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ORIGINAL PAPER

Madreporites of Ophiuroidea: are they phylogenetically informative?

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Abstract The madreporite of brittle stars is poorly studied, and the features of its structure are rarely used in the taxonomy. However, it is known that there is diversity in the madreporite structure. But are the ophiuroid madreporites phylogenetically informative? To check this hypothesis, we investigated the structure of the madreporite of 33 species of brittle stars from 4 families of Euryalida and 12 families of Ophiurida. The fixed specimens were processed with sodium hypochlorite using the standard procedure and then studied using SEM. If we combine our results with the modern phylogenetic data about brittle stars (O'Hara et al. in Curr Biol 24(16):1874–1879, 2014), we will find wide morphological diversity of the madreporites present in each of the three clades of Ophiuroidea. The madreporites with numerous pores, the well-developed oral shields in other interradii instead of irregularly arranged plates and the multiple madreporites occur in the representatives of all three clades. Only in Euryalida, which belongs to the clade A as well as the sister clade Ophiuridae + Ophiomusium, the definite oral shields are absent in all interradii except CD. Whereas in the family Ophiuridae (as in the clades B and C), the oral interradial shields are regularly formed. Contrary to this, the multiple madreporites and numerous madreporic pores appear to have evolved several times in different clades. Hence, the hypothesis that madreporite morphology is phylogenetically informative must be rejected since madreporites are highly homoplasious.

Keywords Ophiuroids · Ophiuroidea · Madreporite · Ophiurida · Euryalida · Morphology · Phylogenesis · Evolution

Introduction

The idea of the possible phylogenetically informativity of the madreporite in different families of the recent Ophiuroidea has appeared during the discussion of our earlier hypothesis about the transformations of the ophiuroid axial complex as a result of shifting of madreporic plate along interradius CD from the aboral side to the oral side (Ezhova et al. 2015). The madreporite of brittle stars is poorly studied, and the features of its structure are rarely used in the taxonomy of this class as a key structure. On the species level, when describing the species, it is usually only indicated whether the madreporic oral shield stands out from the other oral shields. However, it is known that there is variety in the structure of the madreporite. And ophiuroid taxonomy is predominantly based on the characters of the calcitic structures (Thuy and Stöhr 2011). Most madreporites of brittle stars have only one madreporic opening. At the same time, there is evidence that some groups of brittle stars have five madreporites (see, for example, Ludwig 1878; Stewart 2000). The number of

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openings in the madreporite may also vary from one to 250 (Ludwig 1878; Cuénot 1888, 1948; Reichensperger 1908; Hyman 1955; Ferguson 1995; Ezhova et al. 2014). We can assume that the structural features of madreporite may be important in the determination of the phylogeny within the class Ophiuroidea.

Thus, the aim of this study is the evaluation of madreporite morphology as a phylogenetically informative character. To check this hypothesis, we carried out a comparative study of the fine morphology of the brittle star madreporites, belonging to different taxa within the class and then compared these data with the modern concepts of the phylogeny of brittle stars.

Materials and methods

We studied representatives of 16 families from the class Ophiuroidea, 4 of which belong to the order Euryalida (6 species) and 12 to the order Ophiurida (27 species). We used the material from the collections of the Zoological Museum of the Moscow State University. To determine the external structure of the madreporite and oral shields, fixed specimens were treated with sodium hypochlorite to remove the outer layers of the integument and reveal the skeleton, then dried and examined with SEM (Camscan-S2 and JSM-6380LA) in the Laboratory of Electron Microscopy of Biological Faculty of Moscow State University.

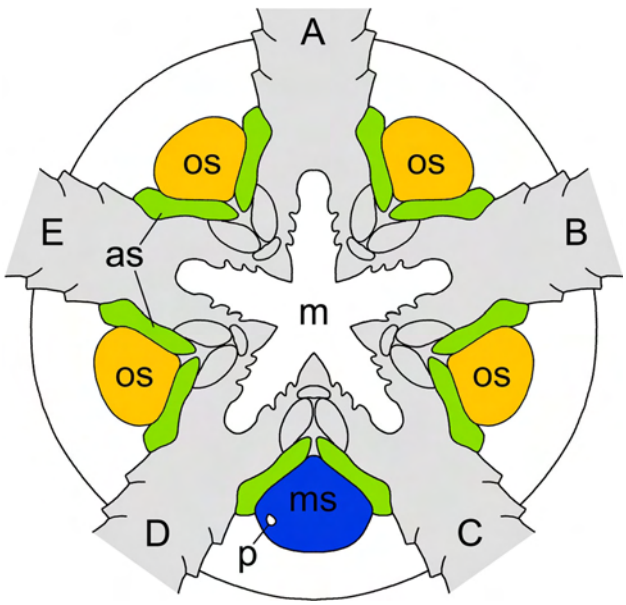


Fig. 1 General scheme of the ophiuroid oral disk. *A*, *B*, *C*, *D*, *E*—radii; *as* adoral shields, *m* mouth, *ms* madreporic shield or madreporite (oral shield of interradius *CD*), *os* oral shields in other interradii (in Euryalida, there are irregularly arranged plates instead of oral shields), *p* madreporic pore

Fig. 2 Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in one species of Asteronychidae and three species of Gorgonocephalidae. *Asteronyx loveni* (**a**–**c**): **b** view of interradius CD with the only madreporite; **c** view of interradius AB. *Gorgonocephalus arcticus* (**d**–**f**); **e** view of interradius CD with the only madreporite; **f** view of interradius DE. *Astrochele* sp. (**g**–**i**); **h** view of interradius CD with the only madreporite; **i** view of interradius DE. *Astrotoma* sp. (**j**–**l**); **k** view of interradius CD with the only madreporite; **l** view of interradius AE

Results

In the description of the results, we use the traditional terminology adopted for Ophiuroidea (Hyman 1955) (Fig. 1). The order of consideration of taxa adopted in the present study corresponds to the classical system (see, for example, Mortensen 1927; Dyakonov 1954; Smith et al. 1995). The dimensions of the structures are indicated when the madreporite significantly differs from the oral shields in other interradii.

Order Euryalida Lamarck, 1816

Family Asteronychidae Verrill, 1899

Asteronyx loveni Müller & Troschel, 1842

The only well-defined madreporite is trapezoidal in shape (Fig. 2a, b). The distal part of the madreporite retains a rounded shape, and the trapezoidal shape in the proximal part is due to the fact that madreporite is not close to the adoral shields, but between them, as though compressed by them. The central part of the madreporite has well-defined pores (the studied specimens have 4 and 11) (Fig. 2b). There are no oral shields in the other interradii. Instead, between the adoral shields there are small, irregularly arranged plates (Fig. 2a, c). There are several plates in the CD interradius, on the sides of the madreporite.

