Filling a blank in bivalve taxonomy: an integrative analysis of Cyamioidea (Mollusca: Bivalvia)

DIEGO G. ZELAYA[1,2*,] MARINA GÜLLER[1,2] and CRISTIÁN ITUARTE[3]

[1]*Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento Biodiversidad y Biología Experimental, Ciudad Universitaria, Pabellón 2, 4° Piso, Buenos Aires, Argentina*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[3]*Formerly: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) at Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina*

This contribution provides the first integrative study of Cyamioidea, a group of bivalves largely neglected in the past. By combining information obtained from shell morphology, gross anatomy, histology, reproductive biology and DNA sequencing, the distinctive characters of Cyamioidea are determined, and the taxa belonging to this superfamily are revised. Gaimardioidea, previously considered as a distinct superfamily, is reduced to family level (Gaimardiidae), which, along with Cyamiidae, are assigned to Cyamioidea. All studied cyamioideans share a common reproductive character: the presence of a true follicular epithelium surrounding each developing oocyte, which persists after spawning, surrounding the developing embryos and participating in their anchorage to the gill filaments. Several morphological and anatomical characters support the distinction of Gaimardiidae and Cyamiidae. Based on that information, the placement of *Gaimardia* and *Kidderia* in Gaimardiidae, and that of *Cyamiocardium*, *Cyamiomactra*, *Cyamium*, *Heteromactra*, *Pseudokelly*, *Ptychocardia* and *Reloncavia* in Cyamiidae, is confirmed. *Jukesena*, historically placed in Veneroidea, is here allocated to Cyamiidae. Neoleptonidae, previously also attributed to Cyamioidea, are reallocated to Veneroidea.

ADDITIONAL KEYWORDS: Cyamiidae – Gaimardiidae – Gaimardioidea – *Jukesena* – Neoheterodontei – Neoleptonidae.

INTRODUCTION

At present, Cyamioidea Sars, 1878 appears to be one of the most poorly understood superfamilies of marine bivalves. This is attributable not only to limited knowledge of their diversity, but also because of their unclear faunistic affinities (Ponder & de Keyzer, 1998). This superfamily has been and continues to be in a state of flux (Morton, 2015), and there is no general consensus among authors about which taxa should be included in this group, which is currently suspected to be polyphyletic (Salas & Gofas, 1998).

Thiele (1934) used Cyamioidea to accommodate Cyamiidae Sars, 1878, Sportellidae Dall, 1899 and Neoleptonidae Thiele, 1934. This proposal received general acceptance and remains as such in the opinion of some authors (e.g. Ponder & de Keyzer, 1998; Morton, 2015), although others proposed the removal of Neoleptonidae from Cyamioidea (e.g. Salas & Gofas, 1998; Mikkelsen *et al.*, 2006). In addition, since Thiele's proposal, several other families have been added by different authors to Cyamioidea: Basterotiidae Cossmann, 1909 (Bieler *et al.*, 2010); Bernardinidae Keen, 1969 (Coan, 1984; Salas & Gofas, 1998); Galatheavalvidae Knudsen, 1970 (Bieler *et al.*, 2010); Turtoniidae Clark, 1855 (Chavan, 1969; Vokes, 1980; Ponder & de Keyzer, 1998); and Perrierinidae Marwick, 1928. Perrierinidae was regarded as a synonym of Cyamiidae by Ponder (1971). The inclusion or exclusion of these families in Cyamioidea did not receive a general consensus.

Cyamioideans, like almost all (if not all) other bivalve taxa, were defined originally based on shell morphology. However, in this case, there was originally an incorrect interpretation in the number of hinge teeth of *Cyamium antarcticum* Philippi, 1845 (the

*Corresponding author. E-mail: dzelaya@bg.fcen.uba.ar

type species of the genus) by Philippi (1845, 1847), which was subsequently followed by Sars (1878) when erecting Cyamiidae. Chavan (1969) provided an alternative interpretation of the hinge teeth of *Cyamium* Philippi, 1845, but this is not correct. The previous (and frequent) misinterpretations of the morphology of the hinge plate of this genus, and the subsequent description of several genera with a hinge morphology identical to that of *Cyamium antarcticum*, seem to be the origin of the current poor understanding of Cyamiidae. There were also discrepant opinions on the affinities of *Cyamium* with these 'morphologically similar' genera. In this regard, a total of 21 (sub) genera attributed to Cyamiidae are mentioned in the literature (summarized in Table 1).

The global confusion surrounding the identity of *Cyamium* and, consequently, the diagnostic characters for Cyamiidae, is also evidenced at higher taxonomic levels. Thiele (1934) regarded Cyamiacea as a different 'stirps' from Gaimardiacea. This conception was followed by Vokes (1980) and Chavan (1969) and is also in agreement with the usage by Bieler *et al.* (2010), Carter *et al.* (2011) and Lemer *et al.* (2018), who referred to them as Cyamioidea and Gaimardioidea, respectively. Alternatively, Ponder (1971) regarded

these taxa as two subfamilies of Cyamiidae: Cyamiinae and Gaimardiinae. Such different opinions have resulted in discrepancies on whether some (sub) genera correspond to Cyamiidae, Gaimardiidae or another family, even to a different superfamily. For instance, this is the case in *Pseudokellya* Pelseneer, 1903, regarded as Cyamiidae by Thiele (1934) and Zelaya & Ituarte (2009), but as Lasaeidae Gray, 1842 (Galeommatoidea) by Chavan (1969) and Engl (2012). Likewise, *Kidderia* Dall, 1876 was historically regarded as closer to *Gaimardia* Gould, 1852 than to *Cyamium* (e.g. Thiele, 1934; Chavan, 1969; Vokes, 1980), but in the most recent views it is mentioned as being closer to *Cyamium* (e.g. Ponder, 1971).

To date, there are no molecular studies focussing on Cyamioidea. Only two cyamioidean species have been studied thus far, with their sequences included in broad-scope phylogenies of bivalves. This has resulted in discrepant opinions. Taylor *et al.* (2007: 587) concluded in the placement of 'the Gaimardiidae group near to the Ungulinidae and not with Cyamioidea where most recently classified'. Bieler *et al.* (2014) and Combosch *et al.* (2017) recovered Cyamioidea, comprising Cyamiidae and Gaimardiidae, as sister to Ungulinidae.

Table 1. (Sub)genera included in the Cyamiidae or Gaimardiidae by previous authors and/or in the present study

Genera	Thiele (1934)	Vokes (1980)	Chavan (1969)	Huber (2010)	Present study
Costokidderia	= *Kidderia*	Gaimardiidae	Gaimardiidae	Cyamiidae*	Gaimardiidae?
Cyamiocardium	–	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Cyamiomactra	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Cyamionema	–	Cyamiidae	Galeommatidae	Cyamiidae*	Gaimardiidae?
Cyamium	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Dicranodesma	–	Cyamiidae	Cyamiidae	–	Lasaeidae?
Eugaimardia (replacement name for *Neogaimardia* Cotton, 1931)	Gaimardiidae	Gaimardiidae	Gaimardiidae	Cyamiidae	Gaimardiidae?
Gaimardia	Gaimardiidae	Gaimardiidae	Gaimardiidae	Cyamiidae	Gaimardiidae
Heteromactra	–	Cyamiidae	= *Cyamiomactra*	= *Cyamiomactra*	Cyamiidae
Jukesena	Veneridae	Veneridae	Veneridae	Veneridae	Cyamiidae
Kidderia	Gaimardiidae†	Gaimardiidae	Gaimardiidae	Cyamiidae	Gaimardiidae
Legrandina	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae‡	Cyamiidae
Lutetina	Neoleptonidae	Cyamiidae	Cyamiidae	Neoleptonidae	Neoleptonidae
Neogaimardia	Gaimardiidae	Gaimardiidae	Gaimardiidae	Cyamiidae†	Gaimardiidae?
Perrierina	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Phaseolicama	Gaimardiidae[2]	= *Gaimardia*	= *Gaimardia*	= *Gaimardia*	Gaimardiidae
Progaimardia	–	–	–	Cyamiidae†	Gaimardiidae?
Pseudokellya	Cyamiidae	Kelliidae	Kelliidae	–	Cyamiidae
Ptychocardia	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Reloncavia (replacement name for *Kingiella* Soot-Ryen, 1957)	–	Cyamiidae	Cyamiidae	Cyamiidae	Cyamiidae
Turtonia	Cyamiidae?	Turtoniidae	Turtoniidae	Turtoniidae	Veneridae *s.l.*

When the name is regarded as a junior synonym, the senior name is provided in the table.
Symbols indicate that the name was considered a subgenus of the following: *Kidderia, †Gaimardia and ‡Perrierina.

Ponder (1971) stated that 'it is only through a study of the soft parts that the correct systematic position of many species [of Cyamioidea] will be found'. However, anatomical studies on this taxon are currently restricted to a limited group of species: *Gaimardia trapesina* (Lamarck, 1819) (Gould, 1852; Rousseau, 1854; Pelseneer, 1903; Igel, 1908a, b), two species of *Neogaimardia* (Odhler, 1924; Morton, 1979), two species of *Pseudokellya* (Pelseneer, 1903; Zelaya & Ituarte, 2009) and five species of *Cyamiocardium* (Soot-Ryen, 1951; Passos & Machado, 2014; Urcola & Zelaya, 2018). Odhler (1924) provided some additional information on the gross anatomy of *Kidderia campbellica* Odhler, 1924, and Ponder (1971) on *Cyamium antarcticum*, *Cyamiomactra problematica* (Bernard, 1897) and *Kidderia auporia* Powell, 1933.

Concerning reproductive biology, the current knowledge of this group is far from being complete. It was assumed that Cyamioidea are usually brooders (Ponder & de Keyzer, 1998). However, detailed information on the reproductive characteristics of representatives of this group is available for only a few species (Pelseneer, 1903; Simpson, 1977; Ituarte, 2009; Zelaya & Ituarte, 2009; Chaparro *et al.*, 2011; Passos & Machado, 2014; Urcola & Zelaya, 2018).

The aim of the present study was to take an integrative approach to the characteristics that allow Cyamioidea to be defined, to re-evaluate which taxa belong to this superfamily and to re-examine the relationship between Cyamioidea and Gaimardioidea. In this context, and considering the previous misinterpretations of the hinge conformation of *Cyamium antarcticum*, the hinge of this species is studied in detail and redescribed.

MATERIAL AND METHODS

The main source of information for the present study comes from the material collected personally during several field trips in the last 15 years, along the coasts of Patagonia, the Scotia Arc Islands and Antarctica, from the intertidal zone to 402 m depth. Additional information comes from the malacological collections at Museo Argentino de Ciencias Naturales, Buenos Aires (MACN); Museo de La Plata, La Plata (MLP); United States National Museum, Smithsonian Institution, Washington (USNM); Los Angeles County Museum of Natural History (LACM); Museum für Naturkunde, Berlin (ZMB); The Natural History Museum, London (NHMUK); Naturhistoriska riksmuseet, Stockholm (NRM); National Museum of Scotland, Edinburgh (NMS) and Museo Nacional de Historia Natural, Santiago de Chile (MNHN). These collections are also the repository of several types, studied here for comparative purposes.

A total of 21 species, representing nine (sub) genera attributed to Cyamioidea *s.l.* (*Cyamium*, *Cyamiocardium*, *Cyamiomactra*, *Gaimardia*, *Heteromactra*, *Kidderia, Neolepton*, *Pseudokellya* and *Ptychocardia*), corresponding mostly to the type species, are studied here (Table 2). We refer to *Heteromactra* as a separate genus, instead of treating it as a synonym of *Cyamiomactra* (as previously proposed by some authors; see Table 1). This is because of the lack of molecular information on the type species of *Cyamiomactra* to confirm this proposed synonymy. Furthermore, *Jukesena foveolata* (Dell, 1964), currently regarded as a member of Veneridae, is also included in this study. Species of *Neolepton* are considered here only for molecular studies; the shell morphology and anatomy of members of this genus are described by Salas & Gofas (1998) and Zelaya & Ituarte (2004).

SHELL MORPHOLOGY

The number and morphology of the hinge teeth, ligaments and their supports are studied in detail. Different interpretations and nomenclatures have been provided in the literature concerning the teeth of cyamioideans (see Bernard, 1897 vs. Lamy, 1917 vs. Ponder, 1971). In this study, we refer to those teeth located immediately under the umbones and radiating from it as 'cardinals'. The cardinals are referred to as 'anterior', 'median' and 'posterior' (see Fig. 1A, C), following the recent usage by Urcola & Zelaya (2018), which is also in agreement with the 't', 's' and 'r' nomenclature by Ponder (1971). Other morphological characters considered herein include the shell outline and sculpture and the position of the umbones.

GROSS ANATOMY

Information on the gross anatomy is derived mainly from dissections of specimens, decalcified by immersion in a solution of 10% formalin with the addition of 5% of acetic acid, performed under a stereoscopic microscope. The number and extension of mantle margin fusions and openings, the presence of tentacles along the mantle margin, the morphology of the foot and its associated musculature, and the characteristics of ctenidia and labial palps are investigated.

Additional observations come from living specimens of *G. trapesina* and *Cyamiocardium rotundatum* (Thiele, 1912) maintained in the laboratory.

HISTOLOGICAL STUDIES

Histological procedures were performed to study the following features: the number and morphology of mantle margin folds, the folds involved in mantle

Table 2. Material considered for the different studies performed

Species	Provenance	Repository/voucher/accession number	Shell morphology	Gross anatomy	Histology	Reproductive characters	DNA 28S	16S
Cyamium (type species: *Cyamium antarcticum* Philippi, 1845)								
Cyamium antarcticum Philippi, 1845	'Magellanstrasse' (= Estrecho de Magallanes)	MNHN without number (possible syntypes)	X					
	'Magellan'	MNHN 5865	X					
	Malvinas/Falkland Islands	MACN-In 10165; MACN-In 10172	X	X				
	Argentina, Tierra del Fuego, Rio Grande (53°47′S, 67°42′W)	MACN-In 30527	X	X				
	Estrecho de Magallanes, Punta Remo, intertidal (52°42′S, 69°39′W)	LACM 54567	X					
Cyamiocardium (type species: *Cyamium denticulatum* E.A. Smith, 1907)								
Cyamiocardium denticulatum (E.A. Smith, 1907)	South Shetland, King George Island (62°15′S, 58°51′W), 50 m	ZMB-Moll 114.653	X	X	5	X		
	South Shetland, 62°36.2′S, 61°39.8′W, 130 m	MACN-In 42737; MN423267, MN423268, MN423280, MN423281	X				X	X
	Antarctica, Ross Sea (77°50′S, 166°39′W)	NHMUK 1905.9.25.25 (lectotype)	X					
Cyamiocardium rotundatum (Thiele, 1912)	South Orkneys, 60°59.19′S, 43°27.42′W, 402 m	MACN-In 41443	X	X				
	Antarctica, Davis Sea (66°12′S, 89°48′E)	ZMB-Moll 63116 (holotype)	X					
Cyamiocardium dahli Soot-Ryen, 1957	Argentina, Tierra del Fuego, Bahía Sloggett, 55°00′S, 66°20.6′W, 15–30 m	MLP-Ma 14505	X	X	7	X		
	Argentina, Tierra del Fuego, 53°07′41.3″S, 65°47′08.4″W, 294 m	MACN-In 42738; MN423270, MN423271, MN423282, MN423283					X	X
	Chile, Golfo de Ancud, 41°44.17′S, 73°15,25′W, 15 m	NRM 3893, NRM 5940, NRM 5941 (holotype and paratypes)	X					
Cyamiocardium crassilabrum Dell, 1964	Burdwood Bank, 53°55′45.6′S, 61°32′42.8′W, 196 m	MACN-In 42739	X	X	2	X		
	Burdwood Bank, 54°45′23.7′S, 59°51′02.9′W, 174 m	MACN-In 42740; MN423269; MN423279					X	X
	Malvinas/Falkland Islands, 50°17′S, 60°06′W, 161–174 m	NHMUK 1962863 (holotype)	X					

Table 2. Continued

Species	Provenance	Repository/voucher/accession number	Shell morphology	Gross anatomy	Histology	Reproductive characters	DNA 28S	DNA 16S
Cyamiocardium chuanisinense Urcola & Zelaya, 2018	Isla de los Estados, 54°51′S, 64°30′W, 60 m	MACN-In 41433	X	X	7	X		
	Isla de los Estados, 54°51′S, 64°30′W, 60 m	MACN-In 41423, MACN-In 41424, MLP-Ma 14510 (holotype and paratypes)	X					
Cyamiocardium namuncurense Urcola & Zelaya, 2018	Burdwood Bank, 54°01,36′S, 62°01.33′W, 272 m	MACN-In 41425 (paratypes)	X	X		X		
Cyamiocardium yeskumaala Urcola & Zelaya, 2018	Cape Horn, 55°44.2′S, 66°14.9′W, 428 m	ZMB-Moll 103.539c (paratypes)	X	X				
	Argentina, Tierra del Fuego, 55°05′56.5″S, 66°26′44.6″W, 67 m	MACN-In 42741	X		2	X		
Cyamiomactra (type species: *Cyamiomactra problematica* F. Bernard, 1897)								
Cyamiomactra problematica Bernard, 1897	Chatham Island (43°58′S, 175°45′W)	USNM 680093	X					
Gaimardia (type species: *Modiola trapesina* Lamarck, 1819)								
Gaimardia trapesina (Lamarck, 1819)	Canal Beagle, Ea El Tunel, 54°49′10″S, 68°10′24.3″W, intertidal	MACN-In 42742; MN423276, MN423288	X				X	X
	Argentina, Santa Cruz Province, Puerto Deseado, 47°45′S, 65°55′W	MACN-In 42743	X	X	10	X		
Gaimardia mesembrina (Melvill & Standen, 1907)	Isla de los Estados, Puerto Vancouver (54°47′S, 64°15′W)	MACN-In 33032	X	X	6	X		
	Malvinas/Falkland Islands, Port Stanley (51°41′S, 57°50′W)	NMS 1921-143-703 (syntypes)	X					
Heteromactra [type species: *Mactra (Heteromactra) laminifera* Lamy, 1906]								
Heteromactra laminifera Lamy, 1906	South Shetland, 63°02.4′S, 60°39.1′W, 132 m	MACN-In 42744; MN423277, MN423289	X	X	1	X	X	X
Kidderia (type species: *Kidderia minuta* Dall, 1876)								
Kidderia minuta Dall, 1876	Isla de los Estados, Puerto Vancouver (54°47′S 64°15′W)	MACN-In 33032-2	X	X	6	X		
	Kerguelen Islands (49°15′S, 69°10′W)	USNM 11908 (syntypes)	X					
Kidderia bicolor (Martens, 1885)	South Georgia, Cumberland Bay (54°14′S, 36°28′W)	MACN-In 13525	X	X	2	X		

