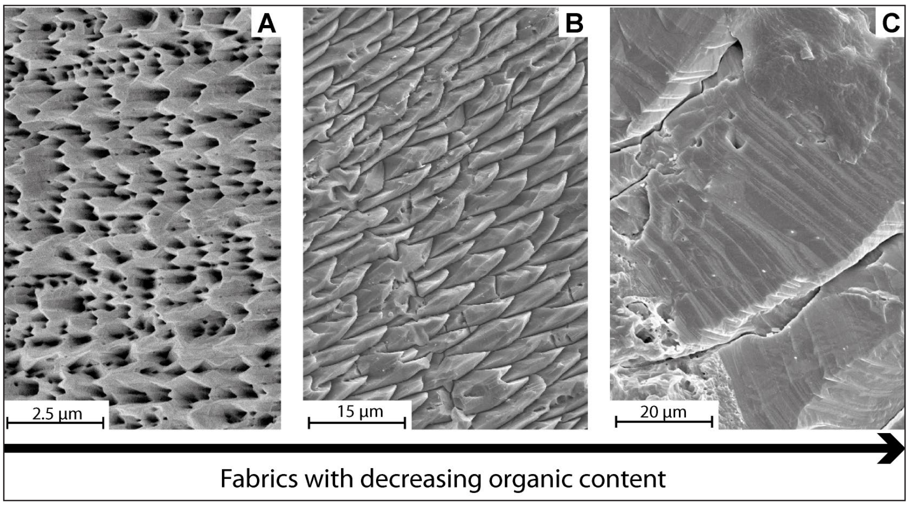

Fig. 10 - Comparison of three different fabric types in which the size of the structural units increases and the ratio of enveloping organic membranes decreases: A) laminar fabric; B) fibrous fabric; C) columnar fabric.

units could lead to the evolution of fabrics, through modifications of the organization of organic membranes, delimiting the structural units. In addition, chemico-structural changes in the organic sheets can be relevant, as it happens in modern taxa, where columns are enveloped in a different organic substrate than the fibers (Williams & Cusack 2007). If the disposition of the organic membranes during the growth is defined by an ontogenetic program, the changes in the overall fabric organization provides important indications about the phylogenetic relationships among brachiopods (Williams 1956; Williams & Brunton 1993). The observation of analogies reveals that the overall shell fabric organization could be useful only at low taxonomic ranks (e.g. genus, subfamily, family).

An important factor that must be taken in account to understand fabric evolution is its impact on the energetic balance of the organisms. The metabolic cost of $CaCO_3$ precipitation is ~5% of the energy that is required to produce proteinaceous organic fractions (per unit of shell; Palmer 1992). This means that the laminar fabric of the Strophomenata Productida and Orthotetida are energetically more expensive than the fibrous fabric of the orders belonging to Rhynchonellata, since the laminae require a larger fraction of organic membrane to be enveloped, because they are composed of small structural units, i.e. the blades (Fig. 10). Additionally, the columnar layer shows a microstructure more similar to an inorganic precipitate (Goetz et al. 2009; von Allmen et al. 2010); a shell containing a columnar layer can be produced at a lower metabolic cost, because there is less production of organic membranes. Pseudopunctae and tubular hollow spines represent an additional complexity and cost in laminar fabrics; also punctae add a cost to fibrous fabrics, but, from a metabolic perspective, they could provide some advantages, since they operate as storage compartments. On the contrary, tubular hollow spines and pseudopunctae are just structures for the shell functionality.

Therefore, each different type of shell entails a different metabolic investment, which plays a critical role in survivorship, especially during events such as the end Permian global mass extinction (Clapham & Payne 2011; Garbelli et al. 2017), when the marine carbonate system was strongly perturbed and the sweater temperature raised (Kump et al. 2009; Brand et al. 2012; Clarkson et al. 2015; Brand et al. 2016; Garbelli et al. 2016).

Outlining the differences in the cost of production of the shell could lead to a better understanding of the paleobiology of extinct groups. For example, productid brachiopods preferred low nutrient settings, instead spire bearing brachiopods, like athyridids and spiriferids, proliferated in high nutrient settings, suggesting that they have different physiologies (Pérez-Huerta & Sheldon 2006). In fact, these brachiopod groups show the following differences:

(1) their secondary layer fabrics are different;

(2) the concavo-convex (most of the productids) and biconvex (spiriferids and athyridids) brachiopods have a different ratio between body volume and shell: this could mean that the repartition of costs between shell biocomposite and the soft-body parts is different between productids and spiriferids/ athyridids, with the former having a lower ratio;

(3) productids show a higher frequency of intercalations of laminar secondary layer in the columnar layer than the other orders;

(4) productids present pseudopunctae and

variably developed tubular hollow spines; spiriferids do not have any complication of the shell structure, being impunctated and showing spines composed only of primary layer;

(5) athyridids present a more complex situation, with some clades evolving punctae and others developing spines, which grow in a different way if compared with those of productids (Alvarez & Brunton 2001).

These differences suggest that productids had assigned a larger fraction of their limited energy budget to shell building compared to spiriferids and athyridids. Since re-allocating energy from shell formation to support increase of metabolic cost during ocean warming and acidification affects survivorship ability (Mackenzie et al. 2014), the productids could have been more sensitive to this environmental perturbations than spiriferids and athyridids.

The mechanical characteristics conferred to the shell are another important factor to understand fabric evolution. Studying modern Rhynchonelliformea, Goetz et al. (2009) reported that different fabrics affect the mechanical proprieties of the shell, pointing out that this feature easily undergoes selective pressure related to environmental and ecological conditions. Noteworthy the most derived brachiopods lineage of the Strophomenata, i.e. the Productida, which proliferated in the semi-infaunal environment, bear a laminar fabric coupled with a shell that is commonly concavo-convex to planoconvex (Brunton et al. 2000). Excluding older stocks of Strophomenata, i.e. the Plectambonitoidea, which may have a fibrous fabric with a semi-infaunal habitus (Brunton 1972; Congreve et al. 2015), the unique condition of the Productida could indicate that the laminar fabric confers mechanical advantages related to the substrate interactions of this life-style.

CONCLUSION

This study has shown that there is variability in the shell structure of Rhynchonelliformea during the Lopingian, and this is related to:

- the type of structures crossing the shell substance, which have their origin in the deep evolutionary history of the analyzed taxa;

- the type of fabric succession composing the shell, i.e. the presence/absence of the columnar layer;

- the pattern of change in the secretory mechanism and the relative alternation between fibrous/laminar and columnar layers;

-the amount of intercrystalline organic matter, i.e. the organic membranes separating the structural units.

The mechanism producing/evolving the differences in shell structure remains unclear. It can be argued that there are some ontogenetic constrains in the development of shell fabric which are common to all Rhynchonelliformea brachiopods. On the other hand, there should have been some selective environmental force causing the differences in shell structure and acting on the evolutionary timescale. The effects of these factors are difficult to be evaluated in short time laboratory experiments (i.e. Cross et al. 2016), which test the response of single organisms during their lifetime, or even smaller intervals. From this point of view, the analysis of shell fabric evolution in fossil brachiopods provides important information to understand how and why their fabric changed in different environmental conditions. In particular, at lower taxonomic levels, shell microstructure may be useful to infer phylogenetic relationships.

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