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Biology, functional morphology and genetics of the Australian endemic bivalve *Ciboticola lunata* (Bivalvia: Mytiloidea: Ciboticolinae): a new subfamily showing epizoic commensalism

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ABSTRACT

In terms of its overall shell shape, the Australian marine mytiloidean *Ciboticola lunata* strongly resembles the Asian freshwater mytiloidean *Sinomytilus harmandi* which, superficially, may indicate a close phylogenetic affiliation between these two species. This study demonstrates, however, that this is because both taxa are 'shell-clinging shells' – the former byssally nestling on *Anadara pilula,* the latter on *Corbicula fluminea* that live as epizoic commensals tapping into the inhalant streams of their hosts. Their similar shell shape simply results from a similar lifestyle – a deeply concave shell ventrally, enabling firm byssal attachment to their curved hosts, and thus is an evolutionary convergence. In terms of shell morphology the two species are distinct. *Ciboticola lunata* possesses a nepioconch, byssal setae attached to the shell posteriorly, long labial palps and the possibility of a vestigial anterior adductor muscle positioned on its internal umbonal septum. *Sinomytilus harmandi* possesses none of these characters. Genetic evidence supports this distinction; *S. harmandi* along with *Limnoperna fortunei* being contained within their own subfamily, Limnoperninae, and associated with other mytilines. Conversely, *C. lunata* is herein shown to be affiliated with the Modiolinae, as the shell morphology also suggests, yet situated in its own subfamily, Ciboticolinae subfam. nov.

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Introduction

Representatives of the Mytiloidea Rafinesque, 1815 are widely distributed in our modern seas where many live byssally attached in a variety of marine (and some freshwater) habitats from the intertidal to the deep sea. Some taxa are habitat-forming and are among the most ecologically, and in some cases commercially, important bivalves (Gosling 2021). While colonial large-bodied taxa form reef-like structures on sedimentary seabed substrata (Buschbaum et al. 2009), small-bodied species often attach to hard geogenic or biogenic substrata. Some of the latter live as epizoic commensals on interspecific hosts (e.g., Dinesen and Ockelmann 2005), or bore into corals (Kleemann 1990; Valentich-Scott and Dinesen 2004) or adhere to the curved shell exterior of other molluscans (Hedley 1902; Morton and Dinesen 2010; Morton et al. 2020b). This research is concerned with such representatives of the Mytiloidea and in particular with the subfamilies Mytilinae and Modiolinae G. & H. Termier, 1950. We refer to the species of *Mytilus* Linnaeus, 1758 and *Perna* Philipson, 1788 in the case of the former subfamily and *Modiolus* Lamarck, 1799 in the case of the latter. The Mytiloidea is currently considered monophyletic, and basal to the suborder Pteriomorphia Beurlen, 1944, whereas the lower order taxonomy of this superfamily remains unsettled (Morton 2015a).

Molluscan commensals live associated with a diverse array of hosts, such as annelids, crustaceans and echinoderms (Stanley 1970; Morton 1988; Li et al. 2016), however, relatively few form partnerships with other molluscs. Vermeij (2020) showed that a variety of small patelliform gastropods have specialised to commensal life on the curved surfaces of larger gastropods, whereas others are epibiontic generalists kleptofeeding from the inhalant current of their larger suspension feeding bivalve

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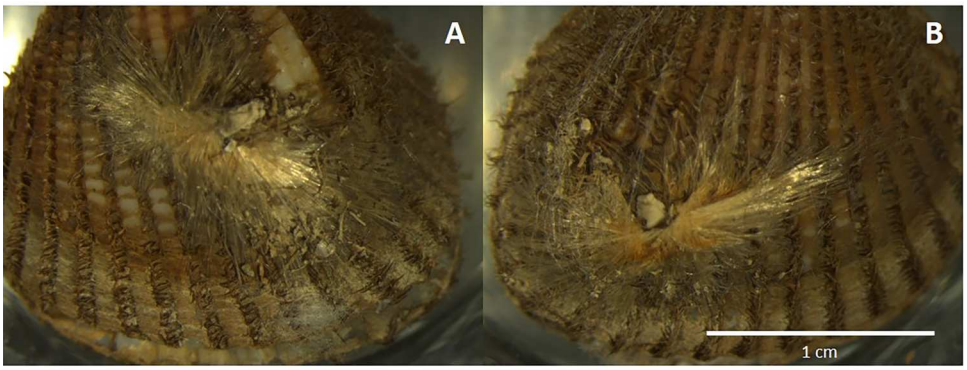

Figure 1. *Anadara pilula*. The left (**A**) and right (**B**) shell valves of the larger (29.0 mm shell length) individual showing characteristic byssal masses of *Ciboticola lunata* in the same approximate positions on both shell valves.

(pteriomorphian) hosts (Sharman 1956; Thieltges et al. 2003). Some ostreids, anomiids and pectinids are facultative, interspecific commensals of other pteriomorphians and may benefit metabolically from their hosts' flow field (Stanley 1970). Most mytiloideans are suspension feeders (Morton 1996), although some representatives of the subfamily Bathymodiolinae Kent & B.R. Wilson, 1985 have evolved to harbour symbiotic chemosynthetic carbon-fixing bacteria in their gill tissue (Duperron et al. 2009). Deposit and carnivorous feeding has been reported upon in a few species (Davenport et al. 2000; Lehane and Davenport 2002; Ockelmann and Dinesen 2011), but none are considered obligatory carnivores (Morton and Machado 2019). Settling larvae of aggregating mytiloideans may risk predation, whereas juveniles are likely to benefit from the feeding currents of their adult conspecifics (Bayne 1976), and smaller commensals nestling on ascidians may benefit from the feeding streams of their host (Morton and Dinesen 2011).

There are few examples of mytiloideans specialised to commensal life on the shell surface of a larger bivalve. One is the freshwater *Sinomytilus harmandi* (Rochebrunne, 1882), which in the Mekong River lives attached to shells of living endobenthic corbiculids, *Corbicula fluminea* (O.F. Müller, 1774) (Morton and Dinesen 2010). This species is virtually unstudied and believed to be endemic to the Mekong River (Thiele 1934; Morton and Dinesen 2010). It has, moreover, recently been shown to be related to the monospecific freshwater taxon *Limnoperna fortunei* (Dunker, 1857) and thereby situated with it in the subfamily Limnoperninae Scarlato & Starobogatov, 1979 (Morton et al. 2020b). As of recent years, *L. fortunei* has become highly invasive and has become an important aquatic pest in its conquered habitats (Boltovskoy 2015).