Table 2. Continued

Species	Provenance	Repository/voucher/accession number	Shell morphology	Gross anatomy	Histology	Reproductive characters	DNA 28S	16S
Pseudokellya (type species: *Kellia cardiformis* E.A. Smith, 1885)	South Georgia (54°19'S, 36°39'W)	ZMB-Moll 37486 (syntypes)	X					
Pseudokellya cardiformis (E.A. Smith, 1885)	South Orkneys, 60°53'S, 44°33'W, 218 m	MACN-In 42745	X	X	1	X		
Pseudokellya inexpectata Dell, 1964	South Shetland, 64°24.7'S, 64°19.2'W, 165 m	MACN-In 42746	X	X	1	X		
	South Georgia (54°19'S, 36°39'W)	NHMUK 1962885/1/2 (holotype and paratype)	X					
Pseudokellya franki Zelaya & Ituarte, 2009	South Shetland, 62°10.19'S, 58°35.58 W, 100 m	ZMB-Moll 114680-a, ZMB 114680-b; MLP 12997, MACN-In 37535 (holotype and paratypes)	X	X	6	X		
Ptychocardia (type species: *Ptychocardia vanhoeffeni* Thiele, 1912)								
Ptychocardia georgiana (Dell, 1964)	South Georgia, 54°30'S, 35°50'W, 94 m	MACN-In 42747	X	X	1	X		
Jukesena (type species: *Psephis foveolata* Cooper & Preston, 1910)								
Jukesena foveolata (Cooper & Preston, 1910)	Canal Beagle, Playa Yámanas, 54°50'17.3796''S, 68°21'38.4552''W, intertidal	MACN-In 42748	X	X				
	Canal Beagle, Bahía Golondrina, 54°49'59''S, 68°18'55''W, intertidal	MACN-In 42749; MN423278, MN423287	X			X	X	X
	Malvinas/Falkland Islands, 52°00'S, 65°42'W	LACM 118041	X					
Neolepton (type species: *Lepton sulcatulum* Jeffreys, 1859)								
Neolepton hupei Soot-Ryen, 1957	Isla de los Estados, 54°50.96'S, 63°52.016'W, 330 m	MACN-In 42750; MN423272, MN423273, MN423284, MN423285	X				X	X
Neolepton falklandicum Dell, 1964	Banco Burdwood, 54°35'50.2''S, 61°06'51.6''W, 168 m	MACN-In 42751; MN423274, MN423275, MN423286	X				X	X

Coordinates in parentheses indicate an external source of information. Numbers in the Histology column indicate the number of specimens sectioned.

margin fusions, the glandular elements of the foot, the structure (fusions and ciliature) of ctenidia and the reproductive characteristics of the species.

Specimens processed for histology were fixed in Bouin's solution, embedded in Historesin (Leica) and sectioned at a thickness of 3.5–4.5 µm with a motorized rotary microtome (Leica RM2255). Slides were stained with Haematoxylin and Eosin, Groat's hematoxylin, modified Masson's trichrome, periodic acid–Schiff (AB/PAS) or Toluidine Blue (Gabe, 1968). The numer of specimens studied histologically for each species is indicated in Table 2.

Molecular studies

Information on the taxa used for molecular studies is provided in Table 2.

Total DNA was extracted from foot and/or adductor muscles from ethanol-fixed specimens by using a cetyl trimethyl ammonium bromide (CTAB)/proteinase K protocol. Fractions of the nuclear ribosomal gene 28S (~1100 bp) and of the mitochondrial ribosomal gene 16S (~500 bp) were amplified with routine polymerase chain reaction (PCR) protocols using the primer pairs LSU5/D3R (Littlewood, 1994) and 16Sar/16Sbr (Palumbi et al., 1996), respectively. The PCR products were sent out to Macrogen Inc. (Korea) for sequencing in both directions. Sequences were trimmed and refined with chromatogram guidance before contig-building in MEGA v.10.0.5 (Kumar et al., 2018).

Molecular studies were performed for each marker individually, including additional sequences of the Neoheterodontei clade of Combosch et al. (2017), in addition to sequences of Ungulinidae and *Gomphina undulosa* (Lamarck, 1818), downloaded from GenBank. Species of Semelidae and Donacidae were selected as the outgroup to root the trees (based on the phylogeny provided by Combosch et al., 2017).

Alignments were performed with online MAFFT (Katoh et al., 2017) using default settings and viewed in MEGA for manual refinement by end trimming. Those taxa with sequences shortly overlapped (< 200 bp), sites with a majority of gaps (> 90% of sequences) and one 20-bp-long hypervariable region in 28S were deleted. The final alignments consisted of 45 taxa with 908 bp length for 28S and 51 taxa with 391 bp for 16S.

Maximum likelihood (ML) reconstructions were performed in PAUP* alpha-test v.4.0a164 (Swofford, 2003) through heuristic search, with the tree bisection and reconnection (TBR) branch-swapping algorithm. Node support was evaluated through 1000 fast bootstrap replicates performed ten times each and visualized in a 50% majority-rule consensus. Bayesian inference (BI) analyses were performed in MrBayes v.3.2 (Ronquist et al., 2012), with four simultaneous runs of 100 generations each and a sample frequency of 100, until the average standard deviation of split frequencies reached ≤ 0.001. Stationarity was also evaluated in TRACER v.1.6 (Rambaut et al., 2014), visualizing log-likelihood (LnL) vs. number of generation (state) plots and checking ESS values (>> 200). Phylogenetic trees were summarized in a 50% majority-rule consensus, with a 10% burn-in value. The best-fitting evolutionary models for each marker were selected under the Akaike information criterion (AIC), using jModelTest v.2.1.10 (Darriba et al., 2012) and MrModeltest v.2.4 (Nylander, 2004) to obtain the blocks for implementing the analyses in PAUP* and MrBayes, respectively. The model GTR+I+G was selected for 28S and TPM2uf+I+G (ML) and HKY+I+G (BI) for 16S.

RESULTS

Shell morphology

The hinge of Cyamium antarcticum

Cyamium antarcticum has a strong hinge plate, with three well-developed cardinal teeth in the left valve and two well-developed cardinals in the right valve, without distinct lateral teeth (Fig. 1A–C). The left valve cardinal teeth comprise a strong, triangular median tooth that is markedly enlarged ventrally, flanked by a somewhat strong, short anterior tooth and by a thin, low posterior tooth (Fig. 1A). The two cardinals of the right valve, similar in length, are fused dorsally; the posterior is uniform in width, the anterior distally enlarged (Fig. 1C). The left median tooth and the right anterior tooth are somewhat bifid. There is an internal and an external ligament (Fig. 1A, C). The external ligament, attached to a strong, projected nymph, is mostly opistodethic, slightly surpassing the umbones anteriorly. The internal ligament, which contacts the external ligament immediately below the umbones, is large and placed in an elongate, obliquely directed resilifer.

The hinge of the other taxa studied

Considering the general conformation of the hinge, the following four groups are recognized.

1. *Heteromactra* (Fig. 1D, F), *Cyamiomactra* (Fig. 1G, I), *Cyamiocardium* (Fig. 2A, C) and *J. foveolata* (Fig. 1J, L) closely resemble *Cyamium*. As in that genus, there are three well-developed cardinal teeth in the left valve and two in the right valve. The anterior cardinal of the left valve is short and strong, the median cardinal is strong and triangular, and the posterior cardinal is thin and low. In the right valve, the teeth are similar in length and appear
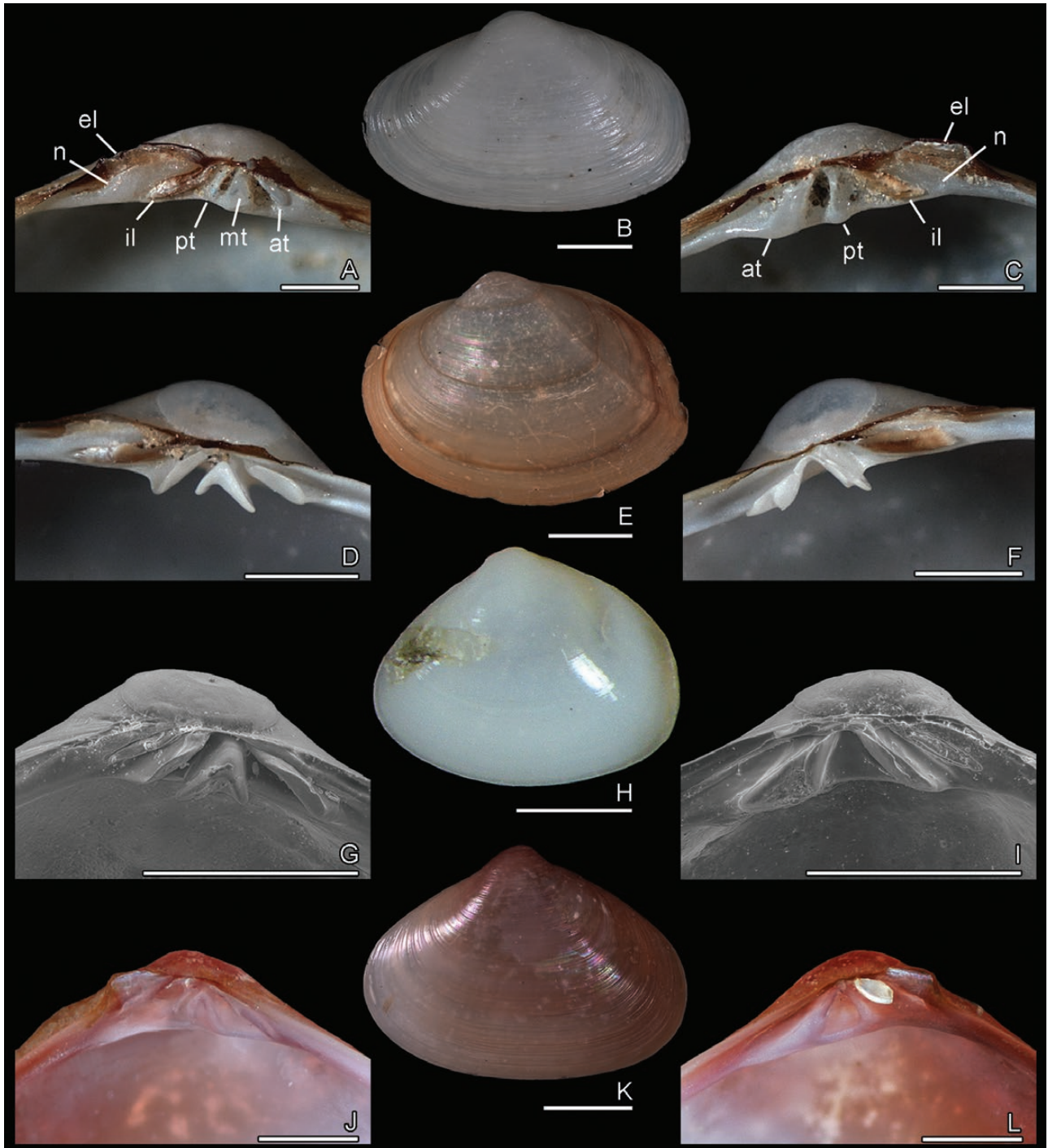
Figure 1. Shells of *Cyamium antarcticum* (A–C), *Heteromactra laminifera* (D–F), *Cyamiomactra problematica* (G–I) and *Jukesena foveolata* (J–L). A, D, G, J, detail of hinge plate, left valve. B, E, H, K, outer view, left valve. C, F, I, L, detail of hinge plate, right valve. Scale bars: 500 µm (A, C, D, F, G, I, J, L); 1 mm (B, H, K); 2 mm (E). Abbreviations: at, anterior tooth; el, external ligament; il, internal ligament; mt, median tooth; n, nymph; pt, posterior tooth.

fused dorsally. The left median tooth and the right anterior tooth are bifid; in *Heteromactra*, the posterior tooth of the right valve also shows this condition. There is an external and an internal ligament; the former is attached to a projected nymph, the latter placed in an elongate, obliquely
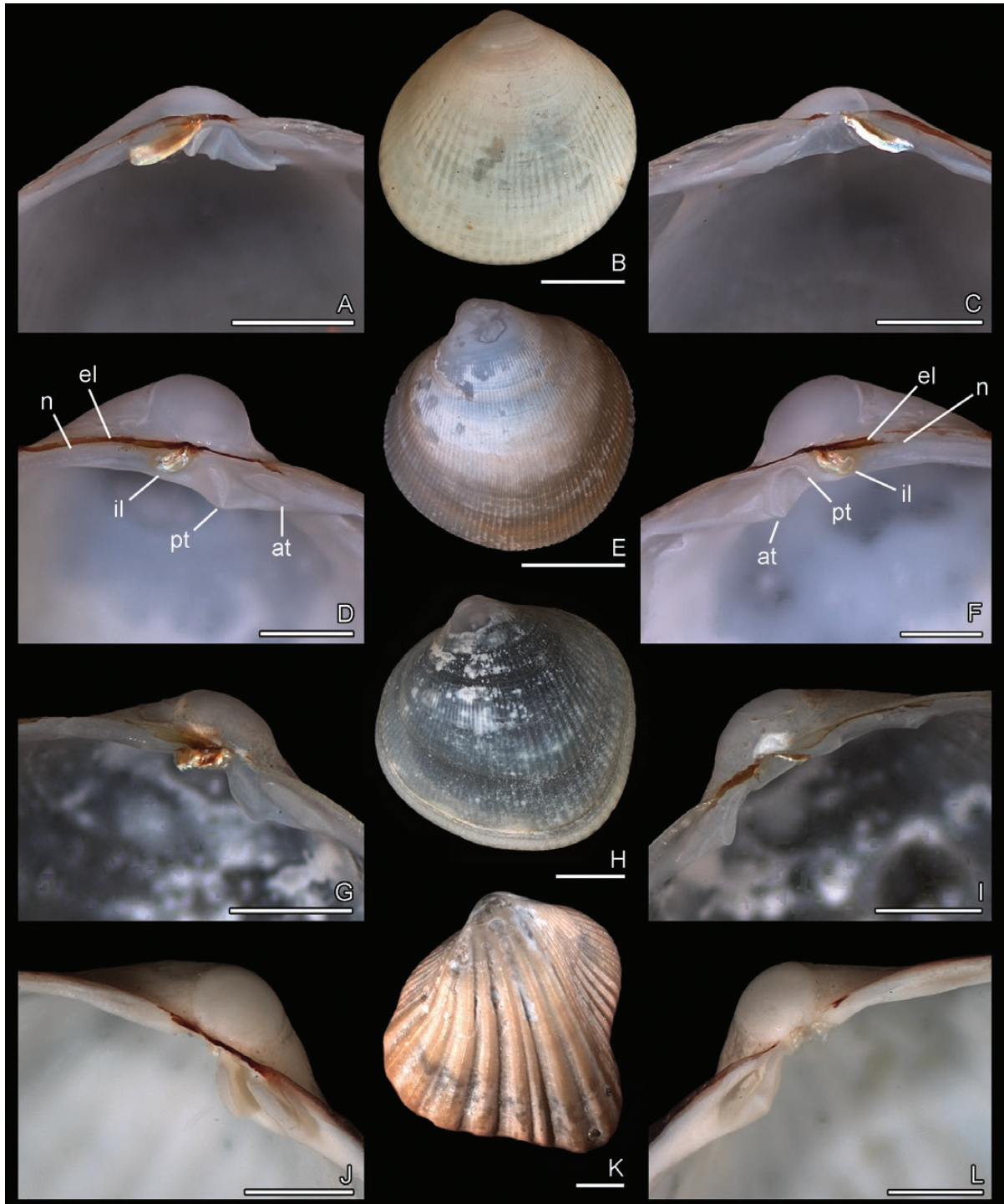
Figure 2. Shells of *Cyamiocardium yeskumaala* (A, C), *Cyamiocardium dahli* (B), *Pseudokellya cardiformis* (D–F), *Pseudokellya inexpectata* (G–I) and *Ptychocardia georgiana* (J–L). A, D, G, J, detail of hinge plate, left valve. B, E, H, K, outer view, left valve. C, F, I, L, detail of hinge plate, right valve. Scale bars: 500 µm (A, C, D, F, G, I, J, L); 1 mm (B, H, K); 2 mm (E). Abbreviations: at, anterior tooth; el, external ligament; il, internal ligament; n, nymph; pt, posterior tooth.

directed resilifer. Both ligaments are in contact immediately below the umbones. According to our observations of *Cyamiocardium*, both internal and external ligaments are formed by two main layers each, with the external ligament having an additional layer of periostracum (Fig. 4E, F).

2. *Pseudokellya* (Fig. 2D, F, G, I) and *Ptychocardia* (Fig. 2J, L) show two cardinal teeth in each valve. Left cardinals are large, elongate, (sub)parallel or divergent; those from the right valve are united dorsally to form a hook, sometimes with the posterior branch extremely reduced in size, appearing to be missing [as in *Pseudokellya inexpectata* Dell, 1964 and *Ptychocardia georgiana* (Dell, 1964)]. In this group, only the anterior tooth of the right valve is bifid. There is also an internal and an external ligament, although here the internal ligament is relatively small and located in a short resilifer; the external, slender ligament is attached to a flat nymph. According to the observations in *Pseudokellya*, the structure of the internal and external ligaments does not differ from that of the previous group.