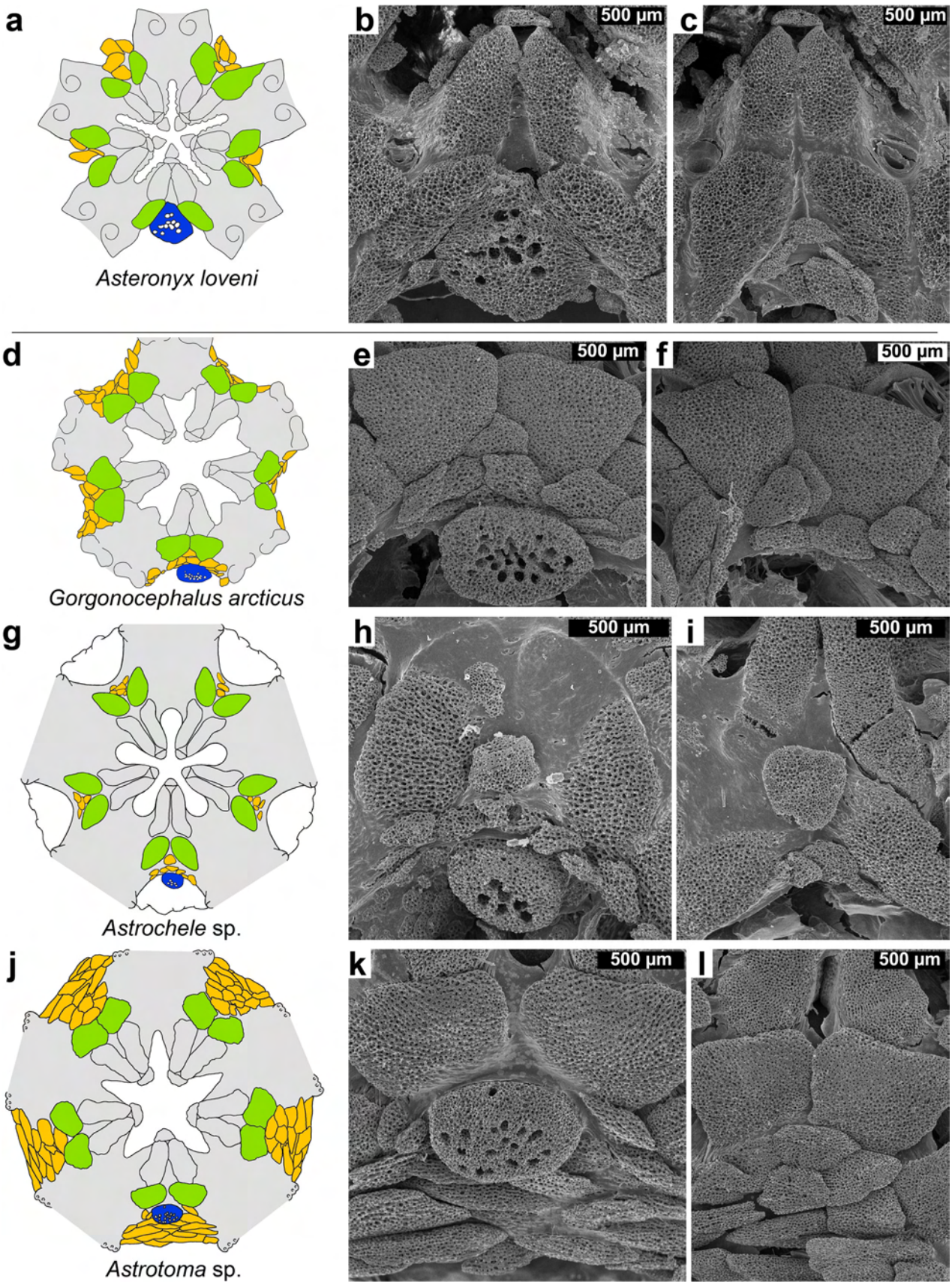
Family Gorgonocephalidae Ljungman, 1867

Gorgonocephalus arcticus Leach, 1819

There is a well-defined madreporite, which is a separate plate oval in shape and thick (Fig. 2d, e). The madreporite is riddled with numerous pores (the studied specimens had from 13 to 23 pores), concentrated near the center of the plate (Fig. 2e). However, in all interradii (including interradius CD), there are numerous small plates of irregular shape (Fig. 2d–f). At the same time, the madreporite is far from the adoral shields, and between these, there are small irregularly arranged plates (Fig. 2e).

Astrochele sp.

There is a well-defined single madreporite, which is round and thick, but rather flat in shape (Fig. 2g, h). The studied



specimen had six large pores in the madreporite, concentrated in the center. The madreporite is somewhat remote from the adoral shields, between it, and the adoral shields lie small irregularly arranged plates (Fig. 2h). The same plates are found in all other interradii, though there are no oral shields in other interradii (Fig. 2i).

Astrotoma sp.

The only madreporite is well defined (Fig. 2j, k). Like the above-described members of the family, it is penetrated with multiple (in this case 17) randomly spaced pores (Fig. 2k). The pores are mainly concentrated in the central region, but there are also some on the edges. Oral shields are lacking, but in all interradii there are numerous small irregularly arranged plates (Fig. 2l). The madreporite is located on the edge of the disk between the oral and the side surfaces and thus is located even closer to the adoral shields. In addition, there are no additional small plates between the madreporite and the adoral shields (Fig. 2k).

Family Asteroschematidae Verrill, 1899

Asteroschema inornatum Koehler, 1906

In each of the five interradii, a small cone-shaped madreporite is present, away from the adoral shields and located outside of the actual oral disk (Fig. 3a–e), so when the oral disk is viewed strictly from above, the madreporites are difficult to notice. In one interradius of the studied specimen, the madreporite is somewhat larger than the others and is penetrated by three pores (Fig. 3b). The madreporites in other interradii are penetrated by one pore (Fig. 3c–e). Any other differentiated oral shields in the interradii are absent.

Family Euryalidae Gray, 1840

Euryale aspera Lamarck, 1816

In each interradius, there is a small madreporite with multiple pores (Fig. 3f–k). The studied specimens have from 3 to 9 deep, sometimes slit shaped, pores penetrating the madreporites. Small madreporites are located close to the large adoral shields, adjacent to the oral disk (Fig. 3f). There are no other oral shields; moreover, there are no small irregularly arranged plates found in the interradii.

Order Ophiurida Müller & Troschel, 1840

Family Ophiuridae Müller & Troschel, 1840

Ophiura robusta Ayres, 1854

Regularly formed oral shields shaped similar to the shell of an oyster lie in the interradii (Fig. 4a–c). The CD interradius, in place of the oral shield, lies the madreporite, which is indistinguishable from the oral shields except by the presence of a well-defined pore (Fig. 4b, c). The pore is located on the edge of the madreporite on the side of the D radius (Fig. 4b).

Fig. 3 Schemes of general view of the oral disk (*above*), and the external structure of the madreporites in different interradii in *Asteroschema inornatum* (Asteroschematidae) and *Euryale aspera* (Euryalidae). *Asteroschema inornatum* (**a–e**): **b** madreporite with three openings; **c–e** view of madreporites with one opening in different other interradii. *Euryale aspera* (**f–k**); **g–k** madreporites of all five interradii ▶

Ophiura sarsii Lütken, 1855

The madreporite does not differ by shape and size from the oral shields in other interradii (Fig. 4d–f). The madreporic pore is visible only from the side—from the radius D (Fig. 4g). From the oral side of the madreporite, only a small lateral indentation is visible in place of the pore (Fig. 4e). Such an indentation can be found on the oral shields, but there is no pore on the side (Fig. 4e, f, h).

Amphiophiura bullata Thomson, 1878s.l.

All oral shields look the same, and it is impossible to identify the madreporite shield by any external characters (Fig. 4). Even when looking at the side edge of the oral shields, the pore is undetectable (Fig. 4k).