There is, however, another mytiloidean that attaches to other bivalves and is also largely unstudied except for descriptions of its shell. That is *Ciboticola lunata* (Hedley, 1902), colloquially referred to as the 'shell-clinging shell'. This is, as far as is known, an Australian (Queensland) endemic and although dry material exists in several Australian museums, the only two intact individuals are held in the collections of the Museum and Art Gallery of the Northern Territory (NTM). Through the courtesy of Dr Richard Willan, the senior author of this paper (BM) received two individuals of *C. lunata* and their two *Anadara pilula* (Reeve, 1843) hosts on loan and for study. Regrettably, the two *C. lunata* individuals had been removed from their respective hosts when they were received at NTM, but with the byssal threads of the former still attached to the latter, it has been possible to identify their respective relationships with one another. The two individuals of *C. lunata* came from the wet collections of the NTM, and have the same registration number, P.54467. They came to NTM with their host shells – the arcid *Anadara pilula*. The two *C. lunata* were collected from Hay Point on the coast of central Queensland, and each was attached to a living *A. pilula*. Both specimens of the host have the registration number P.54466. These bivalves were collected during a survey for introduced marine species in the areas proposed for dredging and ocean disposal at the Port of Hay Point, in October 2014.

An interesting point is that the larger (29.0 mm shell length) of the two *A. pilula* shells had characteristic *C. lunata* byssal thread masses in approximately the same position on both shell valves (Figure 1). Since only one of the *C. lunata* individuals presently under study can be associated with this larger *A. pilula* shell, then it must be assumed that either: (i), an earlier occupant on the other valve had recently died; (ii), a second living individual was knocked off during the collection process, or (iii), it had been lost subsequent to collection.

The first aim of this research was to identify morphological and anatomical features of the remarkable *C. lunata* to define the relationship between this epibiont and its (as far as we know sole) host *A. pilula*. The second aim was to investigate the phylogenetic relationship of *C. lunata* with representatives of the other three known internal-anteriorly septate mytiloidean genera, *Mytilisepta* Habe, 1951, *Sinomytilus*

Thiele, 1934 and *Septifer* Récluz, 1848. For this purpose, new gene sequences were provided for *C. lunata* and molecular-based phylogenies were established to clarify a potential convergent evolution of morphological shell characters within the Mytiloidea.

Materials and methods

The two *Ciboticola lunata* individuals were examined using a dissecting microscope and the internal tissues were removed from the shells. The shells were measured, and drawn from various aspects. Subsequently, the two *C. lunata* and one of the *A. pilula* shells were examined under a stereomicroscope (Leica M165 C) to further clarify details. The internal tissues of the two *C. lunata* individuals were dissected to identify details of their anatomy and once this was completed, the mid-section of one individual was sent to the University of Split (Croatia) for sectioning. Following routine histological procedures, the resulting sections, cut at 6 µm, were mounted on microscope slides and stained in Ehrlich's haematoxylin and eosin. These slides are now deposited in NTM.

Finally, the posterior regions of the two individuals of *C. lunata* were put into separate vials with 100% ethyl alcohol and sent for genomic DNA analysis by coauthors (JW, PTYL and SSWC) in Hong Kong.

Phylogenetic analyses

Total genomic DNA was isolated from either the adductor muscle or mantle tissue of each of the two individuals of *C. lunata* using a TaKaRa MiniBEST universal genomic DNA extraction kit v5.0 (Takara Bio Inc.) following the manufacturer's instructions. Fragments of cytochrome *c* oxidase subunit I (COI), 18S rRNA, 28S rRNA and histone H3 (H3) were amplified and sequenced as previously described (Morton et al. 2020a, 2020b). Information on the primers used in this study and the GenBank accession numbers of the *C. lunatica* sequences are available in Table S1 and Table S2, respectively (Supplementary data).

The placement of *Ciboticola* within the Mytiloidea was revealed using a phylogenetic tree. The dataset for phylogenetic analyses is mainly derived from the phylogenetic tree described in Morton et al. (2020a). Sequence alignments, saturation tests and the selection of the best-fit partitions and substitution models followed the same procedures as described in Morton et al. (2020a, 2020b) (Tables S3 and S4) (Supplementary data). A final alignment of 3060 bp in a concatenated matrix (1st codon position of COI, 221; 2nd codon position of COI, 221; 18S rRNA, 1607; 28S rRNA, 674; 1st codon position of H3, 113; 2nd codon position of H3, 112; 3rd codon position of H3, 112) was used.

Maximum likelihood (ML) trees of genes and concatenated data were reconstructed using the GTR + G + I model to all partitions with 1000 replicates to estimate bootstrap values, while Bayesian inference (BI) trees were reconstructed in MrBayes v3.2 (Ronquist et al. (2012) following the previously described procedures (Morton et al. 2020b)). The phylogenetic trees were visualised using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree).

Abbreviations used in the figures

A – anus; AAM? – possible anterior adductor muscle (or scar); ALP – anterior labial palp; APRM – anterior pedal retractor muscle; BG – byssal groove; BGC – basiphilic byssal gland; BY – byssus; BYC – byssal chords; BN – byssal notch; CPG – cerebro-pleural ganglion; D – dissoconch; DD – digestive diverticulae; EGC – eosinophilic glands cells; ES – exhalant siphon; F – foot; H – heart; IA – inhalant aperture; ID – inner demibranch; ILP – inner labial palp; IMF – inner mantle fold; K – kidney; L – ligament; LM – lip of mouth; M – mouth; MMF – middle mantle fold; N – nepioconch; O – ovary; OD – outer demibranch; OE/DD – oesophagus and digestive diverticulae; OLP – outer labial palp; OMF – outer mantle fold; P – periostracum; PI – prodissoconch I; PII – prodissoconch II; PAM – posterior adductor muscle; PBRM – posterior byssal retractor muscle; PBRM (1-~7) – posterior byssal retractor muscle (Blocks 1 to ~7); PG – pallial gland; PLP – Posterior labial palp; PM – pedal musculature; PN – pallial nerve; PPRM – posterior pedal retractor muscle; PR1 – prodissoconch 1; PR2 – prodissoconch 2; PRM – pallial retractor muscle; PRT – pallial rejectory tract; R – rectum; S – septum; SM – suspensory membrane; SPIMF – sensory papillae of the inner mantle folds; SS – siphonal septum; TMF – transverse muscle fibres; VM – visceral mass.