3. *Kidderia* (Fig. 3A, C) has two minute ('tubercular') teeth in each valve, which appear to hang from the dorsal margin, owing to the extremely thin hinge plate below the umbones. The external ligament is attached to a strong, projected nymph, and the internal, massive ligament is located on an elongate,
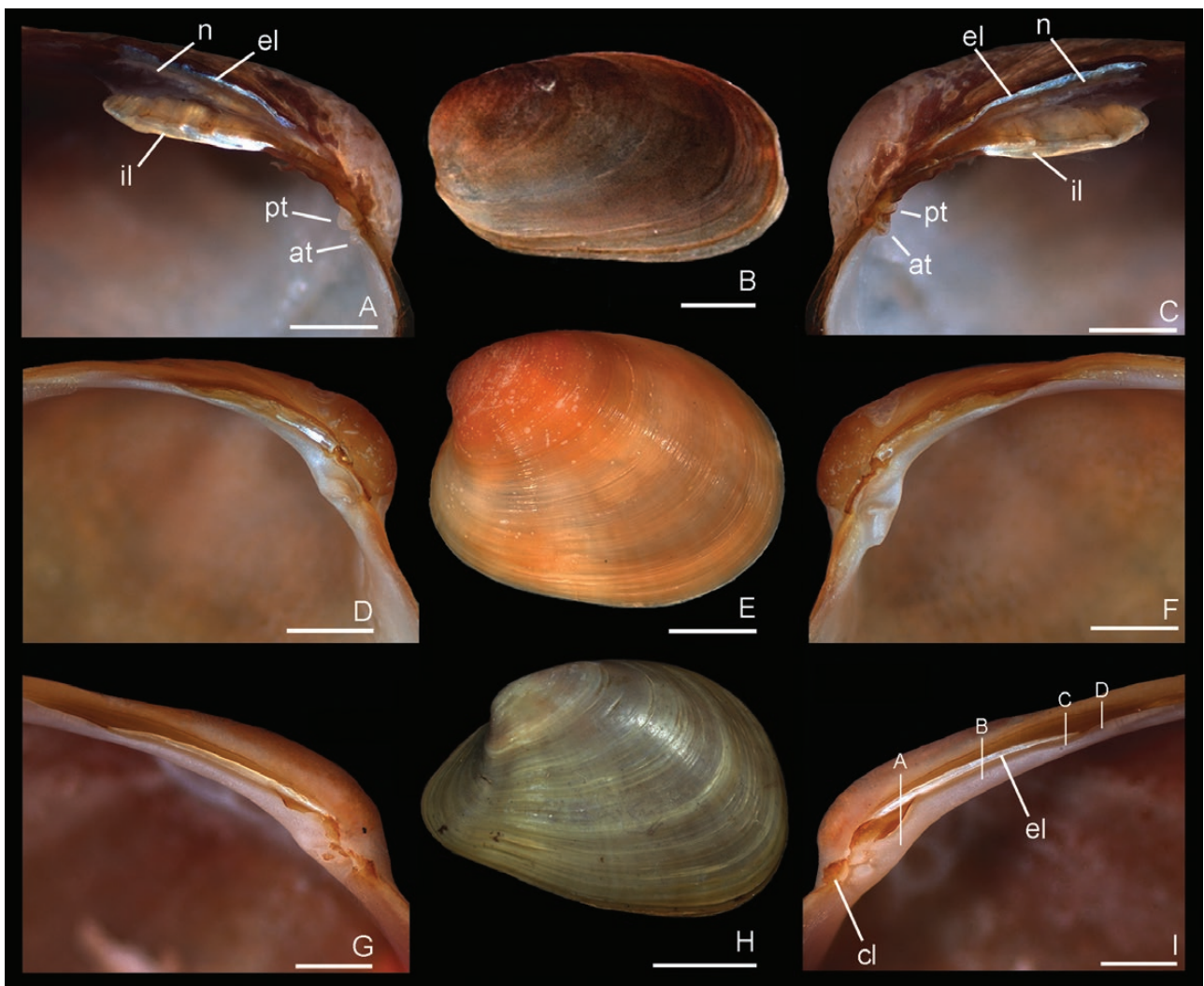

Figure 3. Shells of *Kidderia minuta* (A–C), *Gaimardia mesembrina* (D–F) and *Gaimardia trapesina* (G–I). A, D, G, detail of hinge plate, left valve. B, E, H, outer view, left valve. C, F, I, detail of hinge plate, right valve (capital letters in panel I refer to the details shown in Fig. 4A–D). Scale bars: 1 mm (A, D); 5 mm (G); 500 μm (B, C, E, F, H, I). Abbreviations: at, anterior tooth; cl, cardinal ligament; el, external ligament; il, internal ligament; n, nymph; pt, posterior tooth.
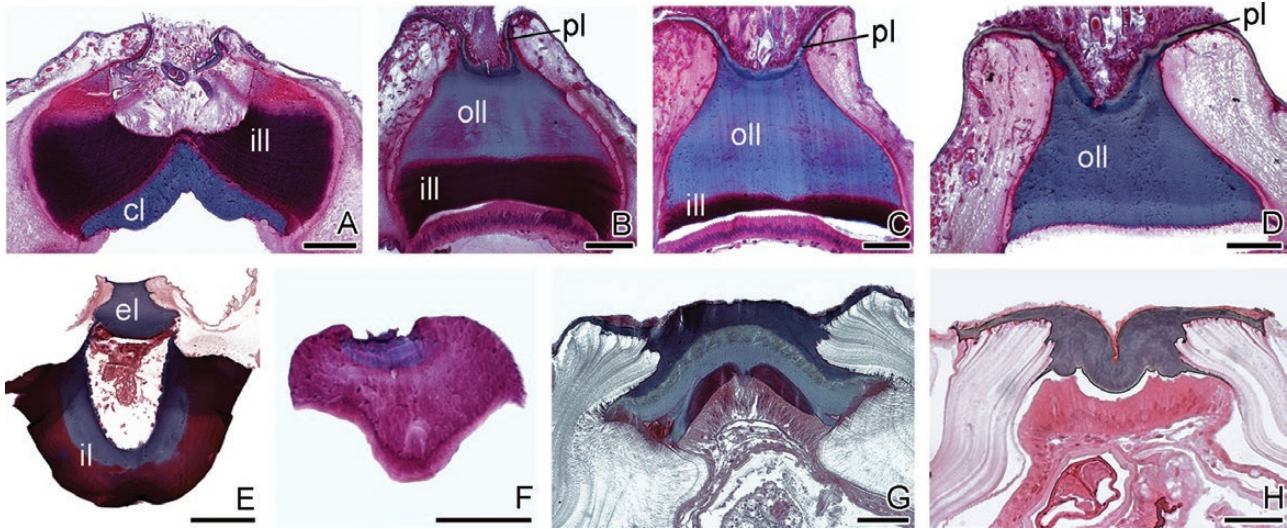
Figure 4. Ligament cross-sections of *Gaimardia trapesina* (A–D), *Cyamiocardium crassilabrum* (E), *Cyamiocardium dahli* (F), *Kidderia bicolor* (G) and *Kidderia minuta* (H). Scale bars: 50 μm (A–D, F–H); 100 μm (E). Histological stains: modified Masson's trichrome. Abbreviations: cl, cardinal ligament; el, external ligament; il, internal ligament: ill, inner ligamental layer; oll, outer ligamental layer; pl, periostracal layer.

obliquely directed resilifer. These ligaments are also similar in structure to those described for the two previous groups (Fig. 4G, H).

4. *Gaimardia* (Fig. 3D, F, G, I) also has two small teeth in each valve, but unlike *Kidderia*, in this case they are located on a relatively strong hinge plate. The external ligament is attached to a strong, projected nymph and, as in all previous cases, it is formed by two main layers, externally covered by the periostracum (Fig. 4A–D). In *Gaimardia*, the thickness of the inner and outer layers varies gradually from the anterior to the posterior ends of the ligament. At the anterior end, only the inner layer is visible, whereas at the posterior end only the outer layer is observed. These two layers disappear immediately below the umbones, where they are replaced by an additional, massive ligamental element (the so-called 'cardinal ligament' of Trueman (1949) or 'anterior outer layer' of Allen (1960); Figs 3I, 4A). The internal ligament is missing in the studied species of *Gaimardia*.

OTHER DISTINCTIVE MORPHOLOGICAL CHARACTERS

When considering the position of the umbo, two main groups are recognized: those taxa in which the umbones are (sub)centrally located, as in *Cyamiocardium*, *Cyamiomactra*, *Cyamium*, *Heteromactra*, *Jukesena*, *Pseudokellya* and *Ptychocardia* (Figs 1B, E, H, K, 2B, E, H, K), and those in which the umbones are strikingly displaced anteriorly, as in *Kidderia* and *Gaimardia* (Fig. 3B, E, H).

The characteristics of the periostracum are in concordance with the delimitation of the above-mentioned two groups: it is extremely thin and single layered in *Cyamiocardium* and *Heteromactra* (Fig. 6D), but thicker and two layered in *Gaimardia* and *Kidderia* (Fig. 6H, L).

MANTLE MARGIN

In the studied species of *Cyamiocardium*, *Cyamium*, *Gaimardia*, *Heteromactra*, *Jukesena*, *Kidderia*, *Pseudokellya* and *Ptychocardia*, the mantle margins are fused to each other (Fig. 5E, L–N), with this fusion involving only the innermost mantle folds (Fig. 6B, F, J; fusion 'type A' according to the nomenclature of Yonge, 1957). Two rows of tentacles are present at the posterior portion of mantle margin in all these taxa (Fig. 5C, F, J, K–N). The tentacles usually arise at the base of the inner mantle fold (Fig. 6B), although in the case of *Jukesena* they arise from the middle mantle fold (Fig. 5J).

Despite the overall similarity, several differences are found in the morphology of the mantle folds, as follows.

1. Number and morphology of the mantle margin folds vary. In *Gaimardia* and *Kidderia*, the inner, middle and outer mantle folds are, in transverse section, relatively short (Fig. 6E, F, I, J). Both genera show a narrow and simple middle fold and two outer wide folds (of-1 and of-2), markedly trigonal in transverse section; the middle fold is much shorter than the outer folds (Fig. 6G, K). The inner fold is
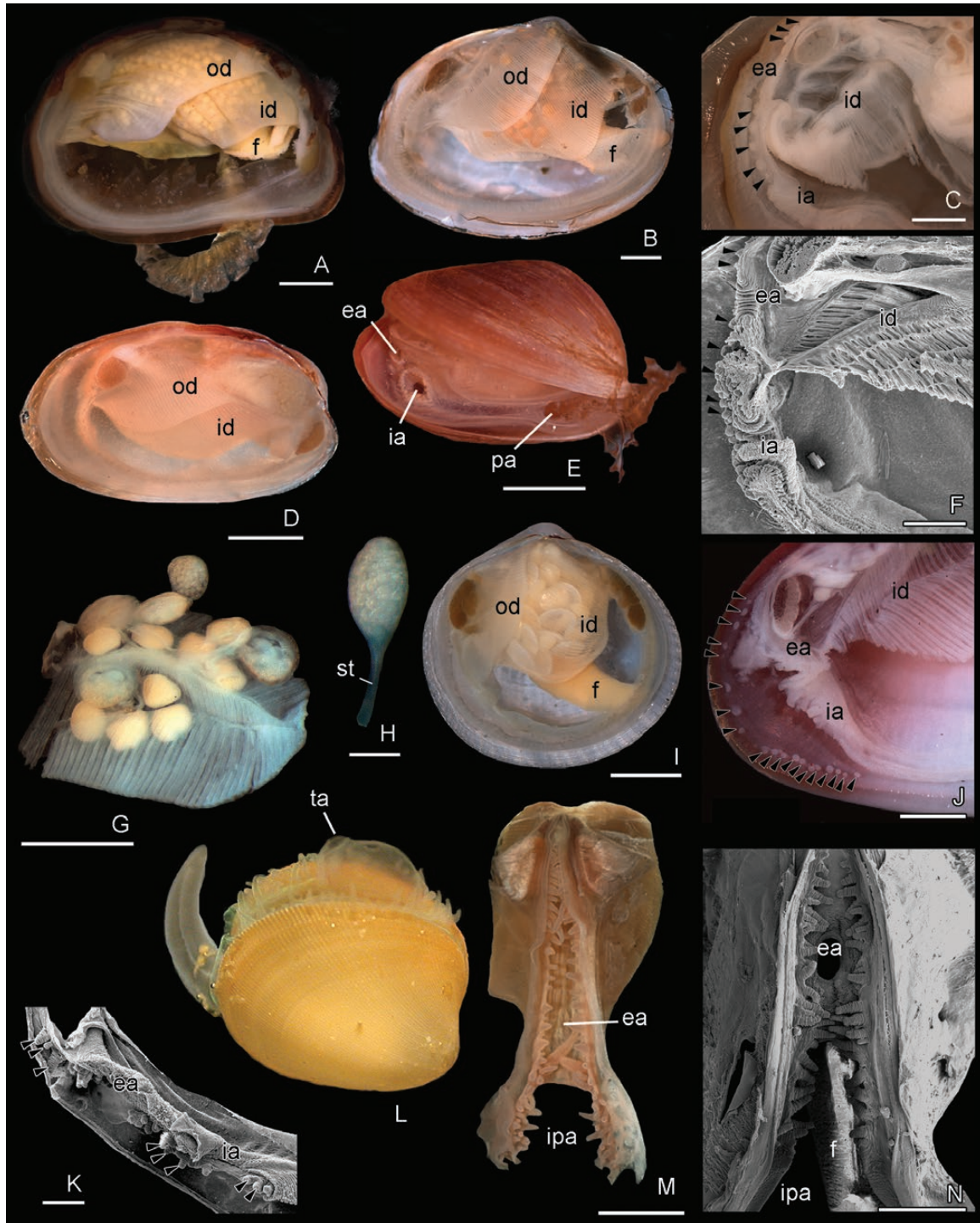
Figure 5. Gross anatomy. A, C, *Gaimardia trapesina*, partly dissected specimen with the right valve removed and posterior fusion of the gill to the mantle margin. B, *Heteromactra laminifera*, partly dissected specimen with the right valve removed. D–F, *Kidderia minuta*, partly dissected specimen with the right valve removed, detail of mantle margin openings (fixed specimen) and posterior fusion of the gill to the mantle margin. G, H, J, *Jukesena foveolata*, detail of a demibranch with brooded embryos, detail of an embryo and posterior fusion of the gill to the mantle margin. I, K, *Cyamiocardium yeskumaala*, partly dissected specimen with the right valve removed and detail of mantle margin openings (fixed specimen). L, *Cyamiocardium rotundatum*, detail of mantle margin openings (living specimen). M, *Ptychocardia georgiana*, detail of mantle
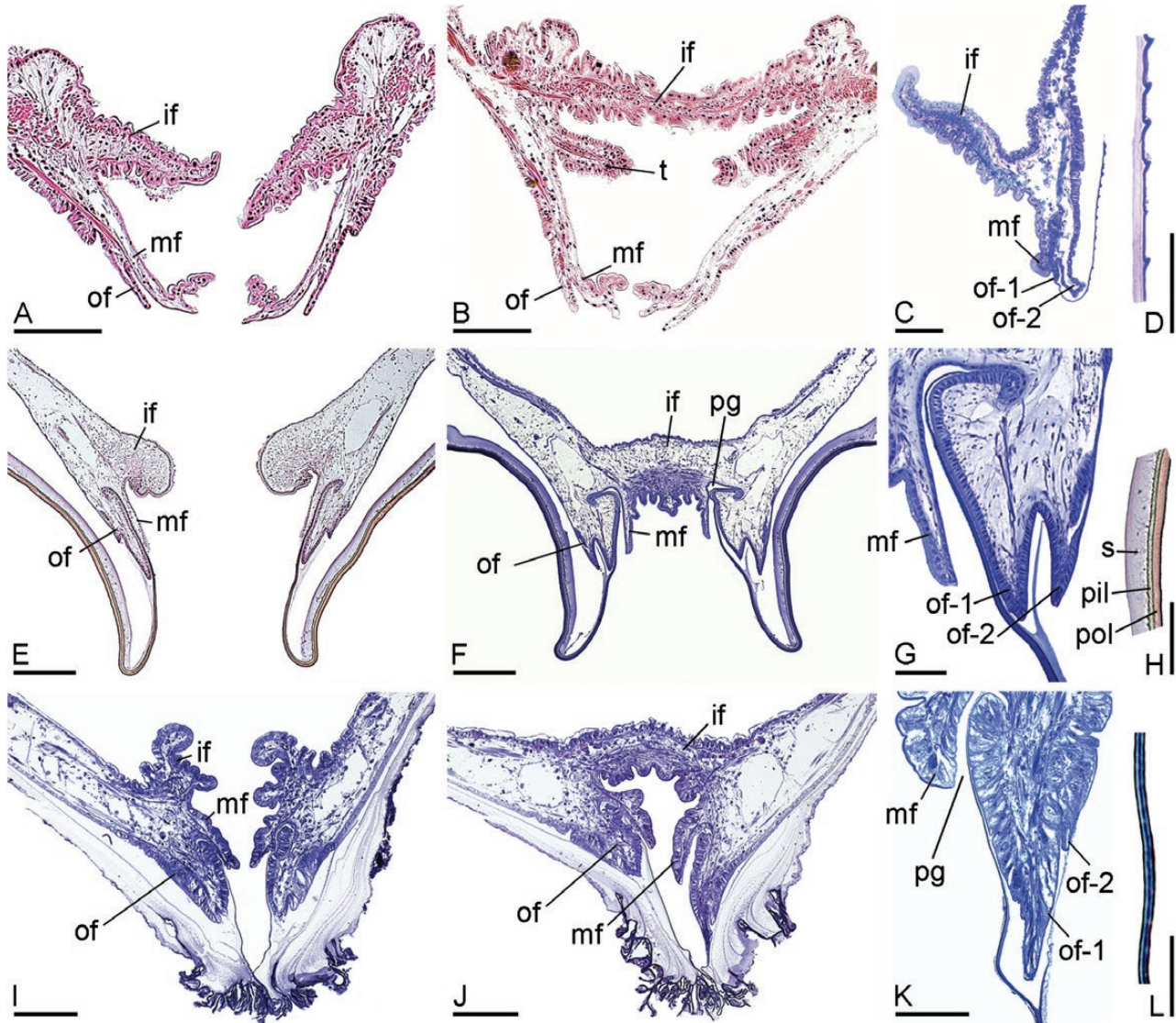
Figure 6. Detail of mantle margin (A–C, E–G, I–K) and periostracum (D, H, L) of *Cyamiocardium chuanisinense* (A, B), *Heteromactra laminifera* (C, D), *Gaimardia trapesina* (E–H) and *Kidderia bicolor* (I–L). A, C, E, I, mantle folds at the pedal aperture. B, F, J, mantle fusion between inhalant and pedal apertures. G, K, detail of the outer mantle fold. Scale bars: 100 µm (A–C, H–J); 50 µm (D, G, K); 200 µm (E, F); 20 µm (L). Histological stains: Haematoxylin and Eosin (A, B); Toluidine Blue (C, D, F, G, I–L); Periodic acid–Schiff (E, H). Abbreviations: if, inner mantle fold; mf, middle mantle fold; of, outer mantle fold(s); pg, periostracal groove; pil, periostracum inner layer; pol, periostracum outer layer; s, shell matrix; t, tentacle.