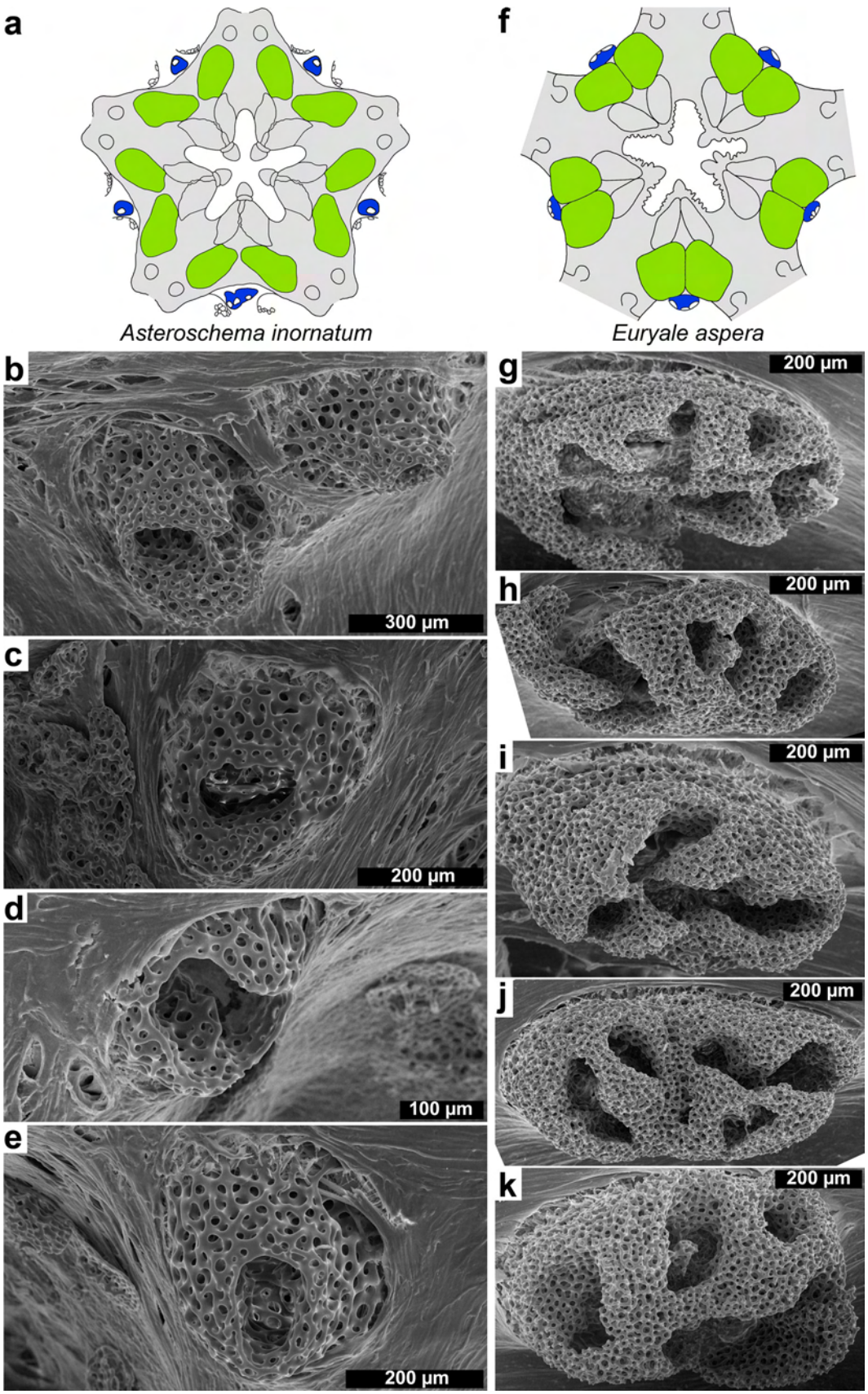
Ophiocten hastatum Lyman, 1878

The madreporite is almost the same in shape and size as the oral shields in other interradii (Fig. 4l–n). The distal part of it is only slightly more rounded compared to other oral shields. However, unlike the other oral shields, the madreporite shield is extremely remarkable due to the presence of numerous (in our case 23), relatively large clearly expressed pores, randomly scattered across the surface of the madreporite (Fig. 4m). Interestingly, the number of madreporite pores in this species is highly variable, and some specimens have fewer pores (in some cases it is impossible to distinguish the madreporite from other oral shields by external examination).

Family Ophiomyxidae Ljungman, 1867

Ophiologimus cf. secundus Koehler, 1914

We obtained a specimen, which had a six-rayed symmetry, hence six interradii. There is a madreporite in each interradius, which is permeated by one or two large, round pores (Fig. 5a–d). In the two interradii, located strictly opposite each other, there are two pores in the madreporite; in one of them, it is even possible to distinguish two madreporites, one pore in each (Fig. 5a–c). The other four interradii also have one pore each (Fig. 5a, d). Most likely, the paired pores in the two opposite madreporites are the results of a beginning autotomy. Therefore, we believe that



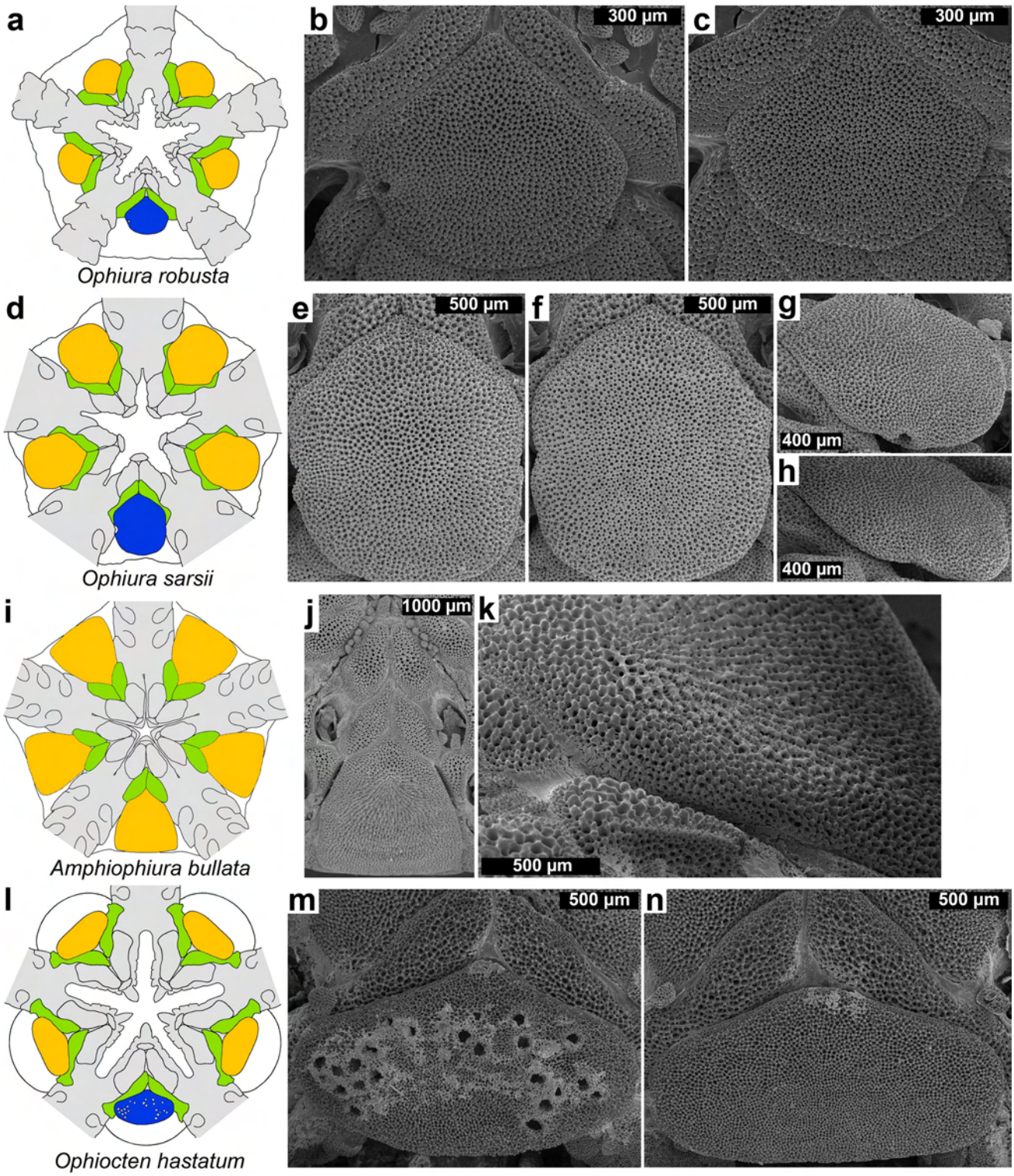


Fig. 4 Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in four species of Ophiuridae. *Ophiura robusta* (**a**–**c**): **b** madreporic shield with the pore on the side of radius D; **c** view of the oral shield in interradius AB. *Ophiura sarsii* (**d**–**h**): madreporic shield from the oral side (**e**), and from the side of radius D (**g**); oral shield in interradius DE from the oral side (**f**), and from the lateral side (**h**). *Amphiophiura bullata* (**i**–**k**): view of oral shield in one of the interradii from the oral side (**j**), and from the lateral side without any pores (**k**). *Ophiocten hastatum* (**l**–**n**): **m** madreporic shield with numerous pores; **n** oral shield in interradius DE