Results

Taxonomy

Hedley (1902, p. 8 + plate I, figs 1–4) first described *Congeria lunata* from a single specimen collected from Keppel Bay, Queensland, by Mr G.L. Pilcher in 1887. The holotype is held in the Australian Museum, Sydney, (Registration Number AMS C.12069). Hedley's description of the shell will be referred to subsequently, but Figure 2 here reproduces the four views of the holotype as described and illustrated by Hedley (1902, figs 1–4), being from left to right: the anterior end of the right valve; an external view of the left valve; a ventral view of the left valve; an internal view of the left valve.

The generic redescription by Iredale (1939, p. 223) is somewhat more revealing, as follows: 'A very pleasing little shell was named *Congeria lunata* by Hedley ('Proc.

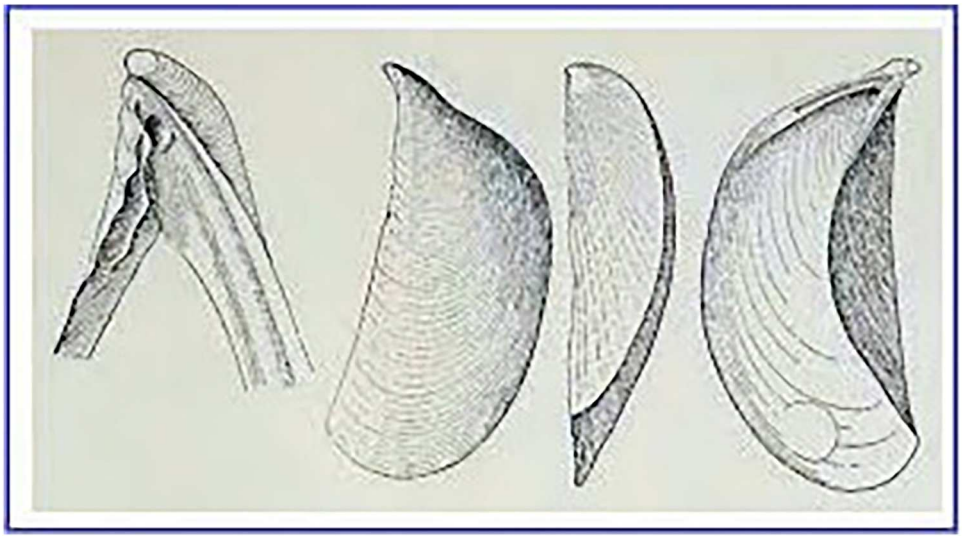

Figure 2. Four views of the specimen of *Ciboticola* as described and illustrated by Hedley (1902, figs 1–4). From left to right: the anterior end of the right valve; an external view of the left valve; a ventral view of the left valve; and an internal view of the left valve.

Linn. Soc. N.S.W.' XXVII, p. 8, pl i, figs. 1–4, 22nd August, 1902) on account of its very peculiar shape. It is a marine species ranging along the Queensland coast, and has been found living on the globose Arks at Keppel Bay and Seaforth, and is obviously a *Modiola* derivative, but nothing whatever to do with *Congeria*, which is made a fossil subgenus of *Dreissena*, a freshwater mussel of Europe. The generic name *Ciboticola* is introduced, the strong curvature of the shell, the concave ventral area, the very short hinge line, the terminal umbones and the small interior umbonal shelf being cumulatively important.' Iredale (1939) further referred to *Ciboticola* as being an obvious derivate of *Modiolus* (Mytiloidea: Modiolinae), unfortunately without substantiating this view.

Newell (1969, p. N861) followed Iredale (1939) in assigning the genus *Ciboticola* to the Mytilidae under the section titled *Bivalve Genera Uncertainly Assigned to Families*. Newell (1969, also on p. N861) further placed another freshwater taxon, *Sinomytilus*, in the Mytilidae list of uncertainly assigned genera. Thiele (1934, p. 801) actually placed *Sinomytilus* as a Section of the subgenus *Chloromya* Mörch, 1853 within the Mytilidae although Brandt (1974) re-assigned it to the Dreissenidae, a conclusion followed by Bogan (2008). This genus was, however, subsequently re-assigned to the Mytilidae by Morton and Dinesen (2010) based on *S. harmandi*, a conclusion confirmed genetically by Morton et al. (2020b). Thiele (1934) noted that other freshwater species from China and Indochina originally described as *Dreissena* also belonged to *Sinomytilus*. In addition to *S. harmandi*, two more species are currently assigned to this genera, that is *S. morrisoni* Brandt, 1974 and *S. swinhoei* (H. Adams, 1870) (Huber 2010; MolluscaBase 2022).

Scarlato and Starobogatov (1979) considered *Ciboticola* to be a member of the mytiloidean subfamily Septiferinae Scarlato & Starobogatov, 1979, and this taxonomic assignment is still followed by the World Register of Marine Species (WoRMS) (MolluscaBase 2022). This emphasises the necessity of using complementary molecular information to resolve phylogenetic relations, especially of highly specialised taxa.

Lamprell and Healy (1998, p. 86–87, species no. 189, + 1 colour plate) provided a description of the genus *Ciboticola* and *C. lunata* and noted that it attains a shell length of 30 mm and lives attached to ark shells in shallow waters off Queensland. These authors followed Newell (1969) and formally assigned *Ciboticola* to the Mytilidae, but noted that '… this genus may be better assigned to the Family Dreissenidae Gray in Turton, 1840.'

Biology

Members of the abyssate Arcidae Lamarck, 1809 (= Anadarinae Reinhart, 1935 – alternative representation) typically lie buried shallowly in soft sediments. Stanley (1970, plates 5 and 6) illustrated the partly buried life positions adopted by *Anadara ovalis* (Bruguière, 1789) (= *Lunarca ovalis* Bruguière, 1789), *Anadara notabilis* (Röding, 1798) and *Anadara chemnitzi* (Philippi, 1851) and showed that they all lay buried shallowly with the antero-margin of their shells downwards and thus with the postero-dorsal region of the shell situated just at and above the sediment surface. Species of *Anadara* do not possess fused siphons so the exhalant aperture is oriented nearly perpendicular to the sediment surface whereas the dorsal end of the inhalant aperture is situated just at the sediment surface and the remainder of its wide gape is situated within the sediment. This orientation has been illustrated for species of *Anadara* by Morton (1996).