bilobed, with the dorsal lobe larger than the ventral one (Fig. 6I). In contrast, in *Cyamiocardium* (Fig. 6A, B), *Heteromactra* (Fig. 6C), *Pseudokellya* and *Ptychocardia* all the mantle folds are relatively long and narrow. The inner fold is also bilobed, showing in transverse section a rounded dorsal portion and a larger, elongated ventral portion; the middle fold is markedly elongated and is bifurcated at the tip (Fig. 6A, B). There is usually a single outer fold (Fig. 6A, B), although *Heteromactra* has two deeply

margin openings (fixed specimen). N, *Cyamiocardium denticulatum*, detail of mantle margin openings. Scale bars: 2 mm (A); 1 mm (B, D, E, G, I, M); 500 µm (C, J, N); 200 µm (F, H, K). Abbreviations: ea, exhalant aperture; f, foot; ia, inhalant aperture; id, inner demibranch; ipa, inhalant–pedal aperture; od, outer demibranch; st, stalk; ta, temporary aperture. Arrowheads indicate the posterior tentacles of the mantle margin.

separated elements (interpreted here as two folds; Fig. 6C). This/these outer fold(s) are consistently shorter than the middle fold.

2. The number of fusions and permanent openings varies. *Ptychocardia* has only two permanent openings (Fig. 5M): a large anteroventral ('inhalant–pedal') and a smaller posterior ('exhalant'). *Cyamium*, *Gaimardia*, *Heteromactra*, *Jukesena* and *Kidderia* have three permanent openings (Fig. 5E): an anteroventral ('pedal') and two posterior (one 'inhalant', the other 'exhalant'). In *Cyamiocardium* Soot-Ryen, 1951, some species show three permanent openings (i.e. *Cyamiocardium chuanisinense*, *Cyamiocardium crassilabrum*, *Cyamiocardium dahli*, *Cyamiocardium namuncurense* and *Cyamiocardium yeskumaala*), whereas others (*Cyamiocardium denticulatum* and *Cyamiocardium rotundatum*) have only two permanent openings (Fig. 5N). However, in the last case the species can form an additional, temporary opening (Fig. 5L). In *Pseudokellya* Pelseneer, 1903, some species have only two permanent openings (*Pseudokellya franki*), whereas others have three openings (*Pseudokellya cardiformis*). In living specimens of *Cyamiocardium rotundatum*, the exhalant aperture projects widely from the valves, forming a 'siphonal membrane' (according to the terminology of Salas & Gofas (1998); Fig. 5L). In living specimens of *G. trapesina*, the mantle margin around the exhalant aperture also projects slightly from the valves. In *J. foveolata*, both inhalant and exhalant apertures project, forming siphons.

3. The relative size of the mantle margin openings and the extension of the fusions vary. In *Cyamiocardium*, *Cyamium*, *Heteromactra*, *Jukesena*, *Pseudokellya* and *Ptychocardia*, the pedal/inhalant–pedal opening is the largest opening, extending from one-half to three-quarters of the total mantle margin length, and it is separated from the inhalant/exhalant opening by a relatively short fused area (Fig. 5L–N). On the contrary, in *Gaimardia*, the pedal aperture is short, extending for less than one-sixth of the total length of the mantle margin, and the inhalant aperture is the largest opening (about one-third of the total mantle margin length). This difference is determined not only by the length of the fusion between the inhalant and pedal apertures, but also by the length of the mantle fusion anterior to the pedal aperture. In *Kidderia*, the pedal opening remains as the largest aperture, although in this case (as in *Gaimardia*) there is a long, fused area between the inhalant and pedal openings (Fig. 5E).

4. The morphology and number of tentacles at the posterior end of the mantle margin vary. In *Gaimardia* and *Kidderia*, the tentacles are short and low in number, never more than ten pairs; they are restricted to the fused areas between the inhalant and exhalant openings and dorsal to the exhalant opening (Fig. 5C, F). In *Cyamiocardium*, *Cyamium*, *Heteromactra*, *Jukesena*, *Pseudokellya* and *Ptychocardia*, there is a relatively large number of elongated tentacles (≤ 40 pairs). In *Cyamium*, *Heteromactra*, *Jukesena* and some species of *Cyamiocardium* (*Cyamiocardium crassilabrum*, *Cyamiocardium chuanisinense*, *Cyamiocardium dahli*, *Cyamiocardium namuncurense* and *Cyamiocardium yeskumaala*), the tentacles are present only dorsal to the pedal opening (Fig. 5K). In *Cyamiocardium denticulatum*, *Cyamiocardium rotundatum*, *Ps. franki* and *Ptychocardia*, the tentacles also appear in the posterior portion of the inhalant–pedal opening (Fig. 5L–N).

5. The presence of additional tentacles around the inhalant/exhalant openings varies. In the species of *Cyamiocardium* with a permanent inhalant opening (i.e. *Cyamiocardium crassilabrum*, *Cyamiocardium chuanisinense*, *Cyamiocardium dahli*, *Cyamiocardium namuncurense* and *Cyamiocardium yeskumaala*), a ring of tentacles surrounds this aperture. In the case of *Jukesena* and *Kidderia*, tentacles also surround the exhalant opening, whereas in *Gaimardia* there are no tentacles around the inhalant or exhalant apertures.

CTENIDIA AND LABIAL PALPS

In all studied species of *Cyamiocardium*, *Cyamium*, *Gaimardia*, *Heteromactra*, *Kidderia*, *Pseudokellya* and *Ptychocardia*, the ctenidia are complete, each being composed of two demibranchs; the inner demibranchs are larger than the outer (Figs 5A, B, D, I, 7A). Each demibranch consists of both ascending and descending lamellae, which are fused dorsally either to the mantle (outer demibranch) or to the visceral mass (inner demibranch). The outer demibranch is also fused posteriorly to the mantle margin (Figs 5F, 7B). Left and right inner demibranchs are fused to each other at their posterior ends (Fig. 7D, G–I). Marginal food grooves along the ventral edges of both inner and outer demibranchs are well developed (Fig. 7C). Lamellae of each demibranch are stabilized by interlamellar junctions (Fig. 7E). Adjacent gill filaments are connected by interfilamental junctions. Each gill filament is supported by chitinous rods, cross-connected by transverse fibres. The ciliature of the gill filaments (studied by light microscopy) comprises frontal, laterofrontal and lateral cilia (Fig. 7F). The lateral cilia are straight and relatively long, the laterofrontal cilia are 'horn-like', and the frontal cilia are much shorter than the laterofrontal and lateral cilia. In transverse section, each filament usually shows a single pair of conspicuous ciliated frontal cells (bearing frontal cilia), one pair of laterofrontal cells (bearing the laterofrontal
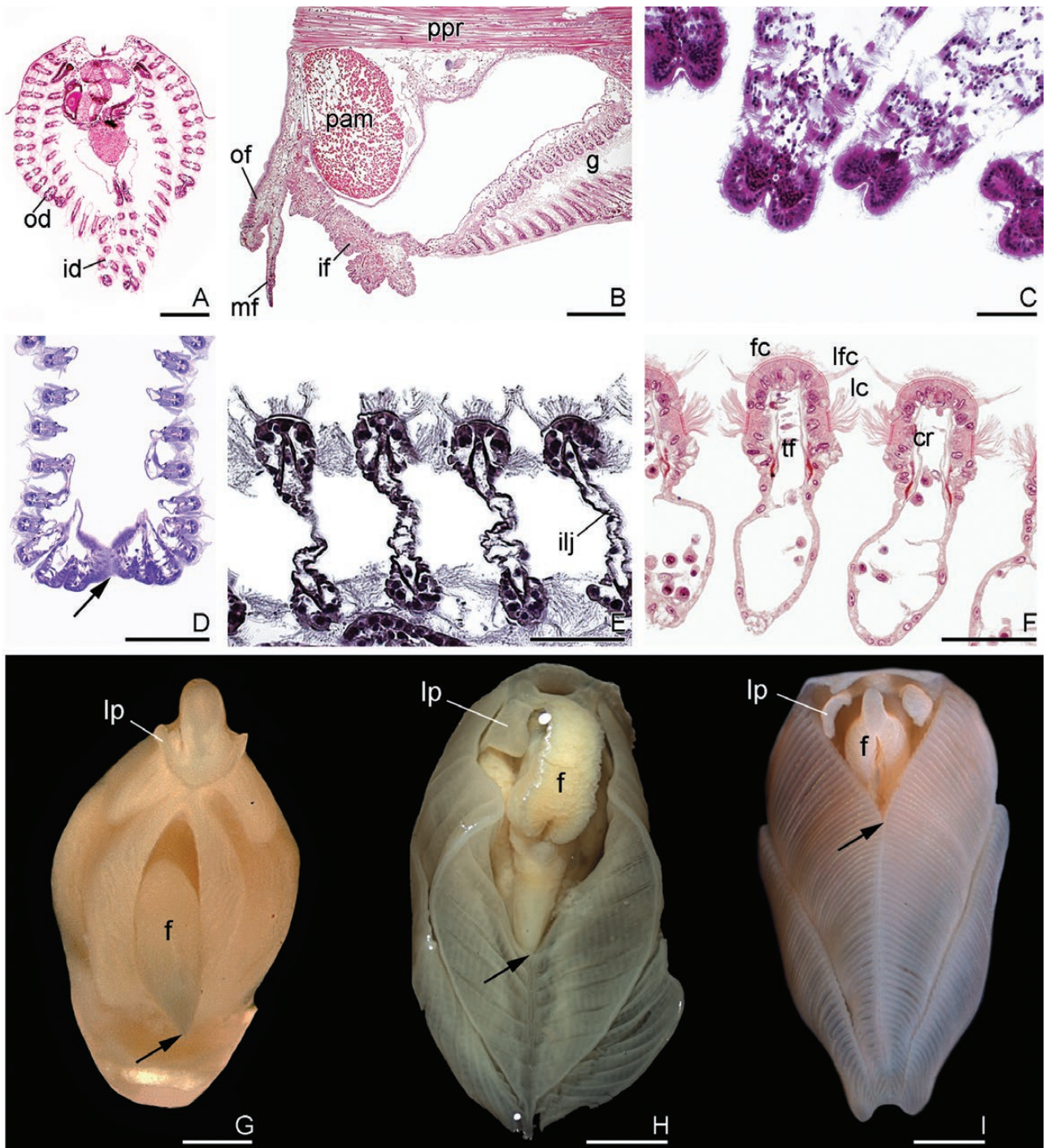
Figure 7. Gills of *Gaimardia mesembrina* (A, D), *Kidderia bicolor* (B), *Gaimardia trapesina* (C, F, H), *Pseudokellya franki* (E), *Cyamiocardium chuanisinense* (G) and *Kidderia minuta* (I). A, transverse section of a specimen, showing the two complete demibranchs. B, posterior fusion of the gill to the mantle margin. C, food grooves. D, G–I, posterior fusion of left and right inner demibranchs. E, interlamelar junctions. F, detail of gill filaments. Scale bars: 200 µm (A, B, D); 100 µm (C); 50 µm (E, F); 500 µm (G, I); 2 mm (H). Histological stains: Haematoxylin and Eosin (A, B, C, F); Toluidine Blue (D); Groat's hematoxylin (E). Abbreviations: cr, chitinous rods; f, foot; fc, frontal cilia; g, gill; id, inner demibranch; if, inner mantle fold; ilj, interlamellar junctions; lc, lateral cilia; lfc, laterofrontal cilia; lp, labial palp; mf, median mantle fold; od, outer demibranch; of, outer mantle fold; pam, posterior adductor muscle; ppr, posterior pedal retractor; tf, transverse fibres. Arrows indicate the fusion of left and right inner demibranchs.

cilia) and three pairs of lateral cells (bearing lateral cilia). However, in the case of *G. trapesina* there are three pairs of frontal cells. In this species, there is a pair of non-ciliated cells between laterofrontal and lateral cells (protolateral cells?). The abfrontal part of each filament is composed of flat, non-glandular and non-ciliated abfrontal cells (Fig. 7F). No differences were observed in the gill filament structure of male and female specimens; a fact to be taken into account owing to the brooding habits of the species studied (see below: Reproduction: structural aspects).

Some differences were found in the length of the fusion of the left and right inner demibranchs at their posterior ends. In *Cyamiocardium*, *Heteromactra*, *Pseudokellya* and *Ptychocardia*, the fused portion is extremely short, restricted to the posteriormost part of the demibranchs (i.e. immediately behind its junction to the mantle margin; Fig. 7G). In contrast, in *Gaimardia* and *Kidderia*, the fused portion extends farther anteriorly, comprising from one-third to one-half of the total length of the ctenidium (Fig. 7H, I).

Another distinctive feature arises from the morphology of the labial palps. In *Cyamiocardium*, *Heteromactra*, *Pseudokellya* and *Ptychocardia*, the anterior and posterior labial palps are minute, similar in shape, narrow and elongate (Fig. 7G). In contrast, in *Gaimardia* and *Kidderia*, the anterior labial palps are subquadrate and large (similar in size to the transverse section of the anterior adductor muscle), and the posterior labial palps are smaller and elongate (Fig. 7H, I).

Like all previously described taxa, *J. foveolata* has two demibranchs at each side, with the inner being larger than the outer, and the outer being fused posteriorly to the mantle margin (Fig. 5J). In this species, as in *Cyamiocardium*, *Heteromactra*, *Pseudokellya* and *Ptychocardia*, the left and right inner demibranchs are fused by a short distance at their posterior ends. However, contrary to the above-mentioned taxa, *J. foveolata* has large, subquadrate anterior labial palps, and small, narrowly elongated posterior labial palps.

FOOT AND BYSSUS GLAND COMPLEX

In all studied species, the foot is composed of a stalk, a posterior 'heel' and a markedly projected anterior 'toe' (Fig. 8A–E); it has a distinct sole and two or three
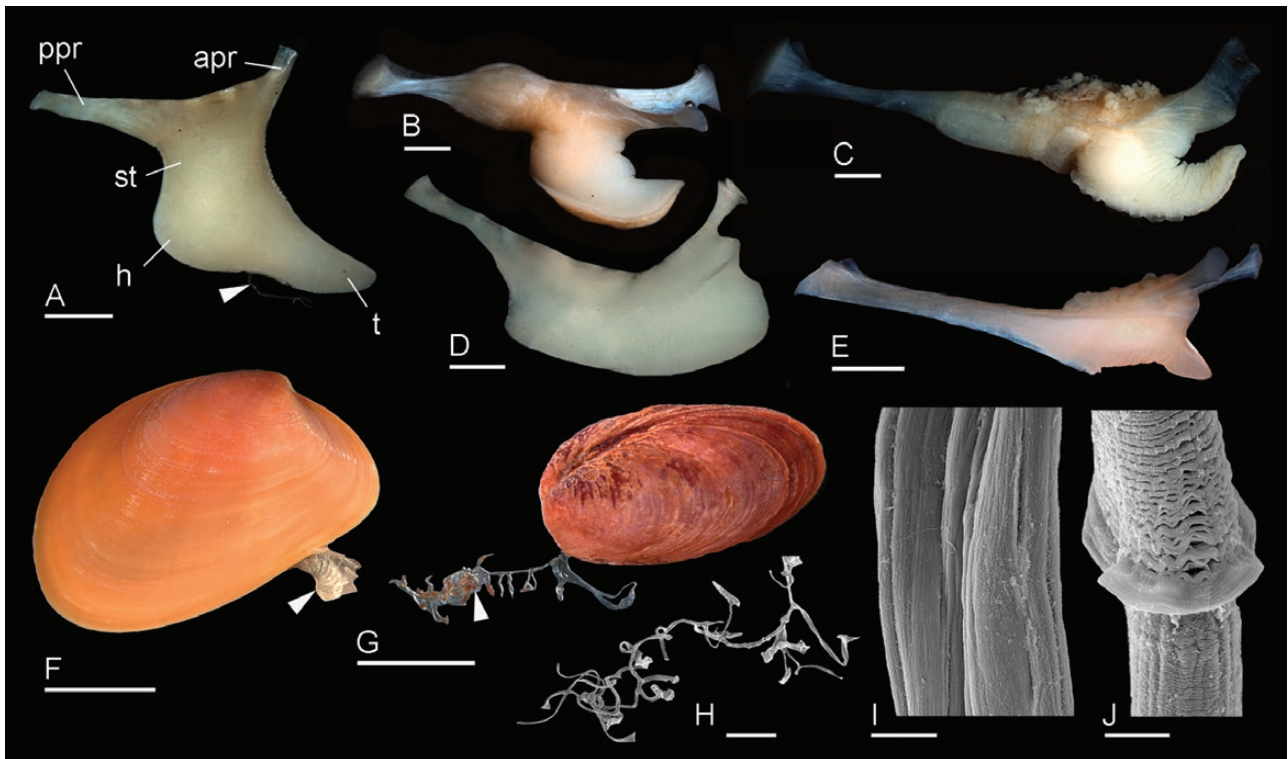

Figure 8. Foot (A–E) and byssus (F–J) of *Cymiocardium chuanisinense* (A), *Gaimardia trapesina* (B, C, F, I), *Jukesena foveolata* (D) and *Kidderia minuta* (E, G, H, J). A, C–G, adult specimen. B, juvenile specimen. Scale bars: 500 μm (A, D, E, H); 1 mm (B, C); 2 mm (F, G); 20 μm (I, J). Abbreviations: apr, anterior pedal retractor; h, heel; ppr, posterior pedal retractor; st, stalk; t, toe. Arrowheads indicate the byssus.

main glandular components responsible for the byssus secretion. Furthermore, there is a pair of anterior and posterior pedal retractors (Fig. 8A–E), with the latter being integrated with the posterior byssus retractor. Despite the overall similar structure, the length of the stalk, the morfology of the 'heel', 'toe' and sole, the morphology of the different foot glands and the size of the anterior and posterior pedal retractors vary considerably among some of the taxa considered.