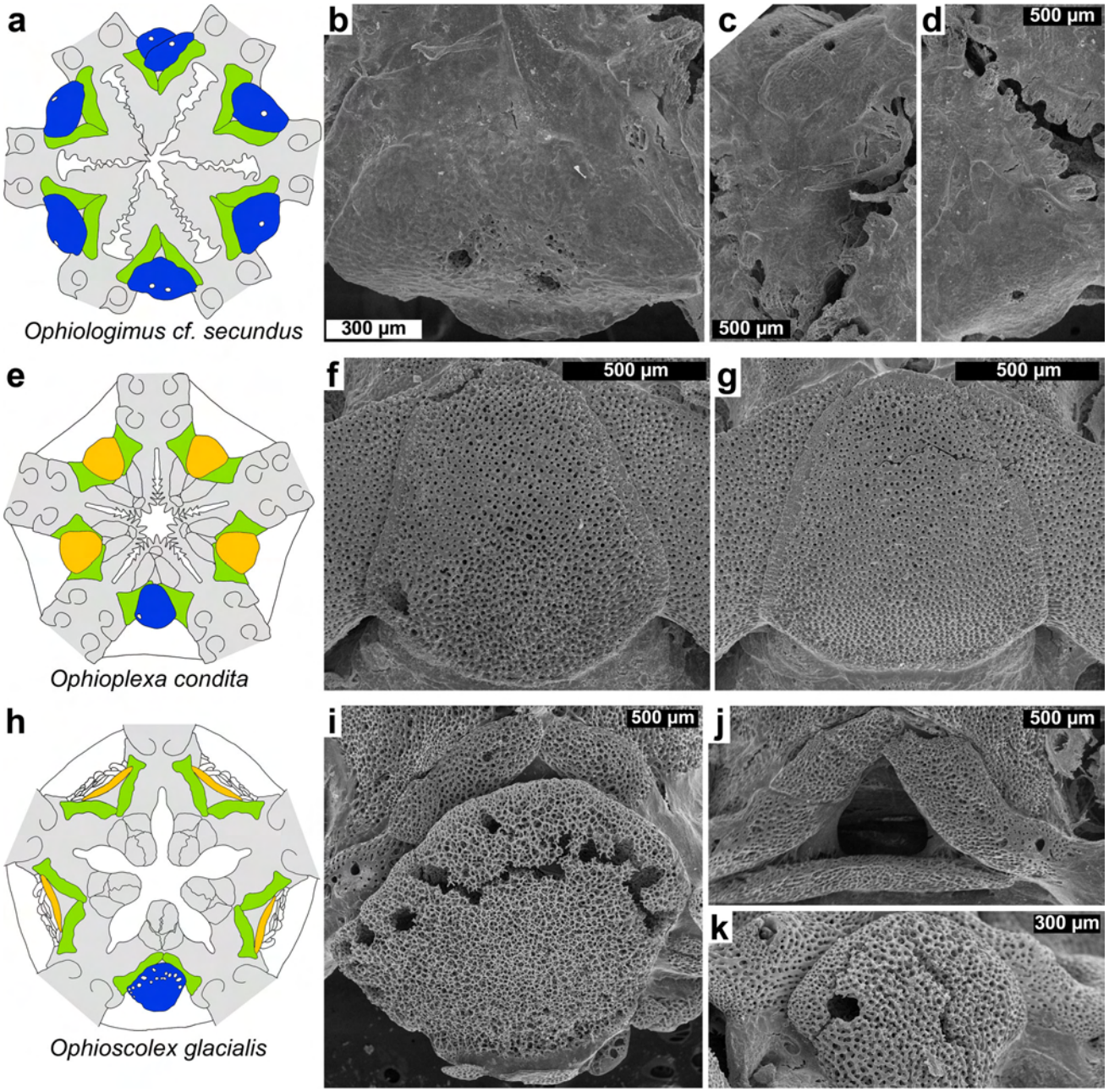
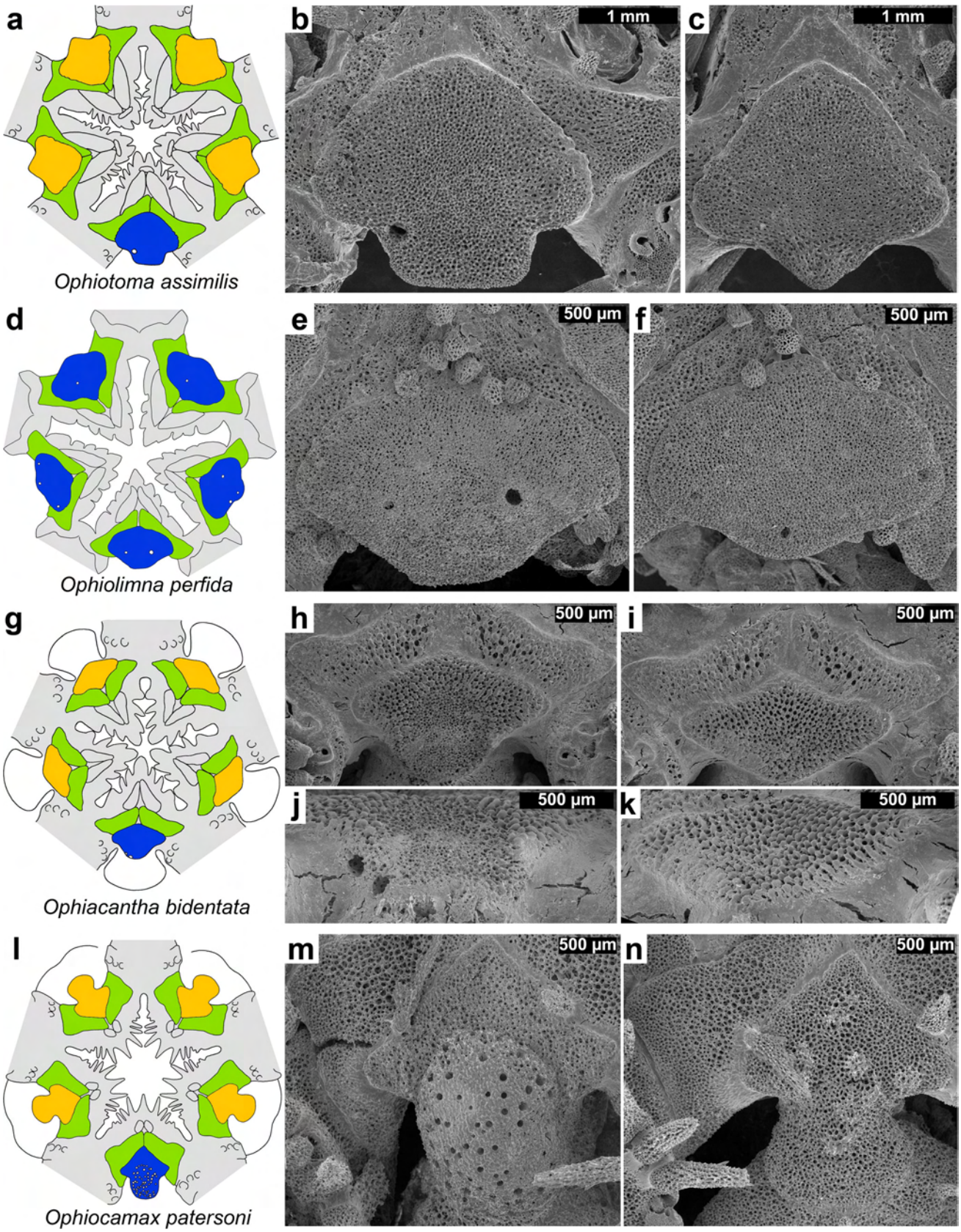


Fig. 5 Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in three species of Ophiomyxidae. *Ophiologimus cf. secundus* (**a**–**d**): **b** one madreporite with two pores (in one interradius); **c** interradius with two madreporites with one pore in each; **d** madreporite with one pore from another interradius. *Ophioplexa condita* (**e**–**g**); **f** madreporic shield with one pore on the side of radius D; **g** oral shield in interradius AE. *Ophioscolex glacialis* (**h**–**k**): **i** view of the madreporic shield with numerous pores from the oral side of the animal; **j** view of the oral shield in interradius DE from the oral side of the animal; **k** view of the madreporic shield in more little specimen with single pore on the side of radius D

it is normal for this species to have one pore in every madreporite. All madreporites are approximately the same in size and irregularly shaped (Fig. 5a). There are no additional oral shields in any other interradii.

Ophioplexa condita Martynov, 2010

The only madreporite is the same in shape and size as the oral shields in other interradii and differs slightly only by a



◀**Fig. 6** Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in four species of Ophiacanthidae. *Ophiotoma assimilis* (**a**–**c**): **b** madreporic shield with the pore on the side of radius D; **c** oral shield in interradius BC. *Ophiolimna perfida* (**d**–**f**): madreporic shields of different interradii (**e**, **f**). *Ophiacantha bidentata* (**g**–**k**): view of the madreporic shield from the oral side (**h**), and from the lateral side (**j**), there are two madreporic pores on the side of radius D; view of the oral shield in interradius DE from the oral side (**i**), and from the lateral side (**k**). *Ophiocamax patersoni* (**l**–**n**): **m** madreporic shield with numerous pores; **n** oral shield in other interradius

round distal edge (compared with a flattened distal edge of the oral shields) (Fig. 5e–g). There is a single large pore, located on the edge of madreporite from the side of the radius D (Fig. 5f).

Ophioscolex glacialis Müller & Troschel, 1842

The madreporic shield is slightly larger than other oral shields and has a different orientation in relation to the oral surface (Fig. 5h–j). The madreporite is located on the edge of the oral disk, and its lobed distal part extends over the edge of the disk (Fig. 5i). The madreporite is permeated by from 1 to 13 pores in different specimens (Fig. 5i, k). If there is a single pore, it lies closer to the D radius (Fig. 5k). If there are several pores, they are located on the proximal edge of the madreporite (Fig. 5i).

Family Ophiacanthidae Ljungman, 1867

Ophiotoma assimilis Koehler, 1904

The only madreporite differs slightly from the oral shields in other interradii by a bigger size and the shape of the distal part: in the madreporite, it is trapezoidal, while in the other oral shields—sagittate (Fig. 6a–c). The proximal region of the madreporic shield has a single, well-defined pore, lying in the radius D side (Fig. 6b).