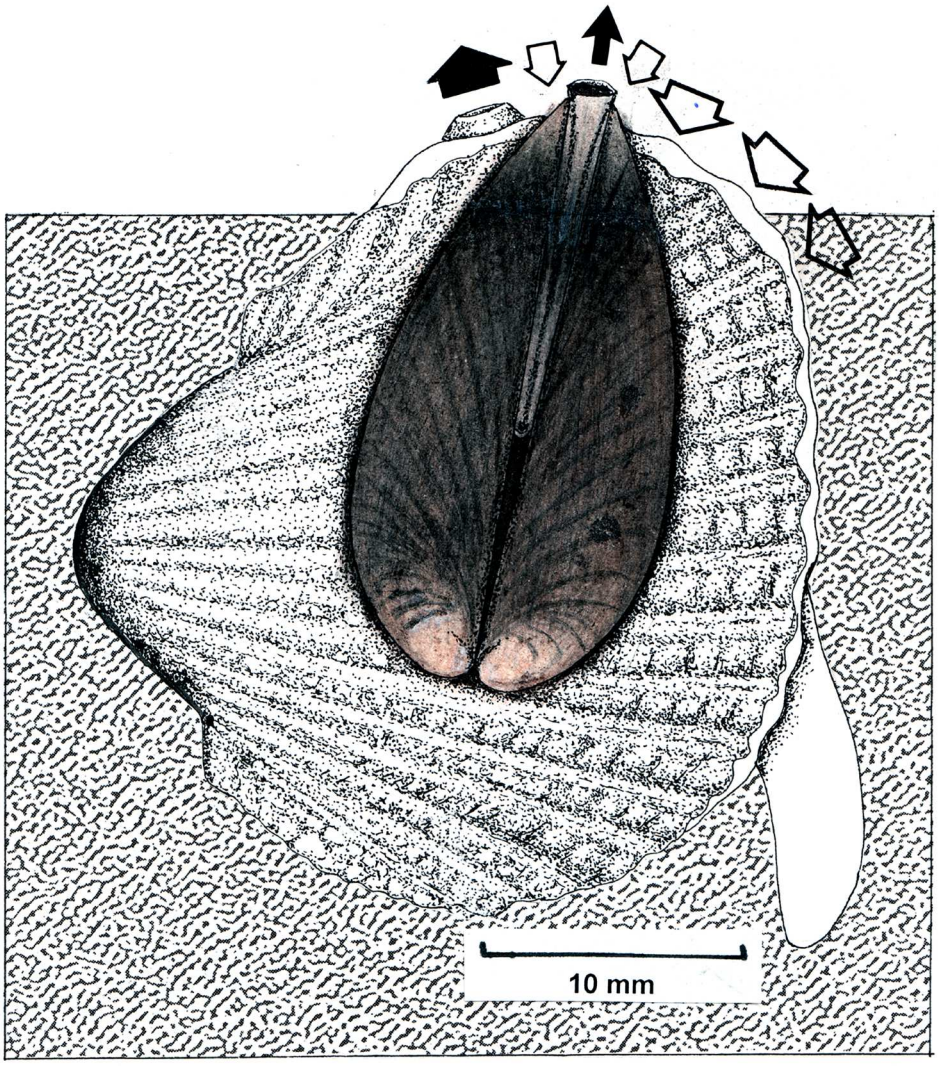

Figure 3. An illustration of an *Anadara pilula* shell in its postulated position in the sediment and the left valve colonised by an individual of *Ciboticola lunata*. Large open and closed arrows indicate the inhalant and exhalant currents, respectively, of *A. pilula*. Smaller arrows indicate those of *C. lunata*.

Both individuals of *Ciboticola lunata* studied here were attached to their *Anadara pilula* hosts positioned approximately at the mid-points of the latter's right valves. Based on the above descriptions and the locations and orientation of the byssal masses still attached to the two *A. pilula* shells, Figure 3 is an illustration of the ark shell individual in its postulated position in the sediment and colonised by one individual of *C. lunata*. Open and closed arrows indicate the pairs' inhalant and exhalant currents, respectively.

Morphology

The adult shell

The dimensions of the two individuals of *Anadara pilula* studied here are given in full in Table 1. The larger had a shell length of 26.9 mm and the smaller 21.4 mm. The shell dimensions of the two individuals of *Ciboticola lunata* are also given in full in Table 1, with the maximum length of the largest individual being 29.0 mm and with a width of 13.7 mm – this being slightly narrower than the second shorter individual. The shell height difference between the overall height and the height between the apex and the ventral concavity (Table 1) demonstrates just how deeply ventrally concave is the shell of *C. lunata*.

The adult dissoconch shell of *C. lunata* (Figure 4) is plain dark brown dorsally and lighter brown ventrally

Table 1. *Anadara pilula* and *Ciboticola lunata*. Dimensions of the two individuals of each species under study. Shell height 1 refers to the overall height of *C. lunata*; Shell height 2 refers to the height between the apex and the ventral concavity of *C. lunata*.

Dimension (mm)	Shell length	Shell width	Shell height 1	Shell height 2
Anadara pilula				
Specimen 1	26.9	23.3	26.5	
Specimen 2	21.4	19.2	21.5	
Ciboticola lunata				
Specimen 1	29.0	13.7	12. 4	9.9
Specimen 2	26.7	14.1	11.4	9.6