In *Cyamiocardium*, the stalk is strong and relatively long (Fig. 8A). The 'heel' is rounded, the 'toe' is subcylindrical, dorsoventrally elongated in transverse section, and the base is narrow and slightly flattened, with an elongated long byssus groove extending for one-third of the total foot length (Fig. 10A–F). The anterior and posterior pedal retractor muscles are equally well developed, although the posterior ones are slightly longer than the anterior ones. The byssus gland complex is represented by three components (Fig. 10A). The main glandular component ('byssus gland 1') is placed at the posterodorsal part of the foot. It is formed by a relatively small, hollow, bulbous structure surrounded by a cortical zone of glandular cells, with the cytoplasm filled with a fine granular, strongly basophilic secretion (Fig. 10C). Towards the lumen of the bulb, the epithelium is folded in a few dorsal, lateral and ventral short, conical crests (seen as triangular in transverse section; Fig. 11A, B). Between contiguous crests, a single filament originating from the gland cells of the cortical zone is moulded and directed to the lumen of the bulb, where they fuse together to form a single, narrow byssus thread. The lumen of the bulb continues anteriorly in an oblique duct that opens at the posterior end of the byssus groove (Figs 10A, B, 11L); the opening of the duct is not visible macroscopically. Both the lumen of the bulb and the duct are lined by a single-layered epithelium of densely ciliated cuboidal cells (Fig. 11A, B, L). The second glandular component of the byssus gland complex ('byssus gland 2') is located at the anterior third of the foot and consists of a large mass of cells with vesicular cytoplasm (Fig. 10A, B, D–F); the cell content is colourless when stained with Haematoxylin and Eosin, pale blue with AB/PAS and slightly metachromatic with Toluidine Blue. These cells form a distinct mass without a defined lumen and/or duct (Fig. 11G). This glandular component opens ventrally, through 'multiple ways' at the anterior part of the byssus groove (Figs 10E, 11H). Between the opening of the 'byssus gland 1' and the 'byssus gland 2', deep basophilic gland cells also secrete their content into the byssus grove ('byssus gland 3'; Figs 10A, B, D, 11M). The byssus groove extends from the opening of 'byssus gland 1' to the anterior part of 'byssus gland 2'. The characteristics described above for the foot of *Cyamiocardium* were also observed in *Heteromactra*, *Pseudokellya* and *Ptychocardia*.

In *Gaimardia*, the foot stalk is stout, shorter than in *Cyamiocardium* (Fig. 8B, C). The 'heel' is truncate, the 'toe' is subrectangular in transverse section, and the sole is flat and wide (Figs 8B, C, 10J–L). Two conspicuous openings are visible macroscopically between the 'heel' and 'toe': the anterior, corresponding to the 'byssus gland 2' ('bg2-o'), is smaller than the posterior, which corresponds to the 'byssus gland 1' and 'byssus gland 3' opening ('bg1, 3-o'; = byssus groove?; Fig. 10H). The posterior pedal retractors are markedly longer and stronger than the anterior ones; this difference is more evident in larger specimens (Fig. 8B, C). The byssus gland complex, as in *Cyamiocardium*, is represented by three components. However, in this case the entire complex is restricted to the posterior part of the foot (Fig. 10G). Several differences are noted with respect to the complex described for *Cyamiocardium*, as follows. The main glandular component ('byssus gland 1') in *Gaimardia* is represented by a conspicuous, large bulbous structure occupying a large volume at the posterior half of the foot (Fig. 10G). Towards the lumen of the bulb, the epithelium is folded in multiple dorsal and laterodorsal lamellate crests. In transverse section, the structure appears to be bilaterally symmetrical (Figs 10J, 11C, D). The crests, and the spaces between them, converge to the centre of the lumen in a wide central chamber that communicates to the exterior through a short and wide duct (Fig. 10G). This duct opens to the exterior by a large opening located between the 'heel' and the 'toe' ('bg1, 3-o'; Fig. 10G, H). Both crests and 'ducts' are lined by a single-layered epithelium of cubic cells, with short cilia (Fig. 11E). Between each pair of crests, a single secretion filament originates from the gland cells of the cortical zone (Fig. 11C, D). These filaments converge in the 'ducts', and the secretions of the different ducts fuse together to form a single byssus filament entering the central chamber (Fig. 10G). The second component of the byssus gland complex consists of cells whose morphology and staining properties closely resemble the described above for 'byssus gland 2' of *Cyamiocardium* (Fig. 11J, K). However, in this case, the cells arrange around a long, wide and somewhat curved duct, which projects dorsally from the foot base (Figs 10G, 11I). The cells surrounding the lumen of the duct are densely ciliated (Figs 10L, 11K). The duct opens to the exterior at the foot sole through the small anterior opening described above, visible macroscopically in dissected specimens ('bg2-o'; Figs 10G, H, 11I). The third component of the byssus gland complex ('byssus gland 3') is similar in cell morphology and staining properties of the secretion product to that described for the third glandular component of *Cyamiocardium* (Fig. 11N).

However, in this case, the cells release their secretions through a common opening with 'byssus gland 1' ('bg1, 3-o'; Fig. 10G). The duct of 'byssus gland 3' is lined by a single-layered, folded epithelium, composed of high columnar, ciliated cells (Figs 10K, 11P).

In *Kidderia*, the foot exhibits only slight ventral projection, appearing to lack a stalk (Fig. 8E). The 'heel' and 'toe' are similar in morphology to those of *Gaimardia*, although the latter is smaller. In addition, between the 'heel' and the 'toe' a single opening is present (Fig. 10O, byssus groove?). The posterior retractor muscles are considerably longer and stronger than the anterior ones (Fig. 8E). Histological sections show several similarities to *Gaimardia*, including the arrangement and morphology of 'byssus gland 1' and 'byssus gland 3' of the byssus gland complex (Figs 10M, N, 11O, Q). The main difference from *Gaimardia* is that, for the most of its extent, the ventralmost duct of the 'byssus gland 1' in *Kidderia* is wider and has a higher columnar epithelium, with longer cilia (Fig. 11F). This differentiated part seems to be responsible for secreting the outermost layer of the byssus filament (see below: byssus). Furthermore, *Kidderia* differs from *Gaimardia* and other genera considered in the present study by lacking cells that resemble those described for the 'byssus gland 2' of the byssus gland complex.

In addition to the glands associated with the byssus formation, in *Gaimardia* and *Kidderia* two types of subepithelial mucous gland cells are present. These discharge their secretions through narrow, elongated ducts. One type, which stains reddish with Haematoxylin and Eosin, appears to be restricted to the anterior part of the foot ('mucous glands type 1'; Fig. 9A–C, E). In fact, in *Kidderia* these glands form a massive structure (Fig. 9F). The second type ('mucous glands type 2'), which stains deep purple with Haematoxylin and Eosin, discharges its secretion inside the 'bulb' of the 'byssus gland 1' (Fig. 9D). A similar structure to 'mucous glands type 1' is also apparent in *Cyamiocardium* (Fig. 10A).

In *J. foveolata*, as in all previously mentioned taxa, the anterior and posterior retractor muscles are well developed, with the former being shorter than the latter. The foot is distally differentiated into a 'heel' and 'toe', and there is a well-developed byssus groove at the base. As in *Kidderia*, it appears to lack a stalk. Unlike all other studied taxa, in this species the foot is markedly compressed, bluntly pointed at the toe and has an extremely narrow base (Fig. 8D). Unfortunately, histological information on the foot of this species could not be obtained.

BYSSUS

The above-mentioned differences in the type, number and disposition of the glands associated with the byssus secretion are also related to some differences
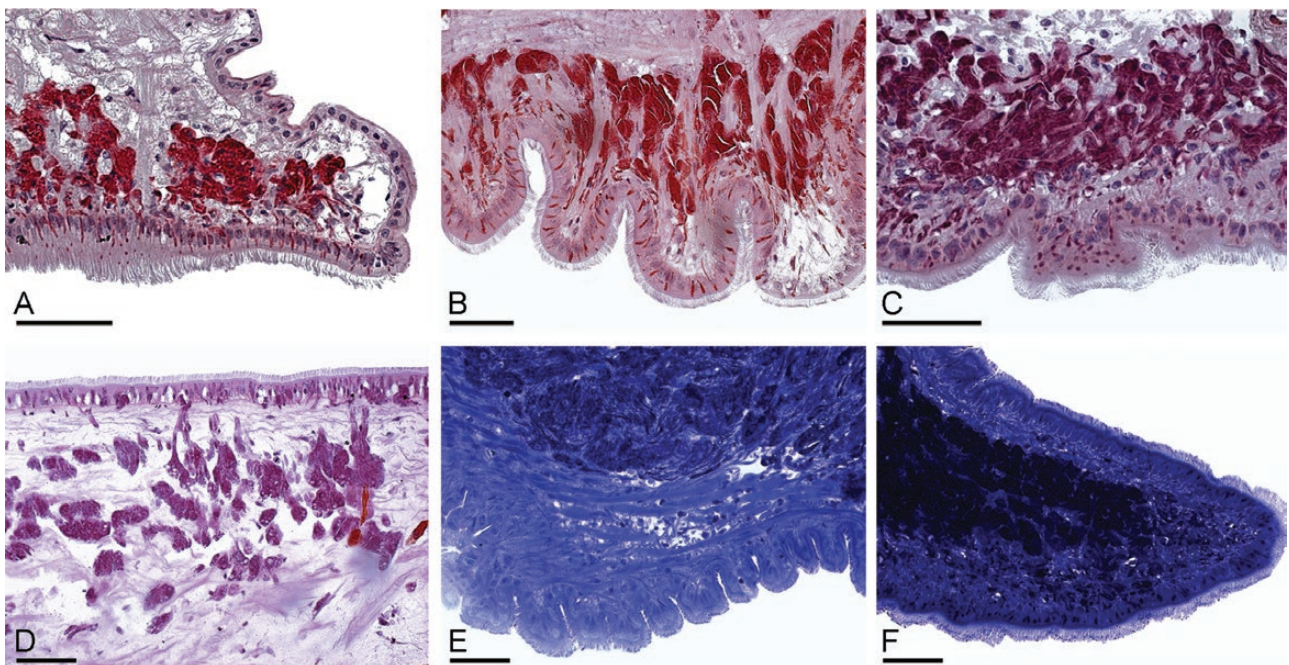