Ophiolimna perfida Koehler, 1904

Each of the five interradii has a madreporite with 1–3 openings (Fig. 6d–f). Some of these openings (even within the same madreporite shield) look like pores penetrating the thick stereom; others are like grooves in the stereom, not penetrating the plate (Fig. 6e, f). The shape and size of the madreporites are roughly similar, so it is impossible to confidently identify the CD interradius.

Ophiacantha bidentata Bruzelius, 1805

The only madreporite is of similar size with oral shields in other interradii but has a slightly different form—the madreporite has a prominent distal blade (Fig. 6g–i). There are two large pores on the lateral edge of the madreporite from the side of the D radius (Fig. 6j).

Ophiocamax patersoni Martynov & Litvinova, 2008

The madreporite is very different from the oral shields in other interradii (Fig. 6l–n). Oral shields are shaped like spades and are 1.9 × 2.3 mm in size (in our case) (Fig. 6n). The madreporite is somewhat larger (in our case 2.2 × 2.4 mm), but the main difference is that the distal part of the madreporite significantly stands out in the size and structure of the stereom, which is swollen in the form of a hemisphere and riddled with numerous pores (Fig. 6m). The proximal part of the madreporite (the spike of the spade) has the same form as in the oral shields. Pores of the madreporite are positioned randomly across the surface of the hemispherical bulge (the base of the spade). All oral shields and the madreporite are adjacent to adoral shields by their sagittate proximal portions, but not by the distal bases (Fig. 6l–n).

Family Ophiocomidae Ljungman, 1867

Ophiocoma scolopendrina Lamarck, 1816

There is one madreporite which is 2.4 × 2.4 mm in size (in our case) and is much larger than the oral shields in other interradii (1.9 × 2.2 mm) (Fig. 7a–c). Besides that, the madreporite slightly differs from the oral shields by an asymmetrical shape—an extension toward the radius D (Fig. 7b). At the edge of this extension, on the side, there is a single pore (Fig. 7b, d).

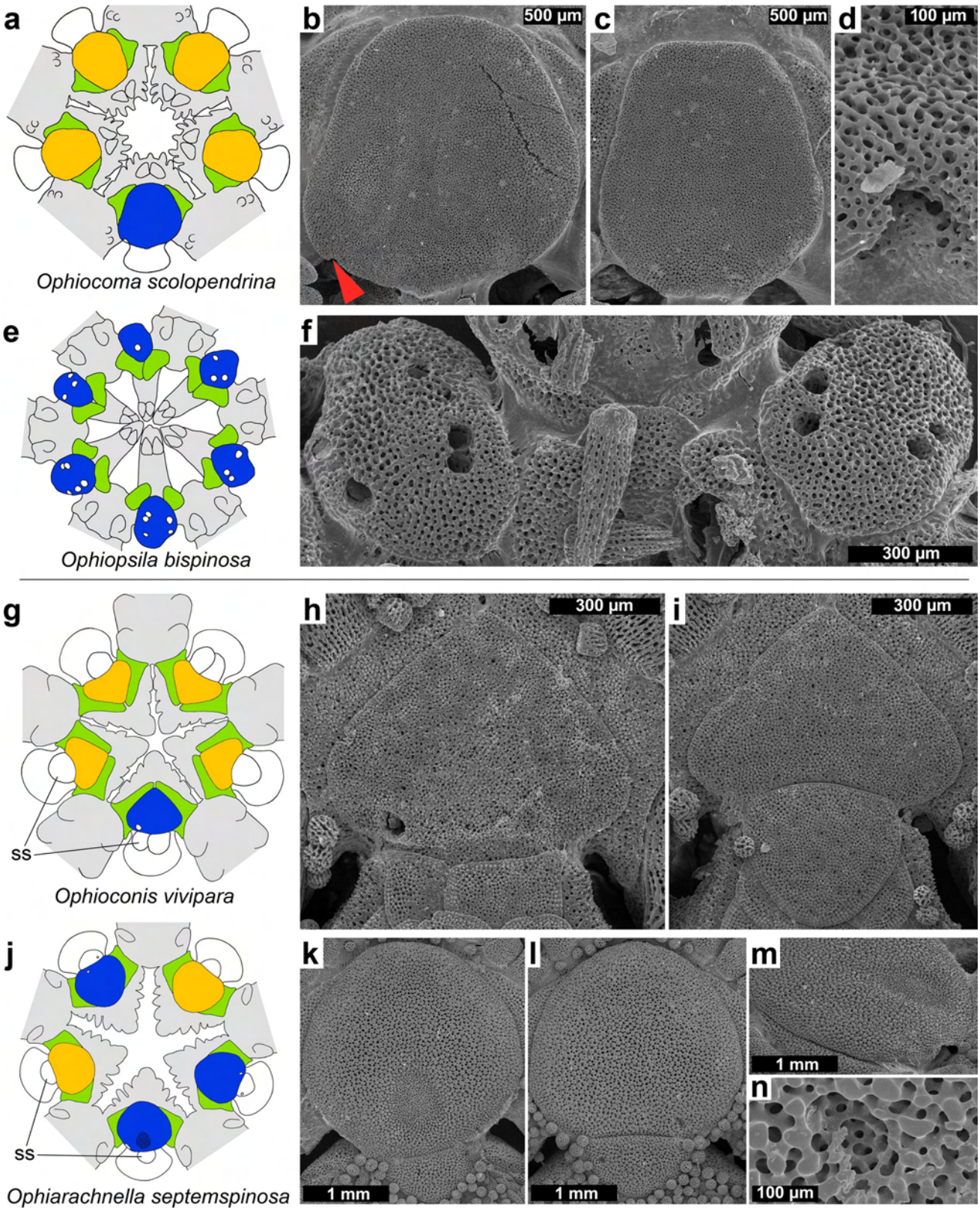
Ophiopsila bispinosa A.M. Clark, 1974

We examined a specimen with six-rayed symmetry; each of the six interradii has a madreporite, pierced by large, rounded pores in an amount of from 1 to 6 (Fig. 7e, f). The pores in each madreporite are arranged randomly, but generally, on the periphery of the shield. The madreporites are more or less oval in shape and approximately of similar size (Fig. 7f). There are no additional oral shields in interradii.

Family Ophiodermatidae Ljungman, 1867

Ophioconis vivipara Mortensen, 1925

The only madreporite has approximately the same size as the oral shields in other interradii (Fig. 7g–i). Oral shields are heart shaped with a proximal tip and a concave distal edge (Fig. 7i). The madreporite, unlike the oral shields, has on the contrary, a convex distal edge (Fig. 7h). Both the madreporite and the oral shields on the distal edge are



◀**Fig. 7** Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in two species of Ophiocomidae and two species of Ophiodermatidae. *Ophiocoma scolopendrina* (**a**–**d**): **b** madreporic shield from the oral side, *arrow* shows the pore on the side of radius D; **c** oral shield in interradius BC; **d** view of the madreporic pore from the lateral side. *Ophiopsila bispinosa* (**e**, **f**): **f** madreporites with numerous pores in two adjacent interradii. *Ophioconis vivipara* (**g**–**i**): **h** madreporic shield with the pore on the side of radius D; **i** oral shield in interradius BC; both the madreporite and the oral shields are adjacent to supplementary shields (*ss* on the scheme). *Ophiarachnella septemspinosa* (**j**–**n**): view of the madreporic shield from the oral side (**k**), and from the lateral side (**m**) with the pore on the side of radius D; **l** view of the oral shield in interradius BC; both the madreporite and the oral shields are adjacent to supplementary shields (*ss* on the scheme); **n** small opening on the oral shield

adjacent to one or two supplementary shields (Fig. 7g–i). A single, well-defined pore, located on the distal edge side of radius D, permeates the madreporite (Fig. 7h).

Ophiarachnella septemspinosa (Müller & Troschel, 1842)

The dimensions of the single madreporite coincide with the size of the oral shields in other interradii (Fig. 7j–l). The shape of the madreporite differs by a more rounded distal edge (Fig. 7k). Both the madreporite and each oral shield are distally adjacent to one semicircular supplementary shield (Fig. 7j–l). The distal region of the madreporite has a rounded area of depression (Fig. 7k, m), devoid of any marked pores. The only large pore is located distally on the side of the madreporite, closer to the radius D (Fig. 7m). The studied specimen had asymmetrically located small openings in the distal part of the oral shields in the BC and AE interradii (Fig. 7j, l). These openings are much smaller than madreporite pores (Fig. 7n). These small openings are also present on the supplementary shields, adjacent to oral shield in these interradii (Fig. 7l).