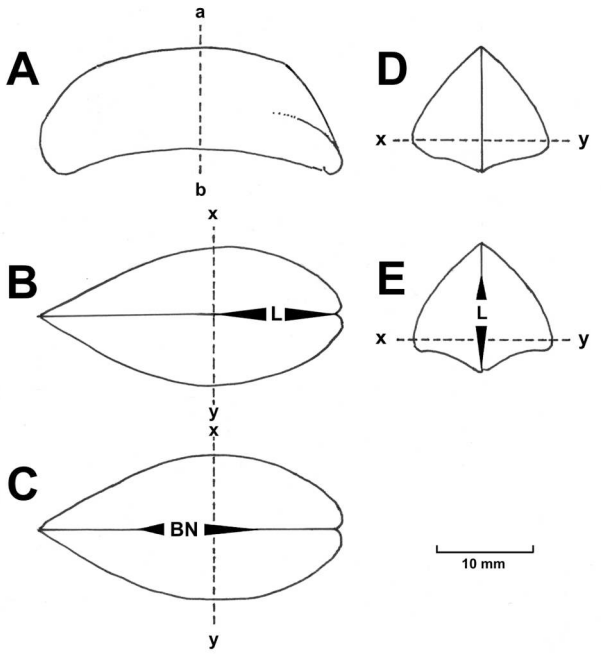

Figure 4. *Ciboticola lunata.* Outline drawings of the shell from various aspects. **A,** from the right lateral; **B,** from the dorsal; **C,** from the ventral, **D,** the posterior; and **E,** from the anterior. a–b, greatest shell height; x–y, greatest shell width. For abbreviations see the list at the end of the Materials and Methods section.)

and ornamented with commarginal growth lines and fine radial rays, the latter most prominent ventrally. When seen from the right side (Figure 4A), the shell is acutely heteromyarian and distinctively inequilateral, the posterior margin being rounded whereas the anterior is pointed and strongly keeled. The shell is therefore pointedly axe-shaped and deeply concave antero-ventrally (a–b, greatest shell height). The umbones lie terminally but the antero-dorsal margin is steep and only slightly curved. Seen from the dorsal aspect (Figure 4B), the shell is widely inflated around the mid-line (x–y, greatest shell width) and narrowly pointed posteriorly. The dorsal valve margins are straight, and are separated almost up to the mid-line by a narrow ligament.

Mid-ventrally (Figure 4C), there is a distinct byssal notch. From the posterior aspect (Figure 4D), the shell of *C. lunata* is squat and sharply triangular in outline and is widest (x–y) towards the lowest one-fifth of the dorso-ventral height of the shell. From the anterior aspect (Figure 4E), with the ligament only slightly visible externally, the shell is also squat and sharply triangular in outline and, again, widest (x–y) towards the lowest one-fifth of the dorso-ventral height of the shell. The light hazelnut-brown periostracum has byssal setae attached to it posteriorly.

The internal anterior hinge region of *C. lunata* is shown in more detail in Figure 4A. The shell septum is distinct beneath the umbo, with the anterior pedal retractor muscle scar separate from it and situated under the ligamental resilifer of each valve. There is no obvious anterior adductor muscle scar although the structure of the septum, with a swollen posterior rim, suggests that if there is such an adductor it is either so small as to be undetectable or is vestigial.

The shell of *C. lunata* (Figure 4A) shows four stages of growth: the embryonic and larval prodissoconch shells (PI and PII), the postmetamorphic juvenile nepioconch (N), and the final (adult) dissoconch (D). The sizes of PI and PII (largest diameter) were approximately 150 and 750 µm, respectively, which strongly suggests larval planktotrophy. The size of the nepioconch was approximately 3200 µm. The growth axis of the adult dissoconch shell is highly angled (approximately 180°) to that of the larval and juvenile shells. As the two specimens of *C. lunata* are old, the teeth of the larval shell stages (potential a- and b-series teeth) are not visible, neither is there any trace of potential c-series teeth.

As noted above, the posterior shell regions of *C. lunata* possess byssal setae with an expanded base and a long tapering point (Figure 4B). These byssal setae are secreted by the pedal byssal gland complex and positioned by the foot. Byssal setae such as these are thought to be most typical of the Modiolinae (Dinesen and Morton 2014) and some Septiferinae, and possibly have a defensive function (Wright and Francis 1984). Their detailed structure in *Modiolus traillii* (Reeve, 1857) has been described by Choo et al. (2014). The pedal byssal gland complex also secretes the byssal threads that attach the bivalve to its *A. pilula* shell host (Figure 4C). The deep convexity of the *C. lunata* shell allows it to affix strongly over the convex shell of its *A. pilula* shell host and such byssal threads are long and widely spaced out in all horizontal dimensions, but especially antero-posteriorly, with a short central apical stalk demonstrating their origin in the byssal apparatus of the foot.

The larval and juvenile shells

Ockelmann (1995) outlined mytiloidean shell ontogeny as a biological trait component important in disentangling the major phylogenetic lines within this superfamily. Fortunately, the two *C. lunata* individuals show well preserved anterior regions (possibly because they were still attached to their host) which has allowed for inspection of the early shell stages, their occurrence, colour, shape and size. Due to the iterative manner of mytiloidean shell growth, in the late adult stage ligaments, teeth series and hinge structures are only visible for the dissoconch.

The *C. lunata* prodissoconch I (Figure 5, PI) is highly convex and transparent with a few thin commarginal lines near the shell edge, while the subsequent prodissoconch II (Figure 5, PII) is 4–5 times larger (its edges invisible as overgrown by the adult shell hinge

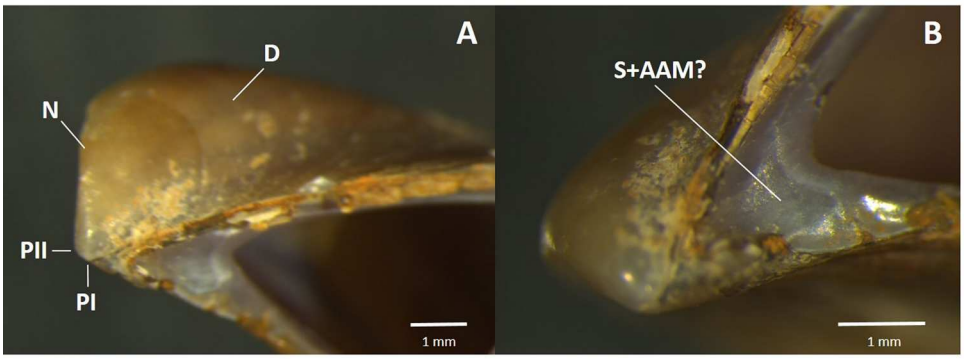

Figure 5. *Ciboticola lunata*. High magnification photographs of the anterior-most end of the shell. **A,** the larval shell stages; and **B,** the internal septum. For abbreviations see the list at the end of the Materials and Methods section.

structures), delicate, and of a non-transparent milky colour with the growth axis oriented at a slight angle to the PI.