Figure 9. Subepithelial mucous glands of *Gaimardia trapesina* (A, B, E) and *Kidderia minuta* (C, E, F). A–C, F, mucous glands type 1. D, mucous glands type 2. Scale bars: 50 μm. Histological stains: Haematoxylin and Eosin (A–D), Toluidine Blue (E, F).
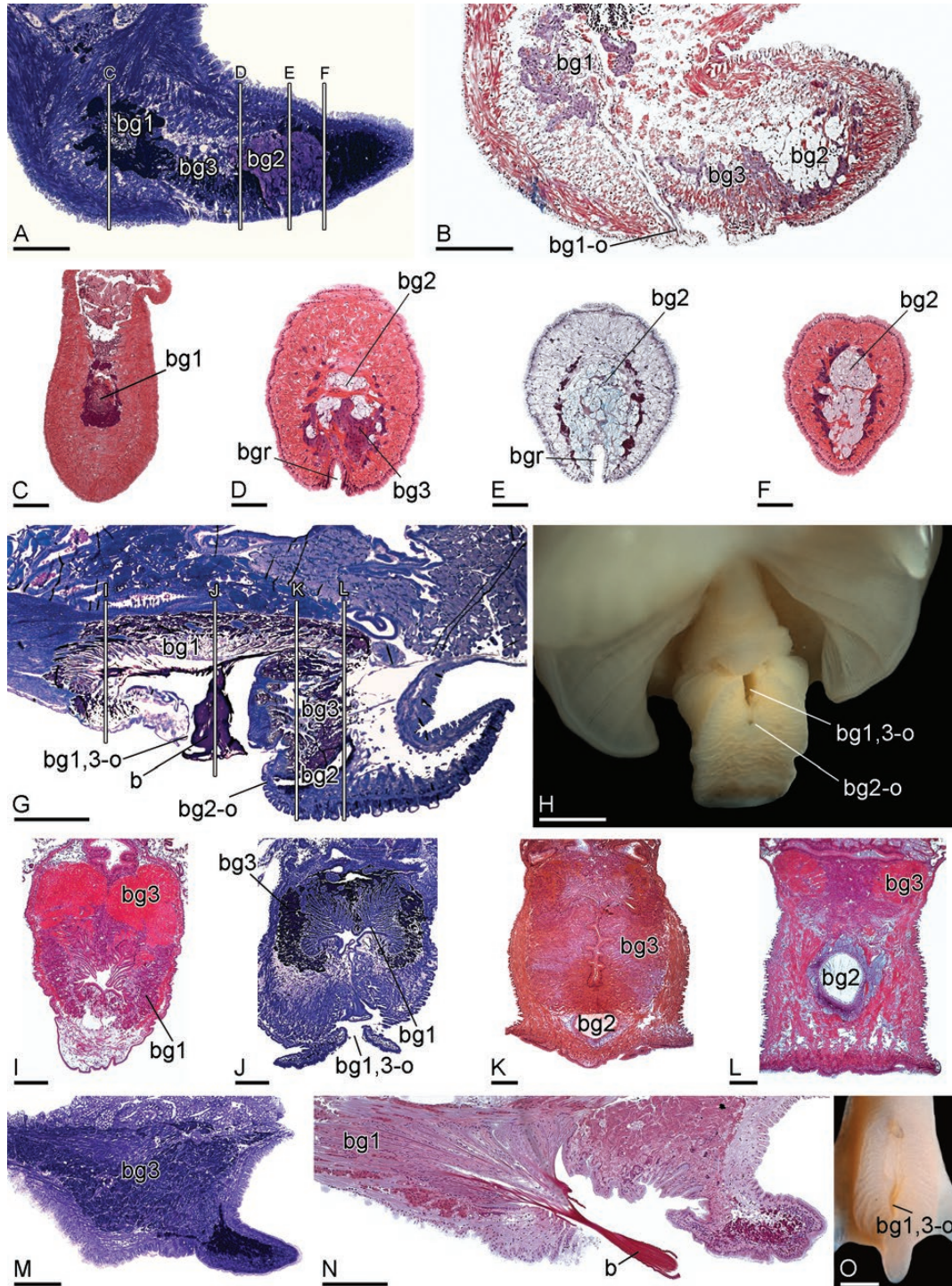
Figure 10. Byssus gland complex of *Cyamiocardium chuanisinense* (A–F), *Gaimardia trapesina* (G–L) and *Kidderia bicolor* (M–O). A, B, G, M, N, sagittal sections of the foot. C–F, I–L, transverse sections of the foot. H, O, byssus gland openings. Scale bars: 200 µm (A–C, I–O); 100 µm (D–F); 1 mm (G, H). Histological stains: Toluidine Blue (A, G, J, M); modified Masson's trichrome (B–D, F, I, K, L, N); periodic acid–Schiff (E). Abbreviations: b, byssus; bg1, byssus gland 1; bg1-o, byssus gland 1 opening; bg1, 3-o, byssus glands 1 and 3 openings; bg2, byssus gland 2; bg2-o, byssus gland 2 opening; bg3, byssus gland 3; bgr, byssus groove.
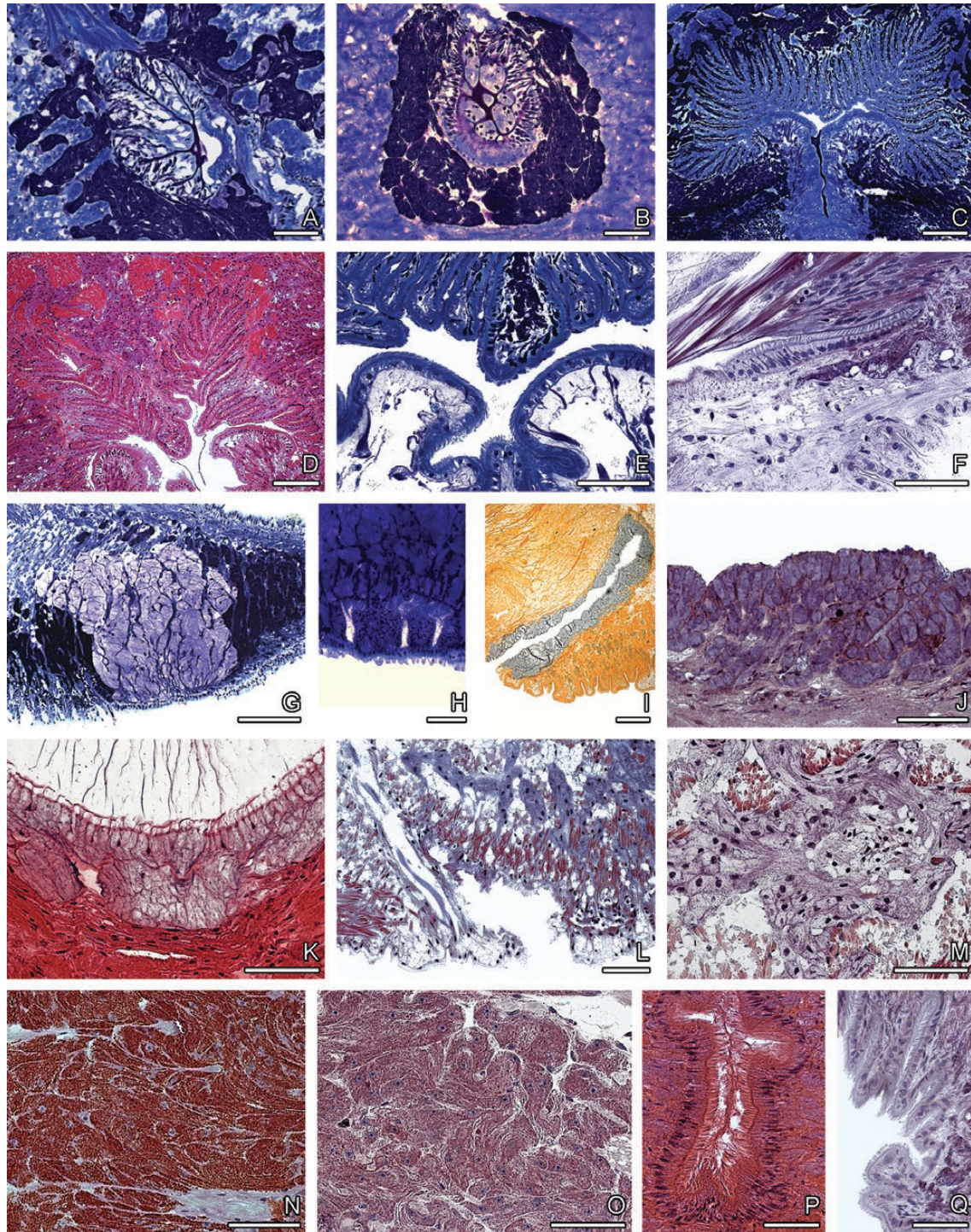
Figure 11. Details of the different byssus glands of *Cyamiocardium chuanisinense* (A, B, G, H, L, M), *Gaimardia trapesina* (C–E, I–K, N, P) and *Kidderia minuta* (F, O, Q). A–E, byssus gland 1 (A, sagittal section; B–E, transverse sections). F, ventralmost duct of byssus gland 1. G–K, byssus gland 2 (G–I, sagittal sections; J, K, transverse sections). L–O, byssus gland 3. P, Q, duct of byssus gland 3 (P, transverse section; Q, sagittal section). Scale bars: 50 µm (A, B, E, F, H, J–Q); 100 µm (C, D, G); 200 µm (I). Histological stains: Toluidine Blue (A–C, E, G, H); modified Masson's trichrome (D, F, J–Q); periodic acid–Schiff (I).
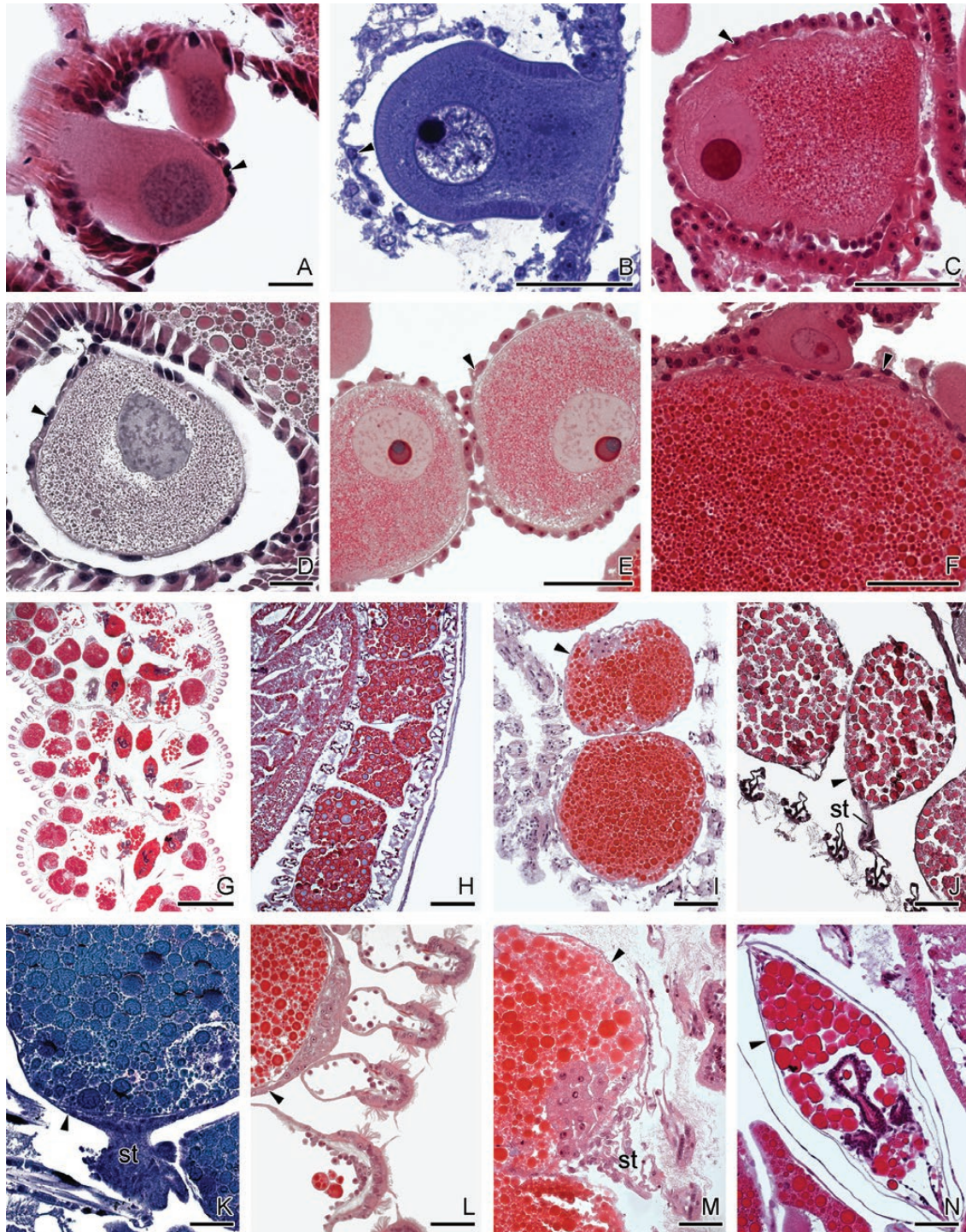
Figure 12. Oocytes and brooded embryos of *Pseudokellya franki* (A, D, J), *Kidderia bicolor* (B), *Gaimardia mesembrina* (C), *Gaimardia trapesina* (E, G, L), *Kidderia minuta* (F), *Cyamiocardium dahli* (H), *Heteromactra laminifera* (I, M) and *Cyamiocardium chuanisinense* (K, N). A–C, early vitelogenic oocytes. D–F, late vitellogenic oocytes. G–N, brooded embryos in the parental gill. Scale bars: 20 µm (A, D); 50 µm (B, C, E, F, J–N); 500 µm (G); 100 µm (H, I). Histological stains: Toluidine Blue (B, K); Modified Masson's trichrome (A, C, E–J, L-N); Groat's hematoxylin (D). Abbreviation: st, stalk. Arrowheads indicate the follicular epithelium cells.
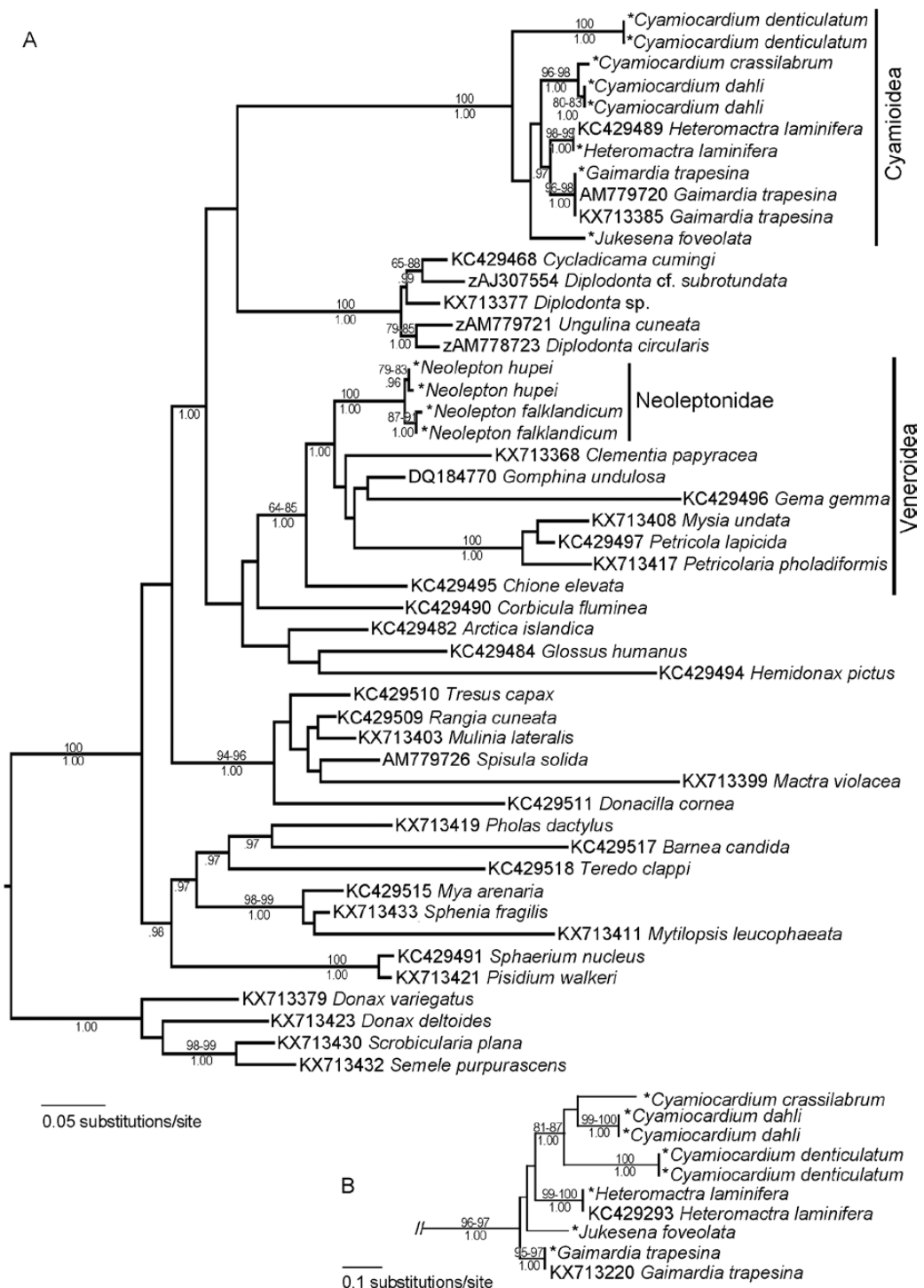
Figure 13. A, phylogenetic tree obtained with maximum likelihood (ML) analysis for 28S (lnL = 12 437.51). B, partial tree obtained with ML for 16S, corresponding to the Cyamioidea clade (lnL = 10 071.55). Numbers at the nodes correspond to the range of bootstrap frequencies obtained (above) and Bayesian posterior probabilities (below); only bootstrap values > 80% and posterior probabilities > 0.95 are shown. Asterisks (*) in terminal labels indicate newly obtained sequences.

in the morphology of the byssus of *Cyamiocardium*, *Gaimardia* and *Kidderia*. In *Cyamiocardium*, the byssus is long, extremely thin and simple (i.e. non-branched; Fig. 8A). On the contrary, in *Gaimardia* and *Kidderia* the byssus is composed of a stem, from which several short and narrow branches radiate (Fig. 8F–H). Both the stem and the branches are composed of several parallel threads, each of which has an adhesive disc at the tip. The stem is short and stout in *Gaimardia* (Fig. 8F), whereas in *Kidderia* it is long and narrow (Fig. 8G, H). Branches are more widely separated in *Kidderia* than in *Gaimardia*. Furthermore, in *Kidderia* (Fig. 8J) the byssus threads are surrounded by a layer of threads arranged in a spiral pattern, which are not present in *Gaimardia* (Fig. 8I). The byssus of *Cyamiocardium*, *Gaimardia* and *Kidderia* remains functional in adults.

REPRODUCTION: STRUCTURAL ASPECTS

All histologically studied specimens of *Cyamiocardium*, *Gaimardia*, *Heteromactra*, *Kidderia*, *Pseudokellya* and *Ptychocardia* were either males or females, and no signals of hermaphroditism were observed. Spermatogenesis and spermiogenesis follow the typical pattern for bivalves and, consequently, are not described herein. However, oogenesis shows a peculiar condition. Early before the start of vitellogenesis, the oocytes become completely surrounded by a single-layered true follicular epithelium (Fig. 12A, B). In this way, each single oocyte becomes 'sealed off' from the acinus lumen. The follicular epithelium persists throughout the development of the oocyte within the acinus (i.e. during the entire vitellogenic process; Fig. 12A–F) and is also present once the ova are released from the gonad, persisting throughout embryonic development, which occurs associated with female ctenidia (Fig. 12G–N). In fact, once fecundation takes place (the site where this process occurs was not observed), at some point the follicular epithelium participates in the formation of a stalk that anchors the embryo to the abfrontal part of a gill filament (Figs 5G, H, 12J, K–M); embryos may be attached to either descending or ascending lamellae. Embryonic development is completed within the female gills, and progeny are released as late pediveligers (Fig. 12G, N). Before being released, pediveligers appear free at the posterior part of the maternal ctenidia, occupying the suprabranchial chamber.

It is possible to recognize embryos at different stages of development being brooded in the female ctenidia, revealing the coexistence of different cohorts or generations (Fig. 12G). This observation is consistent with the simultaneous presence of developing previtellogenic, early vitellogenic and late vitellogenic oocytes within the acini of brooding females.

The total number of embryos brooded per female is considerably higher in *G. trapesina* (Fig. 5A) than in *Gaimardia mesembrina* (Melvill & Standen, 1907), *Cyamiocardium* (Fig. 5I), *Heteromactra* (Fig. 5B), *Kidderia*, *Pseudokellya* and *Ptychocardia* species (> 1000 vs. < 80 embryos). Related to this difference, *G. trapesina* brood the embryos in both inner and outer demibranchs, whereas in all other taxa the embryos are restricted to the inner demibranchs.

The presence of a follicular epithelium and the brooding condition associated with the ctenidia were also observed in *J. foveolata*. In this case, ~30 embryos, anchored to both inner and outer demibranchs by a narrow stalk, were observed (Fig. 5G, H).

MOLECULAR DATA

The topologies recovered for the two markers considered in the present study are mostly in concordance, although the 16S tree provides low support values for the majority of the high taxonomic categories. Regarding the taxa that are the focus of the present study, both 16S and 28S markers show a monophyletic clade including species of *Cyamiocardium*, *Gaimardia*, *Heteromactra* and *J. foveolata* (Fig. 13A, B). This clade, recovered in all molecular analyses with high support [96–100% ML bootstrap support (BS) and 1.00 BI posterior probability (BPP)], excludes the Neoleptonidae, which group among Veneroidea with high Bayesian posterior probabilities (0.98 BPP for 16S and 1.00 BPP for 28S; Fig. 13A).

The relationships among *Cyamiocardium*, *Gaimardia*, *Heteromactra* and *Jukesena* cannot be resolved unambiguously with the markers considered; the topologies obtained from the two analyses are inconsistent in this aspect. The reconstruction based on 16S groups all the species of *Cyamiocardium* (81–87% BS and 1.00 BPP) and places *Gaimardia* as an external group to all other studied taxa, although with low support (Fig. 13B). However, the analyses based on 28S split *Cyamiocardium* species into two groups, one comprising *Cyamiocardium crassilabrum* and *Cyamiocardium dahli* and the other corresponding to *Cyamiocardium denticulatum*, which appears as external to all other studied taxa, although with no support (Fig. 13A).

DISCUSSION

WHAT DO THE STUDIED SPECIES HAVE IN COMMON?