Family Ophioleucidae Matsumoto, 1915

Ophiostriatus sp.

There is a madreporite in each of the five interradii, penetrated by several (5–10) pores (Fig. 8a, b). All madreporites are characterized by a triangular shape and are approximately the same size. No other large oral shields are present in the interradii.

Ophiernus adspersus Lyman, 1883

The only madreporite does not differ from the oral shields in other interradii (Fig. 8c–e). The shape of the madreporite and the oral shields is also similar; the madreporite is just a little more convex on the distal side (Fig. 8d, e). The only pore of the madreporite is located on its side, in its distal part, near the D radius (Fig. 8f).

Family Ophiolepididae Ljungman, 1867

Ophiolepis superba H.L. Clark, 1915

There is one madreporite. In other interradii, there are oral shields that are slightly smaller than the madreporite, but identically shaped like a shield (Fig. 8h–j). In addition, the proximal part of the madreporite has a rounded depression area (Fig. 8i, k). On the edge of the madreporite, on the side of the D radius, there is a single pore in the central area, which is not shifted to the distal edge (Fig. 8k). From the oral side, the pore is noticeable only by a small notch at the edge of madreporite (Fig. 8i).

Family Ophionereididae Ljungman, 1867

Ophionereis schayeri Müller & Troschel, 1844

The single madreporite is slightly larger than the oral shields in other interradii (Fig. 8m–o). Both the madreporite and the oral shields are oval in shape, tapering slightly in the proximal region. The madreporite is permeated with pores (the studied specimen had no less than 17), which are located on the edge of the distal half of the madreporite (Fig. 8n). The pores are arranged more or less evenly by both the radius D and the radius C.

Family Ophiotrichidae Ljungman, 1867

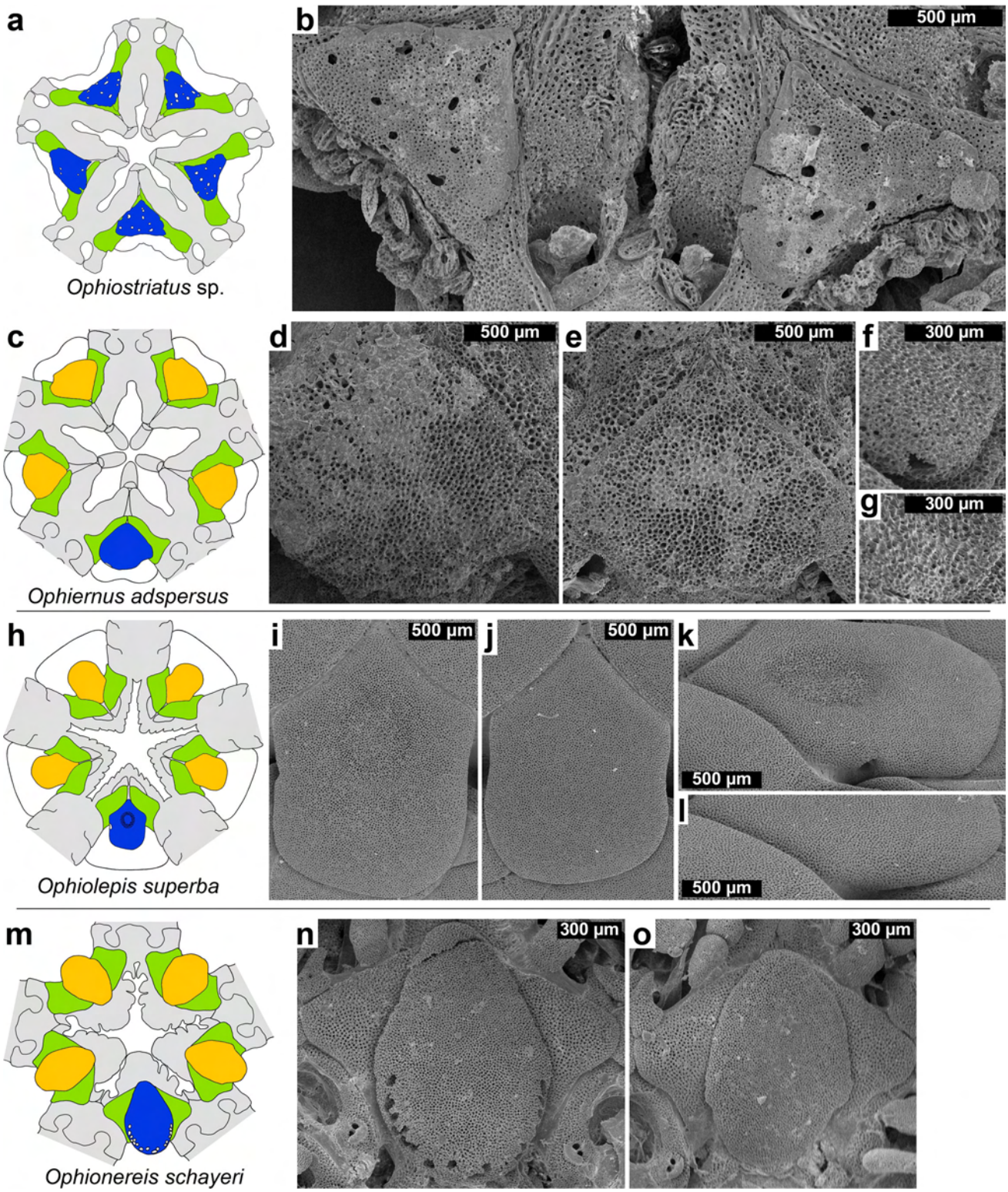
Ophiothrix fragilis Abildgaard, in O.F. Müller, 1789

The only madreporite is slightly different from the oral shields in other interradii (Fig. 9a–c). It is slightly larger and has a bulge on the distal side (Fig. 9b). The only pore in the madreporite lies next to the distal bulge, on the side of the D radius (Fig. 9b).

Family Ophiactidae Matsumoto, 1915

Ophiactis balli W. Thomson, 1840

The only madreporite is much larger than the oral shields in other interradii and has a more rounded shape and is asymmetrical (Fig. 9d–f). There is only one well-defined pore situated at the distal edge of the madreporite closer to the D radius (Fig. 9e, g).



◀**Fig. 8** Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in representatives of three families. *Ophiostriatus* sp. (Ophioleucidae) (**a**, **b**): **b** madreporites with numerous pores in two adjacent interradii. *Ophiernus adspersus* (Ophioleucidae) (**c**–**g**): view of the madreporic shield from the oral side (**d**), and from the lateral side (**f**) with pore on the side of radius D; view of the oral shield in interradius BC from the oral side (**e**), and from the lateral side (**g**). *Ophiolepis superba* (Ophiolepididae) (**h**–**l**): view of the madreporic shield from the oral side (**i**), and from the lateral side (**k**) with the pore on the side of radius D; view of the oral shield in interradius BC from the oral side (**j**), and from the lateral side (**l**). *Ophionereis schayeri* (Ophionereididae) (**m**–**o**): **n** madreporic shield with the pores along the distal edge of the madreporite; **o**, oral shield in interradius BC

Ophiopholis aculeata Linnaeus, 1767

There is one madreporite. It is also larger than the oral shields in other interradii and has a rounded shape with moderate asymmetry (Fig. 9h–j). The distal edge of the madreporite is extended on the side of the radius D. On the side of this expansion, only one pore is located, invisible from the oral side (Fig. 9i, k).

Ophiothamnus affinis Ljungman, 1872

The only madreporite is clearly visible due to its large size and a cone-like elevation of the distal edge, from the side of the D radius (Fig. 9l–n). Oral shields in other interradii have a symmetrical, rounded distal edge (Fig. 9n). At the top of the elevation of the cone-like madreporite opens a single pore (Fig. 9o).

Family Amphiuridae Ljungman, 1867

Dougaloplus derjugini Djakonov, 1949

The only madreporite (in our case 1.2 × 0.9 mm) is much larger than the oral shields in other interradii (0.85 × 0.6 mm) (Fig. 10a–c), although the shape of all five shields is similar and has the same shape as the shell of the *Sphaerium* bivalve. In the distal region of the madreporite on the side of the D radius, there is a single large pore (Fig. 10b).

Amphiura chiajei Forbes, 1843

The madreporite has more rounded shape (especially on the distal side) compared to the other oral shields in other interradii (Fig. 10d–f). Oral shields are smaller and have a strict diamond shape (Fig. 10f). A single small pore lies in the distal part of the madreporite, from the side of the D radius (Fig. 10f, g).