In *C. lunata*, a third shell stage, the nepioconch (Figure 5,N), is clearly present as a light hazelnut-brown shell with a fan-shaped growth pattern extending out from the PII. In mytiloidean species, when present the nepioconch is often relatively thin with either few or no features except for thin commarginal growth lines, which may relate to rapid shell growth during the individual's early benthic life, and immature mantle folds and glands complex responsible for shell growth deposition during the juvenile stage. This shell stage is absent in some taxa, such as representatives of all the Mytilinae, while it forms the juvenile shell in others, such as species of the Modiolinae. The *C. lunata* dissoconch (Figure 5,D) is chestnut brown with a growth direction highly angled to the nepioconch such that the latter almost forms a 'cap' on the antero-dorsal part of the former.

The curvature and concavity of the ventral regions of the shell, in which the shell is ornamented with faint ridges transverse to the commarginal growth lines, is a clear adaptation to its epizoic mode of life on its host and could suggest a use of chemical secretions to shape its habitat, as is known from other mytiloidean species (Kleemann 1990; Valentich-Scott and Dinesen 2004; Ockelmann and Dinesen 2009).

Internal shell structure and musculature

Internally (Figure 6), the shell of *Ciboticola lunata* shows no evidence of d-series teeth along its internal margin. That is, it is uniformly smooth, though partially hidden (especially antero-ventrally) by the thickened periostracum. Each umbo lies anterior to the internal shell septum that is itself situated under the anterior end of the internal ligament.

Internally, the shell of *C. lunata* (Figure 6) is relatively thin and nacreous, and is lighter antero-ventrally and darker elsewhere. The internal shell layer is light brown. The ligament is internal and located along left and right resilifers that are extensions of the dorsal ridge of the septum. The resilial ridge (that is, the subligamental shell layer) that supports the ligament is quite solid with no conspicuous pores.

The musculature of *C. lunata* (Figure 7) comprises a large posterior adductor muscle and, extending

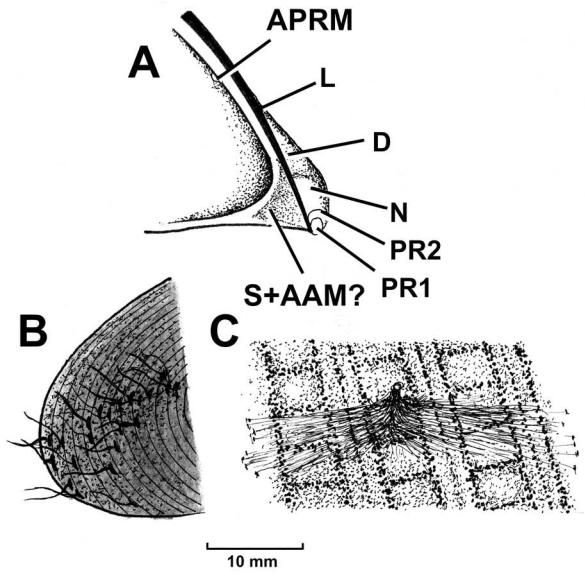

Figure 6. *Ciboticola lunata*. **A,** the anterior end of the left shell valve showing the internal septum and the larval and juvenile shell stages; **B,** byssal setae on the posterior end of the shell; **C,** the byssal plug and its threads on the right shell valve of an individual of *Anadara pilula*. For abbreviations see the list at the end of the Materials and Methods section.

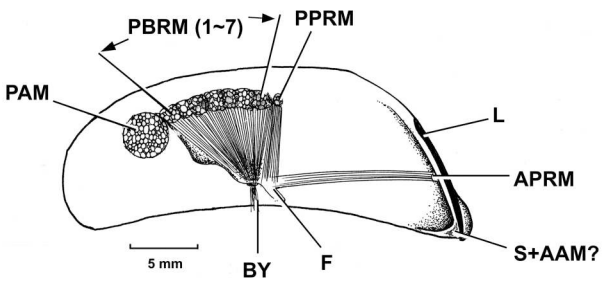

Figure 7. *Ciboticola lunata*. The body musculature as seen from the right side. For abbreviations see the list at the end of the Materials and Methods section.

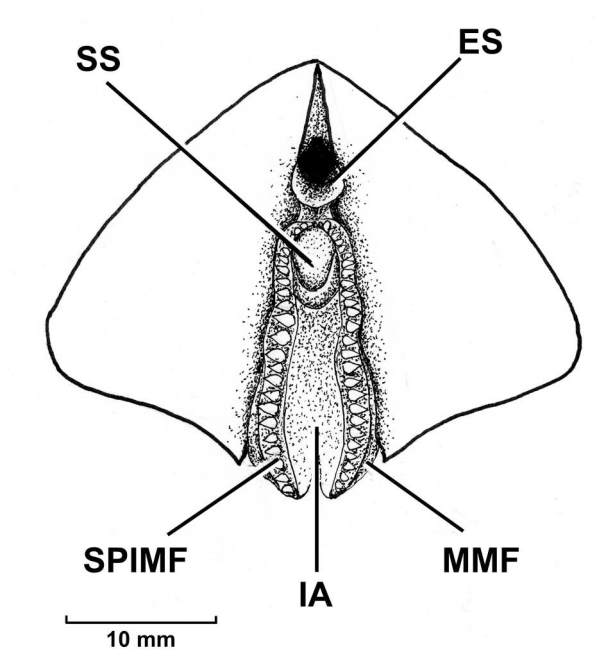

Figure 8. *Ciboticola lunata*. The siphonal apparatus as seen from the posterior aspect. For abbreviations see the list at the end of the Materials and Methods section.

anteriorly from it, a row of some seven byssal retractor muscles. Anterior to these is an unusually large posterior pedal retractor muscle that extends downwards into the foot. As mentioned earlier, there is either no or a vestigial anterior adductor muscle scar, although there is a relatively small anterior pedal retractor muscle with its base located under the ligamental resilifer and somewhat distant from the internal shell septum.

The siphons

The siphonal apparatus of *Ciboticola lunata* is illustrated in Figure 8 as seen from the posterior aspect. There is a low, lightly coloured brown but otherwise unadorned exhalant siphon. Internally, between this and the inhalant aperture is, as in all described mytiloidans, there is a concave siphonal septum or valve. The inhalant aperture is long, not separated from the pedal gape and has a smooth internal margin. The posterior outer margin of the aperture has a strongly papillate margin, but the papillae are small.