All histologically studied species of *Cyamiocardium*, *Gaimardia*, *Heteromactra*, *Jukesena*, *Kidderia*, *Pseudokellya* and *Ptychocardia* share a common reproductive character, the oogenesis of the solitary type, i.e. the presence of a follicular epithelium

surrounding each developing oocyte throughout its entire development. They also share the brooding of embryos within demibranchs attached to the gill filaments by means of a short peduncle derived from the persistent follicular epithelium. The presence of a true follicular epithelium (i.e. a follicle that surrounds each single oocyte) accompanying the entirety of oocyte development is known among molluscs in cephalopods (Bottke, 1974) and polyplacophorans (Selwood, 1968, 1970; Ituarte *et al.*, 2010). Among bivalves, this condition is known only for the genera studied herein. Based on the study of *Ps. cardiformis*, Pelseneer (1903) was the first to describe this epithelium for bivalves. The peculiarity of this finding was highlighted at that time as being the only known case among bivalves (Pelseneer, 1903: 49). Since that publication, this character has been reported for nine other species: *Cyamiocardium domaneschii* (Passos & Machado, 2014), *Cyamiocardium chuanisinense*, *Cyamiocardium dahli*, *Cyamiocardium denticulatum* and *Cyamiocardium namuncurense* (Urcola & Zelaya, 2018), *Gaimardia bahamondei* Osorio & Arnaud, 1984 (Chaparro *et al.*, 2011), *G. trapesina* (Igel, 1908a; Ituarte, 2009), *Neogaimardia rostellata* (Odhler, 1924) and *Ps. franki* (Zelaya & Ituarte, 2009). As part of the present study, we confirm that this epithelium is also found in *Cyamiocardium crassilabrum*, *G. mesembrina*, *Heteromactra laminifera* Lamy, 1906, *J. foveolata*, *Kidderia bicolor* (Martens, 1885), *K. minuta* and *Ps. inexpectata*. This leads us to consider that this peculiar reproductive characteristic does not appear in 'isolated cases', but among closely related taxa; our molecular results support this point of view. Thus, the presence of this follicular epithelium is understood here as a key character to reunite all the above-mentioned taxa into a single group: the Cyamioidea.

Cyamioidea, as defined above, include *G. trapesina*, the type species of the genus *Gaimardia*, upon which Gaimardiidae and Gaimardioidea are based. Consequently, according to the available evidence, we incorporate Gaimardioidea into Cyamioidea.

SHELL MORPHOLOGY AND ANATOMY: HOW DO THEY CONTRIBUTE TO THE DEFINITION OF CYAMIOIDEA?

Besides the reproductive and molecular evidence mentioned above, few anatomical and no morphological characters appear to be shared uniquely among all cyamioidean genera considered in the present study. Shared anatomical characters include the following: the presence of tentacles at the posterior end of the mantle margin; fusions of the mantle margin of type A; left and right inner demibranchs posteriorly fused, and fused to the mantle margin; the foot with differentiated 'heel', 'toe' and byssus groove; and a byssus gland complex that remains functional in adult specimens. However, none

of these characters is exclusive for Cyamioidea, because they are also present in other bivalve superfamilies. Likewise, shell morphology does not allow an unambiguous definition of Cyamioidea. Within this superfamily, shells are usually < 10 mm maximum size, although *G. trapesina* reaches larger sizes (≤ 32.5 mm in length according to Huber, 2010); shell outlines and sculpture are widely variable; there is a different degree of development of the cardinal teeth; and there may be only one (external) ligament (as in *Gaimardia*) or two (an external and an internal ligament).

Despite the restricted significance of morphological and anatomical characters for defining the Cyamioidea as a whole, these characters prove to be useful for delimiting two subgroups in this superfamily: one including *Gaimardia* and *Kidderia* and another including *Cyamiocardium*, *Cyamium*, *Heteromactra*, *Pseudokellya* and *Ptychocardia*. The distinction of these two groups is concordant with the molecular topology obtained with the 16S marker (although without high support therein), and these two groups are regarded as Gaimardiidae and Cyamiidae.

HOW TO DISTINGUISH CYAMIIDAE FROM GAIMARDIIDAE

The shell of Gaimardiidae differs clearly from that of Cyamiidae by having the umbones sharply displaced anteriorly instead of being (sub)central and by having the cardinal teeth greatly reduced in size. The characteristics of the periostracum (thick and two layered in *Gaimardia* and *Kidderia* vs. thin and single layered in *Cyamiocardium* and *Heteromactra*) seem to be an additional character, although the significance of that difference needs to be confirmed by studying the character in the other cyamiids.

Regarding gross anatomy, Gaimardiidae differ clearly from Cyamiidae by having the following: a much shorter pedal aperture, separated from the adjacent posterior (inhalant) aperture by a long mantle fusion; a lower number of tentacles at the posterior part of mantle border; a longer posteroventral fusion of left and right inner demibranchs; a greater development of the posterior pedal retractors; and larger anterior labial palps, which are subsquare instead of elongate. Moreover, in Gaimardiidae the foot stalk is much shorter and massive, the toe is larger and subrectangular in section (instead of subcylindrical), the sole is wider, and the heel is truncated instead of rounded.

Histologically, Gaimardiidae show consistently two wide outer mantle folds, triangular in section (of-1 and of-2), whereas the Cyamiidae usually have a single, narrow and elongated outer fold (of-1). The only exception is *Heteromactra*, which also shows two outer folds (of-1 and of-2), although in this case

the morphology of the folds is the same as that of other Cyamiidae. In addition, in Gaimardiidae the middle mantle fold is simple, whereas in Cyamiidae it is bifurcated at the tip (with the exception of *Heteromactra*). Furthermore, Gaimardiidae have a considerably larger 'byssus gland 1', which has in the bulbous portion a higher number of crests than when compared with Cyamiidae.

The byssus in Gaimardiidae is well developed, branched and bears adhesive discs at the tip of each branch. On the contrary, the byssus is delicate, unbranched and lacking adhesive discs in Cyamiidae. These differences seem to be related to the mode of life of each group: gaimardiids are epifaunal and live permanently (or subpermanently) anchored to the substrate (algae in the case of *Gaimardia*, rocks in the case of *Kidderia*), whereas cyamiids are infaunal, and the byssus seems to represent only a temporary anchorage.

WHAT ABOUT *J. FOVEOLATA*?

Jukesena foveolata was described by Cooper & Preston (1910) based on specimens collected at the Falkland Islands. After its original description, only Dell (1964) reported additional material of this species, also coming from the type locality. Even when rarely sampled, the generic placement of *J. foveolata* has been controvertial. Originally placed in the genus *Psephis* (Veneroidea) by Cooper & Preston (1910), the species was subsequently mentioned under the *Gomphina* subgenus *Acolus* Jukes-Brown, 1913 (Veneroidea) by Melvill & Standen (1914). Iredale (1915) noticed that at the genus level *Acolus* Jukes-Brown, 1913 is preoccupied by *Acolus* Foerster, 1856 (Hymenoptera). Consequently, he proposed *Jukesena* as a replacement name for the former. However, the name *Jukesena* remained largely disregarded, and '*Gomphina* (*Acolus*)' continued to be used in subsequent publications (e.g. Carcelles & Williamson, 1951; Powell, 1951). Powell (1960) recovered the usage of *Jukesena* (as a subgenus of *Gomphina*), subsequently followed by Dell (1964). Despite the different (sub)generic placements for the species, until now its inclusion in Veneridae (Veneroidea) had never been questioned, probably because the species remained known only from its shell morphology for more than a century.

As part of the present study, we had the chance to study one live-collected specimen of this species from Patagonia. This specimen shows great morphological and anatomical similarities to Cyamioidea, particularly by the presence of the follicular epithelium, embryos attached to the gill filaments of the parental specimen by a stalk, and tentacles at the posterior part of the mantle margin. These characters, together with the molecular evidence obtained here (from both 16S and 28S markers), allow us to establish the placement of *J. foveolata* in Cyamioidea and that the species is not closely related to *Gomphina undulosa* (type species of *Gomphina*) or other Veneroidea, as previously thought.

Considering the number, morphology and degree of development of cardinal teeth, the presence of an internal and an external ligament, the numerous tentacles at the posterior part of mantle margin, the long byssus groove and the left and right inner demibranchs being posteroventrally fused by a short distance, *J. foveolata* shows greater similarity to Cyamiidae than to Gaimardiidae. However, it differs clearly from all other Cyamiidae studied herein by having both inhalant and exhalant apertures projected as siphons and crowned by tentacles, related to the presence of a well-marked pallial sinus, and by having the series of posterior tentacles arising from the distal part of the middle mantle fold instead of arising from the base of the inner mantle fold as in *Cyamiocardium*, *Cyamium*, *Gaimardia*, *Heteromactra*, *Kidderia*, *Pseudokellya* and *Ptychocardia*. Furthermore, the anterior labial palps are subquadrate, and the foot appears to lack a differentiated stalk, a condition not seen in any other Cyamiidae. In contrast, unlike any other Cyamiidae and Gaimardiidae, the foot of *J. foveolata* is compressed and has a narrow base. The molecular information obtained from the two markers considered herein does not allow us to be conclusive whether *J. foveolata* is a Cyamiidae or belongs to a different family in Cyamioidea. It is interesting to note the similarity in shell morphology and gross anatomy of this species to *Cyamiomactra problematica* (type species of the genus), although the restricted knowledge of that species does not allow us to be conclusive regarding whether both taxa should be reunited into the same genus.

OTHER TAXA PREVIOUSLY ASSIGNED TO CYAMIOIDEA

Besides the taxa considered above, 11 other (sub) genera (*Costokidderia*, *Cyamiomactra*, *Cyamionema*, *Dicranodesma*, *Eugaimardia*, *Legrandina*, *Lutetina*, *Neogaimardia*, *Perrierina*, *Progaimardia* and *Reloncavia*) were previously mentioned in the literature as belonging to either Cyamiidae or Gaimardiidae, and seven other families (Bernardinidae, Basterotiidae, Galatheavalvidae, Juliidae, Neoleptonidae, Sportellidae and Turtoniidae) were assigned to Cyamioidea/Gaimardioidea.

TAXA HERE REGARDED AS PROBABLY BELONGING TO CYAMIOIDEA

Reloncavia *(type species:* Kingiella chilenica *Soot-Ryen, 1959*)

The hinge of this genus (figured by Soot-Ryen, 1959: figs 14, 15; and Chavan, 1969: fig. E39, 3b, 3c)

shows great similarity with that of *Cyamium*. Soot-Ryen (1957, 1959) described the gross anatomy of *Reloncavia chilenica*, reporting the presence of a large pedal opening and two smaller (inhalant and exhalant) openings, a large foot, small labial palps and small posterior 'papillae'. Gallardo (1993) described how 'each embryo is contained in a small capsule attached to a branchial filament by a short peduncle'. The 'capsule' and 'peduncle' are likely to correspond to the follicular epithelium recognized here as diagnostic for Cyamioidea. All morphological and anatomical characters suggest *Reloncavia* to be a Cyamiidae.

Cyamiomactra *(type species:* Cyamiomactra problematica *Bernard, 1897)*

The description of this genus was not given separately from that of the type species. Bernard (1897) emphasized the similarity of this genus to *Cyamium*, pointing out as the main differences the shell outline ('*Cyamium* is much more elongated') and the presence of posterior cardinal teeth in the left valve (regarded as absent in *Cyamium*, but as a consequence of an erroneous interpretation of the hinge; see above). After studying the gross anatomy of *Cyamiomactra problematica*, Ponder (1971) concluded that it 'agrees closely with *Cyamium antarcticum*'. In fact, the author considered *Cyamiomactra* as a subgenus of *Cyamium*. In its shell outline, *Cyamiomactra* does not show great morphological differences from *Cyamium*.

Perrierina *(type species:* Perrierina taxodonta *Bernard, 1897)*

The genus has the same number, morphology and arrangement of cardinal teeth as *Cyamiomactra problematica*. This fact was pointed out in its description (Bernard, 1897) and ratified by subsequent authors (e.g. Fleming, 1948). The only difference is the presence of several lateral 'crests' along the dorsal margin in *Perrierina* (diversely referred as lateral teeth, marginal denticles, lateral lamellae, taxodont-like laterals or taxodont lamellae). Ponder (1971) studied the gross anatomy of the type species of *Perrierina*, which, although similar to that of *Cyamium* and *Cyamiomactra*, was described as lacking a byssus groove in the foot and tentacles at the posterior end of the mantle margin (two characters present in the other species of Cyamioidea studied herein). Despite that, Ponder (1971) concluded that *Perrierina* 'is clearly derived from a *Cyamium* or *Cyamiomactra*-like ancestor'. In addition, he considered *Legrandina* to be a subgenus of *Perrierina*. We have no additional evidence to confirm or reject Ponder's (1971) opinion. Consequently, we follow the family placement proposed by Ponder (1971) for those taxa.

Neogaimardia *(type species:* Kellia rostellata *Tate, 1889)*

Since its description, *Neogaimardia* has been considered a member of the Gaimardiidae. Odhler (1924) compared it with *Gaimardia*, and Ponder (1971) and Huber (2010) considered it to be a subgenus of *Gaimardia*. Morton (1979) provided valuable anatomical information of *Neogaimardia finlayi* Powell, 1933, a species very similar to *N. rostellata*. The main differences of *Neogaimardia* (with respect to *Gaimardia*) are the presence of a short internal ligament, well-defined posterior lateral teeth, accessory 'marginal teeth' and the presence of only one (the inner) demibranch at each side. Furthermore, judging from Morton's (1979) figure 5, the laterofrontal cilia of ctenidia in *Neogaimardia* do not form horn-like structures. In all other aspects, *Neogaimardia* appears similar to *Gaimardia*. In fact, both taxa also have similar modes of life, attached to floating algae. Odhler (1924) described how *N. rostellata* brood 'large eggs … in follicles which are formed as in *Pseudokellya* (cf. Pelseneer, 1903)'. Considering the very peculiar structure described by Pelseneer (1903), it seems clear that Odhner's description refers to the follicular epithelium considered here as diagnostic for Cyamioidea.

Progaimardia *(type species:* Modiolarca minutissima *Iredale, 1908)*

The general shell morphology of the type species closely resembles that of *Gaimardia*. In fact, this genus was originally proposed as a subgenus of *Gaimardia*, from which it was distinguished by the presence of a large, internal ligament and a strong hinge plate, bearing one large tooth in each valve and moderately long anterior and posterior teeth (Ponder, 1971). No additional anatomical or reproductive information is available for this genus.

Eugaimardia *(type species:* Neogaimardia perplexa *Cotton, 1931)*

Cotton (1931a) described a new species of gaimardiid, erecting *Neogaimardia* as a new genus for its placement, but had overlooked that this name was preoccupied by *Neogaimardia* Odhler, 1924. Cotton (1931b) proposed *Eugaimardia* as a replacement name for the former. Cotton (1931a) distinguished his new genus from *Gaimardia* by the absence of a concavity in the ventral margin and by its different 'dentition of the hinge', namely the presence of a 'U-shaped tooth, and a small tooth between the arms of the U' in the right valve. The morphology of the teeth he described does not appear as discrepant from the one we find in *G. trapesina*. Furthermore, in *G. trapesina*, the

concavity of the ventral margin proves to be variable among specimens. Thus, from a morphological point of view, we find no evidence to consider *Eugaimardia* to be distinct from *Gaimardia*. Unfortunately, nothing is known about the anatomy or reproduction of *Eugaimardia perplexa*. Despite that, *Eugaimardia* was considered a valid genus by Huber (2010).

Costokidderia *(type species:* Kidderia costata Odhler, 1924)

The type species of this genus has a shell outline that closely resembles that of *Kidderia*. Odhler (1924) described for that species the presence of strong radial sculpture and strong cardinals. The first of these characters led Finlay (1926) to propose the genus *Costokidderia*, a name currently regarded as a subgenus of *Kidderia* (e.g. Ponder, 1971).

Cyamionema *(type species:* Cyamium (Cyamionema) decoratum *Melvill & Standen, 1914)*

Despite having originally been compared with *Cyamium*, the general shell outline and morphology of the hinge teeth of *Cyamium decoratum* appear more similar to those of *K. minuta* (type species of *Kidderia*) than to those of *Cyamium antarcticum* (type species of *Cyamium*). *Cyamionema decoratum* differs from *Cyamium antarcticum* (and from other Cyamiidae) by lacking an internal ligament, by having only one tooth in the left valve and by the presence of thin radial sculpture in the central part of the shell (Melvill & Standen, 1914). The significance of these differences and the relationship of *Cyamionema* to *Kidderia* deserve further studies. Scarlato & Starobogatov (1979) proposed a new family, Cyamionematidae (in Galeommatoidea), based on this genus. *Cyamionema* appears listed as a subgenus of *Kidderia* by Huber (2010).

<div align="center">

TAXA HERE EXCLUDED FROM CYAMIOIDEA
</div>

Lutetina *(type species:* Lutetina antarctica *Vélain, 1877)*

Lutetina was included in Cyamiidae by Chavan (1969). Bernard (1898: fig. 1) properly figured the hinge plate of the type species, which shows a morphology and arrangement of teeth consistent with those present in Neoleptonidae. A similar conclusion was reached by Salas & Gofas (1998), who considered *Lutetina* as a possible synonym of *Neolepton*.

Dicranodesma *(type species:* Thecodonta (Dicranodesma) calvertensis *Dall, 1900)*

Described from the Miocene of Maryland, USA, this genus remains at present known only from the type species, which was regarded as Cyamiidae by Vokes (1980) and Chavan (1969). Judging from the descriptions and illustrations provided by Dall (1900: pl. 45, figs 23, 24) and Glenn (1904: pl. 88, figs 14–18), the type species has a short, conical anterior tooth and an elongated, 'lamelliform' posterior tooth in each valve. This hinge morphology does not agree with any Cyamioidea, but resembles the hinge plate of some Lasaeidae (Galeommatoidea).

Juliidae Smith, 1885

The family was originally described as belonging to Bivalvia (Smith, 1885), where it was allocated to Gaimardioidea by Thiele (1934). However, the current conception is that Juliidae is a family of sacoglossan gastropods (Le Renard *et al.*, 1996; Bouchet *et al.*, 2017).