Amphiura diomedeae Lütken et Mortensen, 1899

The single madreporite is significantly larger (in our case 1.50 × 1.56 mm) than the oral shields in other interradii (1.2 × 1.2 mm) (Fig. 10h–j). At the distal edge, the madreporite has several pores (in our case 7 or 8). The pores are located almost symmetrically both from the side of the D radius and the C radius (Fig. 10i).

Family Amphilepididae Matsumoto, 1915

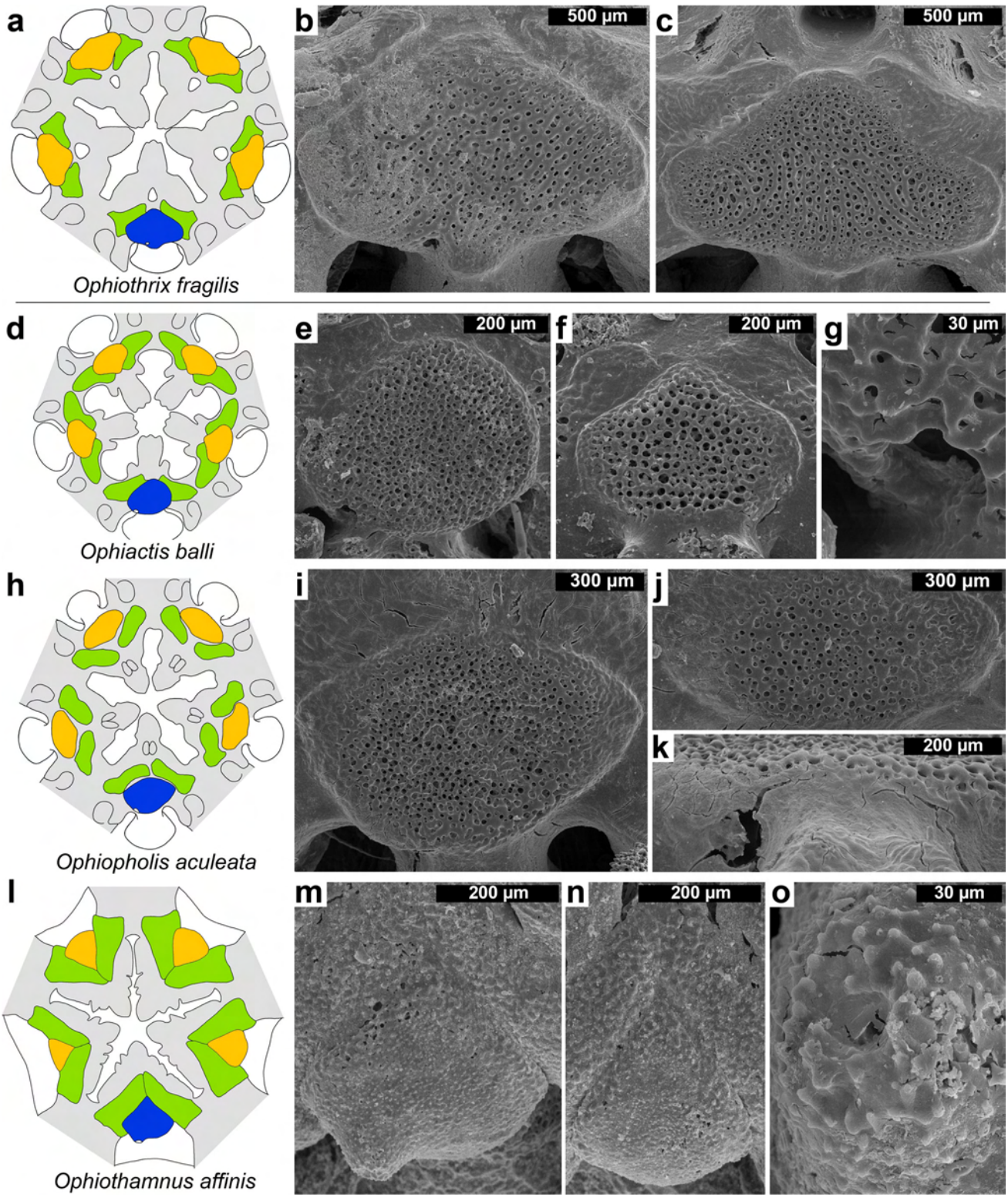
Amphilepis platytata H.L. Clark, 1911

The only madreporite differs slightly in size from the oral shields in the other interradii (Fig. 10k–m). At the distal end of the madreporite, there is a small swelling from the side of the D radius, and the only pore is located there (Fig. 10l, n).

Discussion

If we combine our results with the modern phylogenetic data of brittle stars (O'Hara et al. 2014), we will find that morphological diversity of madreporites in each of the three clades of Ophiuroidea (Fig. 11). The madreporites with numerous pores, the structured oral shields in other interradii and multiple madreporites (i.e., madreporites in each interradii) occur in the representatives of all three clades.

In the clade A, the madreporite is pierced by numerous pores in 7 of 10 studied species. The condition with numerous openings in the madreporite clearly prevails. In two species, the madreporites are in each interradius. In the review by Cuénot (1948, p. 259), it is stated that all brittle stars with multiple madreporites have a separate stone canal extending from each of the madreporite, which all fuse with the ambulacral ring. The number of axial organs is the same as the number of stone canals. Stewart (2000) reported that in *Astrobrachion constrictum* (Asteroschematidae), a stone canal rises from each of the five madreporites to the ambulacral ring, and each stone canal connects on the oral side with the axial coelom. It is significant that only in Euryalida, which is sister clade to Ophiuridae + Ophiomusium, the definite oral shields are absent in all interradii except CD. Whereas in the family