The mantle margin

A transverse section through the mid-ventral right mantle margin of *Ciboticola lunata* is illustrated in Figure 9. The mantle itself is thin and the two epithelia are criss-crossed by fine connectives. Conversely, the mantle margin is much larger and comprises the typical three folds – inner, middle and outer. Where the inner fold connects with the general mantle, there is a ciliated rejectory tract. The mantle is attached to the shell at the pallial line. The line is formed on the inner surface of the shell by the pallial retractor muscles, the other ends of the branches of which extend into all three marginal folds of the mantle. The pallial nerve occurs within these muscle fibres. The periostracum arises between the middle and outer folds, and the middle fold is filled with a dense array of eosinophilic gland cells. Otherwise, the mantle margin has a relatively simple structure.

The organs of the mantle cavity

The organs of the mantle cavity of *C. lunata* are illustrated in Figure 10 as seen from the right side and after removal of the right shell valve and mantle lobe. The dotted lines represent the posterior edges of the suspensory membranes of the labial palps.

There is a large posterior adductor muscle and a similarly large posterior byssal retractor muscle that is divided into some seven units, above which lie the paired kidneys. These large muscle blocks extend into a small visceral mass from which a small foot and a byssus arise mid-ventrally and anteriorly. The heart is located anterior to the byssal retractor muscles and is thus of Category 2 (Morton 2015a). That is, as in representatives of the Mytilinae, including *S. harmandi* (Morton and Dinesen 2010; Morton 2015a) but not *L. fortunei* (Category 1; Morton 2015b). The digestive diverticulae lie under the ligament.

Water enters the mantle cavity via the long inhalant aperture and exits via the small exhalant siphon.

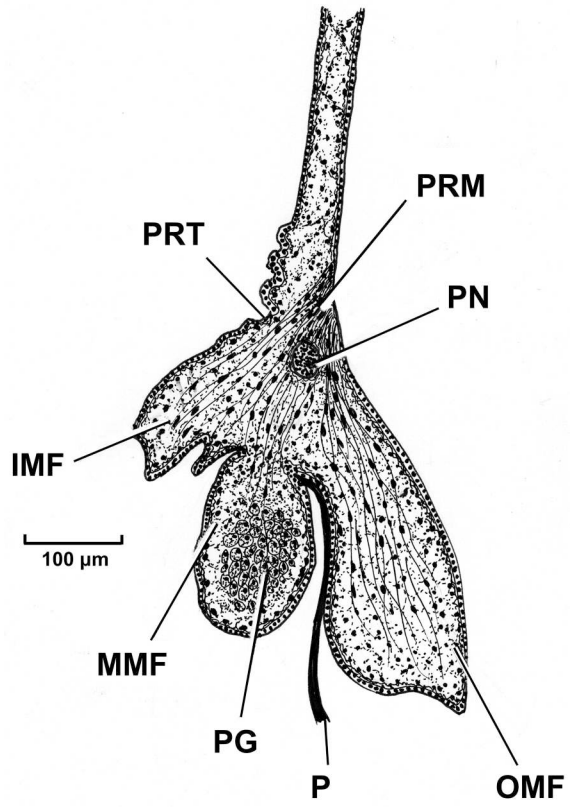

Figure 9. *Ciboticola lunata*. A transverse section through the mid-ventral right mantle margin. For abbreviations see the list at the end of the Materials and Methods section.

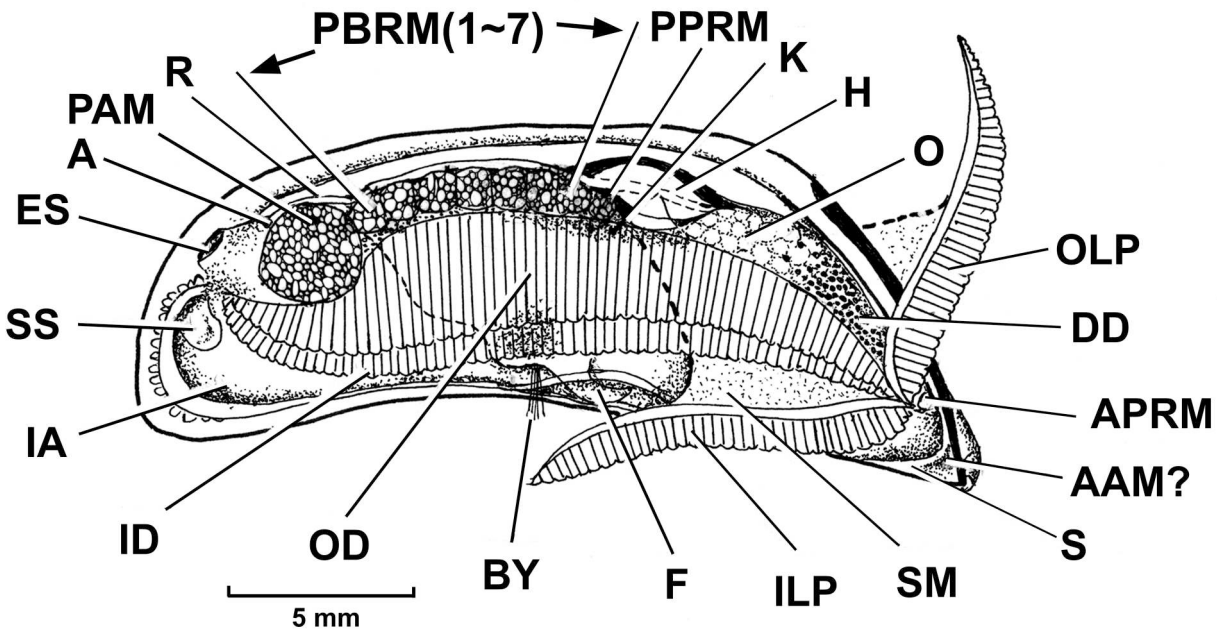

Figure 10. *Ciboticola lunata*. The organs of the mantle cavity as seen from the right side after removal of the right mantle lobe. The dotted lines represent the posterior edges of the suspensory membranes of the labial palps. For abbreviations see the list at the end of the Materials and Methods section.