Neoleptonidae Thiele, 1934

The position of this family has been controversial. Some authors included it in Cyamioidea (e.g. Thiele, 1934; Chavan, 1969; Powell, 1979; Sabelli *et al.*, 1990; Morton, 2015), whereas others regarded it as a (possible) Veneroidea (Ockelmann in Bowden & Heppell, 1968; Salas & Gofas, 1998). Mikkelsen *et al.* (2006) pointed out that this family joined Veneridae in the traditional morphology tree, but fell outside Veneroidea in their all-morphology analysis. The cardinal teeth in *Neolepton* appear translocated with respect to the condition present in *Cyamiocardium*, *Cyamiomactra*, *Cyamium*, *Heteromactra* and *Jukesena* (compare Figs 1A, C, D, F, G, I, J, L, 2A, C of the present study with Salas & Gofas, 1998: figs 1, 2 or Zelaya & Ituarte, 2004: fig. 2). In addition, none of the teeth in *Neolepton* is grooved, and *Neolepton* has strong posterior lateral teeth in both valves, which are not discernible in Cyamiidae. Another difference arises in the degree of development of the external ligament, which is small in *Neolepton* and is equally projected at both sides of the umbones (Salas & Gofas, 1998; D. Zelaya, personal observation), in contrast to the large ligament present in Cyamiidae, which is longer posteriorly. Regarding the anatomy, adult specimens of *Neolepton* (unlike cyamiid species) lack the byssus groove and functional byssus glands. In addition, *Neolepton* lacks tentacles at the posterior part of the mantle margin and lacks the peculiar follicular epithelium characteristic of Cyamioidea (Ituarte & Presta, 2017; D. Zelaya, personal observation). All this evidence pleads for the exclusion of *Neolepton* (and, consequently, the Neoleptonidae) from Cyamioidea. The additional molecular information obtained as part of the present study also supports this hypothesis, at the same time confirming its placement in Veneroidea.

Bernardinidae Keen, 1969

Coan (1984) redescribed and properly figured *Bernardina bakeri* Dall, 1910, the type species of *Bernardina*, considering this family as belonging to Cyamioidea. However, this species has a hinge plate and ligament that are not in agreement with those described above for cyamioideans, but closely resemble those present in *Neolepton* (compare Coan, 1984: figs. 2a, 2b with Salas & Gofas, 1998: fig 1, 2 or Zelaya & Ituarte, 2004: fig. 2). In fact, Bieler *et al.* (2010) regarded Bernardinidae as a synonym of Neoleptonidae. Contrary to *Neolepton* species, *B. bakeri* has massive anterior (instead of posterior) lateral teeth. This difference could justify considering *Bernardina* as a different (valid) neoleptonid genus. However, as stated by Salas & Gofas (1998), the study of living specimens of *B. bakeri* is needed to confirm its correct systematic position.

The same hinge morphology as *B. bakeri* is present in *Bernardina margarita* (Carpenter, 1857) and *Psephis salmonea* Carpenter, 1864 (figured by Coan, 1984: figs 4a, 10a, b, respectively). Morton (2015) provided detailed anatomical information for the last of these species (referred under the genus *Neolepton*). In general, the anatomy of that species closely resembles that of *Neolepton* species (properly described by Salas & Gofas, 1998). However, *Bernardina salmonea* differs clearly by having a byssus gland and byssus groove that remain well developed in large specimens (absent in the adults of *Neolepton*) and by the fact that brooding specimens show the abfrontal cells of the ctenidial filaments greatly glandularized (a condition thus far not observed in any *Neolepton* species). The last condition seems to be related to the fact that *B. salmonea* brood their embryos in the ctenidia, whereas in *Neolepton concentricum* (Preston, 1912) and *Neolepton cobbi* (Cooper & Preston, 1910) the larvae overgo their entire development attached to the external shell margin (Zelaya & Ituarte, 2004 and Ituarte & Presta, 2017, respectively). These anatomical and reproductive differences provide additional evidence to suggest that *Bernardina* is a distinct genus of Neoleptonidae.

Turtoniidae Clarke, 1855

For a long time, Turtoniidae was regarded as a family of Cyamioidea (Chavan, 1969; Vokes, 1980; Ponder & de Keyzer, 1998). After studying the anatomy, reproductive biology and shell morphology of *Turtonia minuta* (Fabricius, 1780), the type species of the genus on which Turtoniidae is based, Ockelmann (1964) concluded that this species corresponds to Veneroidea. Mikkelsen *et al.* (2006) found Turtonidae to be placed outside Veneroidea in all morphological studies they performed, but it was consistently shown to be a member of Veneridae in all their molecular studies.

Consequently, the authors proposed that it should be considered as a subfamily in Veneridae: Turtoniinae. The molecular studies by Combosch *et al.* (2017) support its placement in Veneroidea.

Basterotiidae Cossman, 1909

Even when this family was described, the genus *Basterotia* had generally been included in Sportellidae and was thus regarded as a Cyamioidea (e.g. Thiele, 1934; Chavan, 1969; Vokes, 1980; Coan, 1999). Despite that, and based on morphological, anatomical, ecological and molecular evidence, *Basterotia* was recently moved from Cyamioidea to Galeommatoidea (Campbell, 2000; Giribet & Distel, 2003; Taylor *et al.*, 2007; Goto *et al.*, 2011; Oliver, 2013), in which they appear as a clade (Goto *et al.*, 2012).

Galatheavalvidae Knudsen, 1970

The family was diagnosed by having an internal shell, completely covered by the middle mantle fold; the presence of a single (inner) demibranch; a ventrally displaced anterior adductor muscle; two permanent mantle margin openings (inhalant–pedal and exhalant); and a well developed foot, with byssus. Additional characters reported for *Galathea holothuriae* Knudsen, 1970, the only species thus far known of this genus, include the absence of teeth in the hinge plate and tentacles along the posterior part of the ventral margin, and the presence of a peculiar 'dorsal brood pouch', connected with the mantle cavity and extending beyond the shell. Although the superfamilial placement of this family was not determined by Knudsen (1970) at the time of erecting it, Galatheavalvidae currently appears listed under Cyamioidea by Bieler *et al.* (2010), a placement that appears improbable considering the above-mentioned morphological and anatomical characteristics. Alternatively, Bieler & Mikkelsen (2006) regarded Galatheavalvidae as Galeommatoidea. Huber (2010, 2015) followed this superfamilial placement, but considered Galatheavalvinae to be a subfamily of Galeommatidae.

SPORTELLIDAE: A FAMILY WITH UNCERTAIN AFFINITIES

From Thiele's (1934) classification to the most recent classifications of bivalves (e.g. Ponder & de Keyzer, 1998; Bieler *et al.*, 2010; Huber, 2010), the Sportellidae were always considered as cyamioideans. However, the concept of Sportellidae has varied greatly throughout time. In this regard, and as mentioned before, the genus *Basterotia* Hörnes, 1859 was included in Sportellidae for a long time, although it is currently considered as a different family of Galeommatoidea. Knowledge

about the species that remain at present regarded as Sportellidae appears mostly to be restricted to shell morphology (Coan, 1999). *Sportella dubia* (Deshayes, 1824), the type species of the genus (and the type genus of the family), is a fossil. Consequently, the diagnostic (reproductive, molecular and anatomical) characters used in the present study to define Cyamioidea cannot be studied in that species. The hinge of the specimen described and figured by Deshayes (1824) does not agree with those of Cyamiidae and Gaimardiidae. Ponder & de Keyzer (1998) figured a living specimen of '*Sportella* sp.' as having pedal, inhalant and exhalant apertures, the pedal completely papillate, the exhalant projected in a siphon and with 'well developed tentacles around the posterior inhalant and exhalant apertures'. Furthermore, this figured specimen shows the presence of a long foot, for which Ponder & de Keyzer (1998) described the presence of functional byssus gland(s?) and byssus groove in adults. The presence of a completely papillate pedal aperture and such a long foot are characters not observed in any other cyamioid genus. Some other living species were attributed by different authors to *Sportella*, although most of them were subsequently transferred to other genera, such as *Ensitellops*, *Fabella*, *Neaeromya*, *Paramya* and *Pseudopythina* (all of them currently regarded as Galeommatoidea). The reproductive characteristics of other living species still grouped in *Sportella* have never been investigated. The information available at present does not allow us to draw a conclusion on whether Sportellidae corresponds (or not) to Cyamioidea.

FOCUSING ON CYAMIIDAE: A CASE OF CONSERVED LINAGES OR A FAMILY LESS DIVERSIFIED THAN PREVIOUSLY THOUGHT?

Ponder (1971) pointed out that anatomy is the most useful character for determining the generic relationships of Cyamioidea. To date, anatomical information is known for a relatively reduced group of cyamiid species, as follows: *Cyamiocardium chuanisinense*, *Cyamiocardium crassilabrum*, *Cyamiocardium dahli*, *Cyamiocardium denticulatum*, *Cyamiocardium domaneschii*, *Cyamiocardium namuncurense*, *Cyamiocardium rotundatum*, *Cyamiocardium yeskumaala* (Soot-Ryen, 1951, 1957, 1959; Passos & Machado, 2014; Urcola & Zelaya, 2018; present study), *Cyamiomactra problematica* (Ponder, 1971), *Cyamium antarcticum* (Ponder, 1971; present study), *H. laminifera* (present study), *J. foveolata* (present study), *Perrierina taxodonta* (Ponder, 1971), *Ps. cardiformis* (Pelseneer, 1903; present study), *Ps. franki* (Zelaya & Ituarte, 2009; present study), *Ps. inexpectata* (present study), *Pt. georgiana* (present study) and *Reloncavia chilenica* (Soot-Ryen, 1957, 1959). Considering these species, no major anatomical differences appear among members of *Cyamiocardium*, *Cyamiomactra*, *Perrierina*, *Cyamium*, *Heteromactra*, *Pseudokellya*, *Ptychocardia* and *Reloncavia* to justify their generic separation. Instead, the characters previously regarded as distinctive at the generic level failed to separate groups when considering the intrageneric variability (such as, for instance, the variability observed in the relative size of the demibranchs or the degree of development of the posterior series of tentacles among different species of *Cyamiocardium*), whereas other 'differences' seem to have originated in the state of preservation of the studied material; for instance, the byssus groove of *Cyamiocardium*, which, contrary to the 'absence' mentioned by Soot-Ryen (1951; for *Cyamiocardium denticulatum*) is present in that genus (Urcola & Zelaya, 2018; present study). Consequently, anatomy does not help to separate most of the genera of Cyamiidae considered in the present study.

Thiele (1934) and Chavan (1969) differentiated the genera of Cyamiidae based on the general shell outline and shell sculpture, as follows: *Cyamiomactra* subtrigonal and smooth; *Cyamium* transversely elongated and smooth; *Kingiella* ovate (longer than high) and with strongly radially sculptured; *Perrierina*, *Legrandina*, *Cyamiocardium* and *Pseudokellya* roundish to ovate, smooth or radially ribbed; and *Ptychocardia* oblong (higher than long), sculptured with numerous radial folds, some of them strong and producing undulations in the ventral margin. Undoubtedly, Engl (2012) followed this distinction when he placed *Pseudokellya georgiana* in *Ptychocardia* again. The above-mentioned shell characters seem to be inadequate for a distinction of these genera, because some *Cyamiocardium* species are subquadrate or rhomboidal (*Cyamiocardium chuanisinense*, *Cyamiocardium denticulatum* and *Cyamiocardium namuncurense*), and some species currently regarded under *Cyamiomactra* are not subtrigonal or smooth (e.g. *Cyamiomactra chilensis* Ramorino, 1968 and *Cyamiomactra falklandica* Dell, 1964). Considering shell outline and shell sculpture, *Heteromactra* should be regarded as a synonym of *Cyamium* and not of *Cymiomactra*, as currently considered (e.g. Lamy, 1906, 1910, 1911, 1917; Chavan, 1969). In a similar manner, Zelaya & Ituarte (2009) interpreted the existence of a continuum in the shell outline and sculpture of *Pseudokellya* species, ranging from the rounded and weakly radially sculptured *Ps. cardiformis* to the rhomboidal and strongly radially sculptured *Pt. georgiana*, and also including the ovate and smooth *Ps. franki*.

The number, morphology and arrangement of hinge teeth, ligaments and their supports have been (and still remain at present) used as (some of) the main morphological characters for delimiting genera

of bivalves. However, and surprisingly, only two different hinge conformations are recognized when regarding the Cyamiidae considered in the present study. One conformation is present in *Cyamium* (as redescribed herein), *Cyamiocardium*, *Cyamiomactra*, *Heteromactra*, *Reloncavia*, *Perrierina* and *Legrandina*, the last two taxa with additional 'crests' along the dorsal margin, although these were interpretated as secondary acquisitions by Ponder (1971); in fact, these may be poorly developed in some species, such as *Legrandina harrisonae* Powell, 1935 and *Perrierina matai* Fleming, 1948. The second conformation is present in the hinge of *Pseudokellya* and *Ptychocardia*. This low degree of variability in the overall architecture of the hinge exhibited by cyamiids could reflect: (1) that the general hinge conformation (and the anatomy of the soft part) have remained unchanged ('conserved') among different cyamiid genera; (2) that the hinge conformation (and anatomy) experienced phenomena of parallelism/convergence among different cyamiid genera; or, more probably, (3) that the generic diversity of Cyamiidae is considerably lower than previously thought, with several of the genera that are currently regarded as distinct being synonyms. The previous proposals by Lamy (1917) to consider *Cyamiocardium* as a synonym of *Cyamiomactra* and by Ponder (1971) to regard *Cyamiomactra* as a subgenus of *Cyamium* support the last hypothesis. However, the currently available molecular information is not sufficient to be conclusive about this issue; further taxa and markers need to be studied.

DISCREPANCIES OF THIS STUDY WITH PREVIOUSLY PROPOSED SCHEMES

The present study reveals that Cyamiidae and Gaimardiidae are closely related families. This grouping contrasts with the most traditional point of view, which considered them as belonging to two different superfamilies: Gaimardioidea and Cyamioidea (Thiele, 1934; Chavan, 1969; Vokes, 1980). Although Ponder (1971) had previously suggested that these taxa were closely related (he considered them as two subfamilies of Cyamiidae), this proposal did not receive acceptance by subsequent authors. In fact, in most recent studies both taxa were regarded as belonging to different superfamilies (e.g. Bieler *et al.*, 2010; Lemer *et al.*, 2018).

The difference of the scheme proposed in the present study from that of Ponder (1971) is not restricted to the taxonomic rank assigned to Cyamiidae/Cyamiinae and Gaimardiidae/Gaimardiinae, but also concerns the placement of *Kidderia*. According to Ponder (1971), *Kidderia* is closer to *Cyamium* than to *Gaimardia* (consequently, he regarded it as a Cyamiinae), whereas according to the present study, *Kidderia* is more

similar to *Gaimardia* than to *Cyamium*, consequently being included in Gaimardiidae and not in Cyamiidae. The family placement for *Kidderia* proposed in the present study is in agreement with the previous usages by Odhler (1924), Thiele (1934) and Dell (1964); the placement in all these studies was based upon the characteristics present in the type species of *Kidderia* and the closely similar *K. bicolor*. However, the possibility that some other species currently placed under *Kidderia* could correspond to Cyamiidae cannot discarded, becuse *Kidderia* is presently considered as 'possibly polyphyletic' (Ponder, 1971).

Another point of discrepancy with the previous literature concerns the placement of *Pseudokellya*. This genus was largely regarded as belonging to Lasaeidae/Kelliidae (Galeommatoidea) (e.g. Chavan, 1969; Vokes, 1980; Engl, 2012). The information obtained in the present study (namely, the presence of a follicular epithelium surrounding the oocytes and embryos and the presence of tentacles at the posterior end of the mantle border) allows us to confirm that *Pseudokellya* is a Cyamioidea, as previously reported by Thiele (1934) and Zelaya & Ituarte (2009).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The present study reveals Cyamioidea as a monophyletic superfamily of bivalves. Members of this superfamily vary greatly in shell morphology and anatomy. However, all of them share a reproductive character: the presence of a follicular epithelium, which appears first at the early stages of oogenesis and persists until larvae are fully developed, brooded within the female gills. The follicular epithelium is unusual among bivalves and, in fact, it is not known for any other bivalve family; thus, it is valuable to define Cyamioidea. Curiously, and for the first time among marine bivalves, a reproductive character proves to be more informative than shell morphology and anatomy for the definition of a superfamily.

Despite the restricted significance of morphological and anatomical characters for defining Cyamioidea, they appear to be useful for delimiting families within this superfamily: Cyamiidae and Gaimardiidae, with the latter previously regarded as belonging to a different superfamily (Gaimardioidea). Neoleptonidae, previously regarded as cyamioideans, are definitively removed from this superfamily and reallocated into Veneroidea based on the molecular evidence obtained in the present study. The placement of Sportellidae in Cyamioidea requires confirmation.

As part of the present study, we confirm that *Gaimardia* and *Kidderia* can be reunited in Gaimardiidae and that *Cyamiocardium*, *Cyamium*, *Heteromactra*, *Pseudokellya*, *Ptychocardia* and *Reloncavia* belong to Cyamiidae. Within the latter

group, we also tentatively place *Cyamiomactra* and *Jukesena*, although some anatomical and molecular evidence suggests that they might, in fact, correspond to a different family of Cyamioidea. Likewise, the relationship of *Legrandina* and *Perrierina* to Cyamiidae requires further study.

The synonymy or, alternatively, the eventual validity and relationship among different (sub)genera of Cyamiidae and Gaimardiidae also need to be investigated in more detail, particularly the affinities of *Eugaimardia* and *Progaimardia* with *Gaimardia* and of *Costokidderia* and *Cyamionema* with *Kidderia*. In contrast, cyamiids now appear to be defined mainly upon shell characters. The hinge conformation, usually considered as a key character for defining genera of bivalves, does not provide a clear distinction among *Cyamiocardium*, *Cyamiomactra*, *Cyamium*, *Heteromactra*, *Legrandina*, *Perrierina* and *Reloncavia* or between *Pseudokellya* and *Ptychocardia*. The general shell outline and shell sculpture, currently used to define these genera, prove to be more variable than previously thought, and the same characteristics appear in different taxa. Furthermore, the molecular studies performed in the present study, although providing full support for the entire superfamily, do not resolve the relationships among (sub)genera. All these facts imply either that several of these names are currently being used ambiguously or that some of them correspond to synonyms. The addition of more anatomical and molecular information for other species (in particular, molecular studies involving faster-evolving genes and further taxonomic sampling) appears crucial for an adequate re-evaluation of the validity of the different genera. We prefer to refrain from introducing nomenclatural changes or including new synonymy for these taxa until further study has taken place.

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