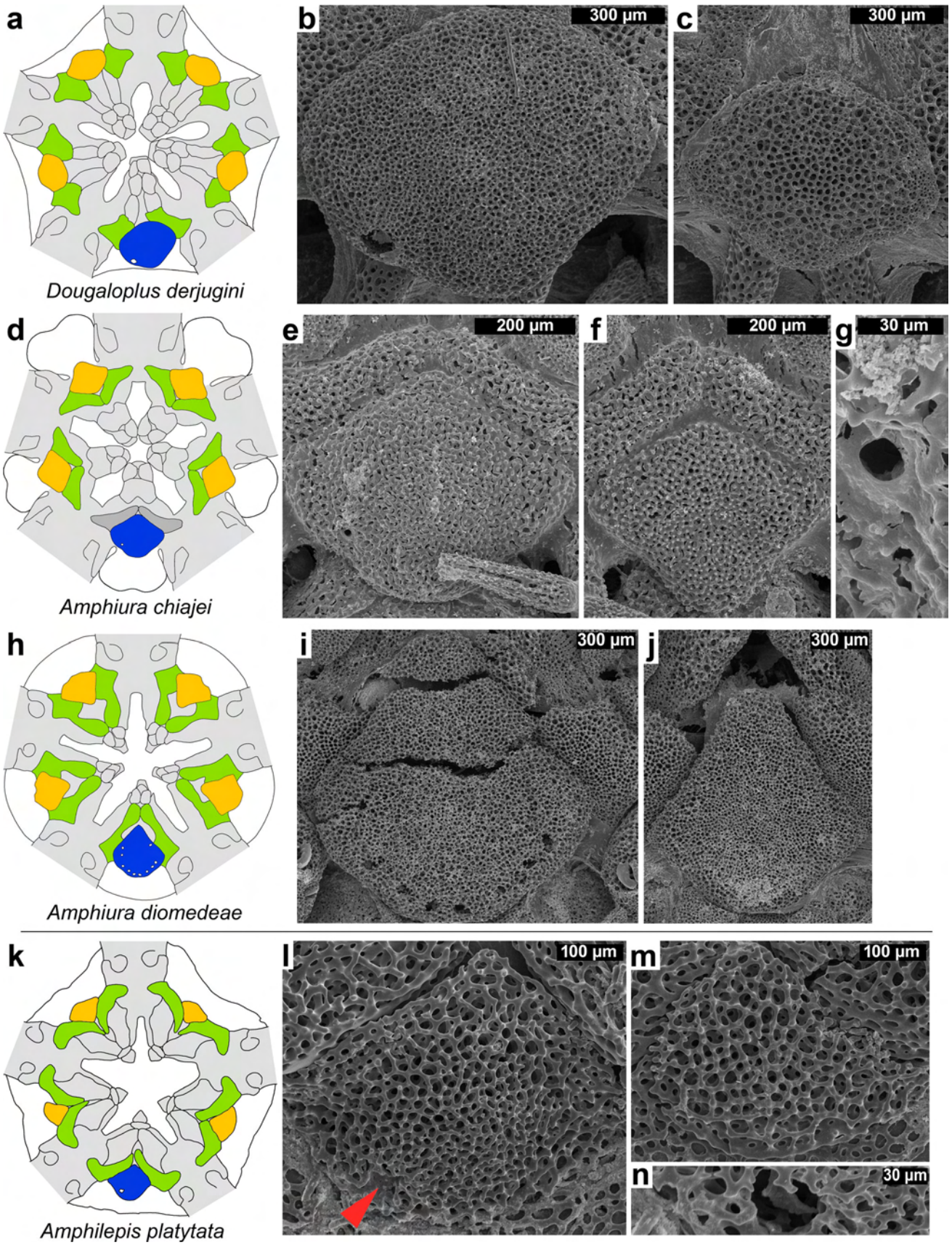


◀**Fig. 9** Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in one species of Ophiotrichidae and three species of Ophiactidae. *Ophiothrix fragilis* (**a**–**c**): **b** madreporic shield with the pore on the side of radius D; **c** oral shield in interradius BC. *Ophiactis balli* (**d**–**g**): **e** madreporic shield with the pore on the side of radius D (**g**); **f** oral shield in interradius AE. *Ophiopholis aculeata* (**h**–**k**): view of the madreporic shield from the oral side (**i**), and from the distal side (**k**) with the pore on the side of radius D; **j** oral shield in interradius DE. *Ophiothamnus affinis* (**l**–**o**): **m** view of the madreporic shield from the oral side; **n** oral shield in interradius; **o** madreporic pore on the cone-like elevation of the madreporite on the side of radius D, view from the distal side

Ophiuridae (as in the clades B and C), the oral shields are well developed. However, a relationship between euryalids and the Ophiuridae was shown on the basis of micromorphological evidence (Martynov 2010). The absence of oral shields in Euryalida could be a plesiomorphic feature as well as an apomorphic loss. A Paleozoic *Aganaster gregarius* from the lower Carboniferous possessed well-formed oral shields (Thuy et al. 2015).

In the clade B, 4 of 11 studied species have madreporites with numerous openings, and in *Ophiacantha bidentata* (Ophiacanthidae), there are two pores in the madreporite. However, in different specimens of *Ophioscolex glacialis* (Ophiomyxidae), there can be one pore as well as numerous openings in the madreporite. In 4 of 11 species within the clade B, there are madreporites in every interradius. At the same time in *Ophiopsila bispinosa* and *Ophiologimus cf. secundus*, the formation of multiple madreporites can probably be attributed to the ability to autotomize. In according to other papers, the madreporite of *Ophiopsila annulosa* (Ophiocomidae) can have from 3 to 12 pores (Reichensperger 1908). For *Ophioderma appressa* (Ophiodermatidae), two pores in the madreporite have been described (Ferguson 1995).

In the clade C, 3 of 12 studied species possess a madreporite with numerous openings and there is only one representative with multiple madreporites. In *Ophionereis annulosa* (Ophionereididae), eight pores in the madreporite have been described (Cuénot 1948). According to our data, another species of this genus—*Ophionereis schayeri*—has at least 17 pores in the madreporite. In *Ophiactis virens* (Ophiactidae), there are up to five madreporites (Hyman 1955), although the studied *O. balli* has the only madreporite with a single pore. However, Hyman (1955) reports that originally these brittle stars had one stone canal and one madreporite, and the formation of multiple madreporites can probably be attributed to the ability to autotomize. A similar phenomenon is also observed in other



◀**Fig. 10** Schemes of general view of the oral disk (the *left column*), and the external structure of the madreporite in comparison with the structure of the other interradius in three species of Amphiuridae and one species of Amphilepididae. *Dougaloplus derjugini* (**a**–**c**): **b** madreporic shield with the pore on the side of radius D; **c** oral shield in interradius BC. *Amphiura chiajei* (**d**–**g**): **e** madreporic shield with the pore on the side of radius D (**g**); **f** oral shield in interradius AE. *Amphiura diomedeae* (**h**–**j**): **i** madreporic shield with the numerous pores along the distal edge; **j** oral shield in interradius BC. *Amphilepis platytata* (**k**–**n**): **l** madreporic shield (the pore is shown by an *arrow*); **m** oral shield in interradius BC; **n** madreporic pore on the side of radius D, view from the distal side

groups of echinoderms such as asteroids (*Coscinasterias tenuispina*, *C. acutispina*, young *Sclerasterias heteropeas* and *S. empleciae*—Hyman 1955; Ivanova-Kazas 1978).

Thus, multiple madreporites and numerous madreporic pores appear to have evolved several times in different clades. This may be an adaptation to ecological conditions or to the behavior of the animals. Hence, the hypothesis that madreporite morphology is phylogenetically informative must be rejected as madreporites are highly homoplasious.

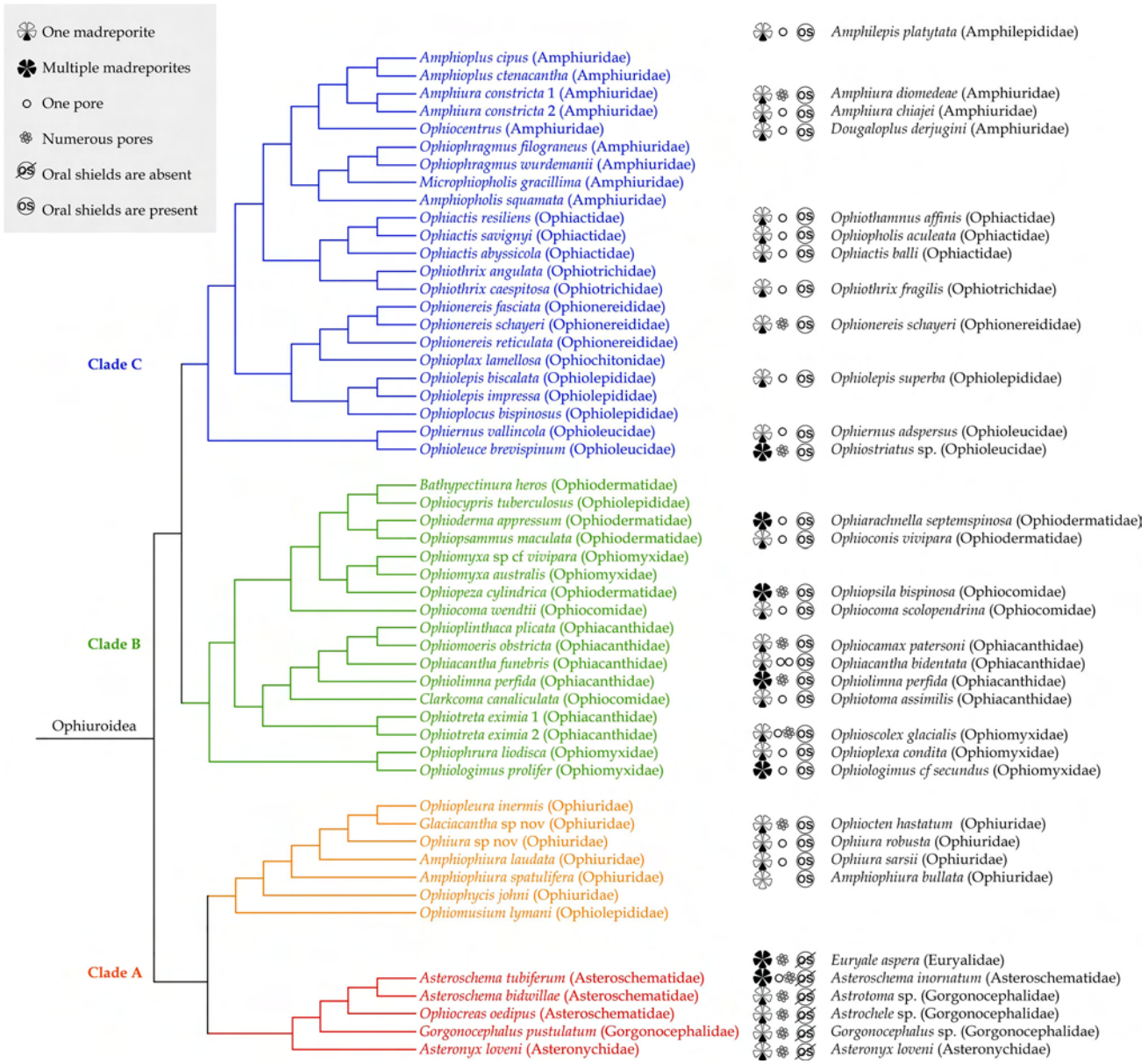


Fig. 11 Combination of our results (the *right column*) with the ophiuroid phylogenomic tree (the *left column*—O'Hara et al. 2014)

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial or non-financial interests.

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