Between the two structures extend the ctenidia, each comprising long, tall, inner and outer demibranchs. These lead to long labial palps. These are exceptionally long, and are supported along half their length posteriorly from the anterior mouth by a membrane that attaches to the mantle in relation to the outer demibranch, and to the dorsal line of the visceral mass in relation to the inner demibranch (Figure 10, dotted lines).

The anterior ends of these structures are further illustrated from the ventral aspect in Figure 11. The mouth with anterior and posterior lips lies between the paired anterior pedal retractor muscles close to their attachment to the shell and anterior to the circum-oesophageal cerebro-pleural ganglia. Also arising posteriorly from the mouth is the oesophagus. The anterior areas of the visceral mass are characterised by the digestive diverticulae that surround the stomach. The gonadal tissues are located everywhere in the mantle and in the posterior regions of the visceral mass, thereby obscuring its internal anatomy. *Ciboticola lunata* is dioecious.

The anatomy of the organs of the mantle cavity of *C. lunata* are, thus, of similar structure to those of *L. fortunei* (Morton 1973), but especially *S. harmandi* (Morton and Dinesen 2010). Notably, when compared to any hitherto studied mytiloideans the lengths of the labial palps are relatively enormous.

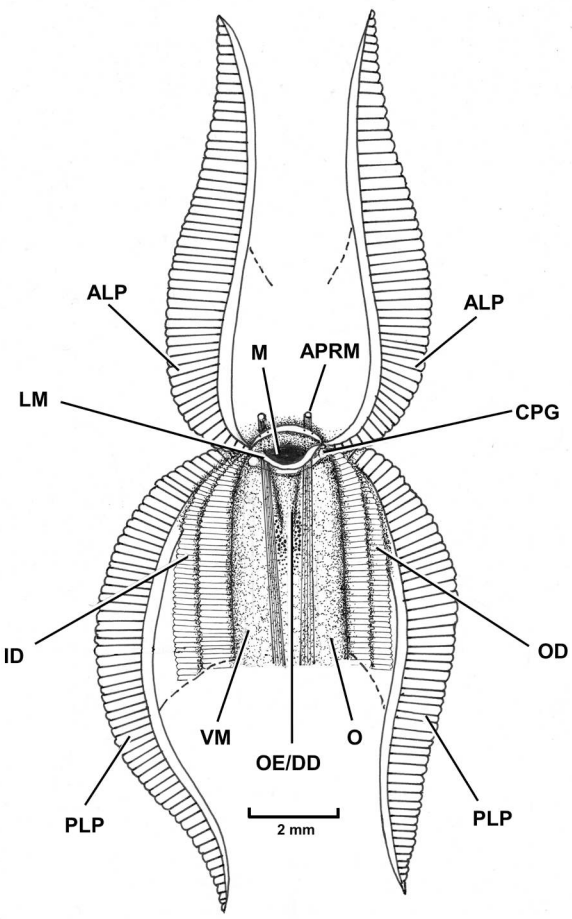

Figure 11. *Ciboticola lunata*. The organs of the anterior-most ends of the mantle cavity. The ctenidia appear dorso-ventrally shorter than they actually are because of the view's foreshortening effect. For abbreviations see the list at the end of the Materials and Methods section.

The byssus

Figure 12 represents a transverse section through the byssal apparatus of *C. lunata*. The large posterior byssal retractor muscle blocks within the visceral

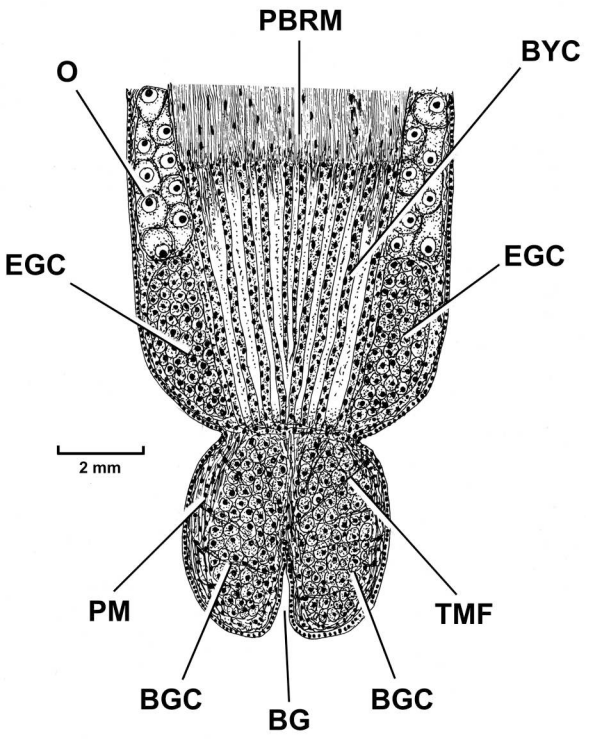

Figure 12. *Ciboticola lunata*. A transverse section through the byssal apparatus. For abbreviations see the list at the end of the Materials and Methods section.

mass end in an array of byssal cords, each separated by a thin space. Within these spaces are secreted the individual byssal threads, and this whole structure is surrounded by a large group of eosinophilic gland cells. At the point where the foot joins the visceral mass, the now more solid byssal threads come together to emerge from the ventral surface of the foot at the byssal gape. Within the foot too can be seen the fibres of the pedal musculature and transverse muscle fibres that criss-cross from the outer epithelium to sustain the integrity of the foot and assist in its extension. Compact basiphilic secretory cells surround the byssal groove.

Phylogenetic analyses

ML and BI trees constructed from the four genes individually are only partially resolved (Figure S1) (Supplementary data), indicating that each gene alone is not informative enough to reconstruct a robust topology for the Mytiloidea. Because of this, the phylogenetic relationships within the Mytiloidea were resolved based on the concatenated data trees (Figure 13). The resulting ML and BI trees resolved identical topologies with well-supported nodes.

The resolved phylogenetic tree depicted in Figure 13 identified two major clades (Clades A and B) among the analysed species of Mytiloidea. Clade A comprised the Modiolinae, Bathymodiolinae, Xenostrobinae, Limnoperninae, and species of *Leiosolenus* and *Ciboticola*. Clade B comprised the Mytiliseptinae, Brachidontinae, Arcuatulinae, Musculinae, Septiferinae, Mytilinae,

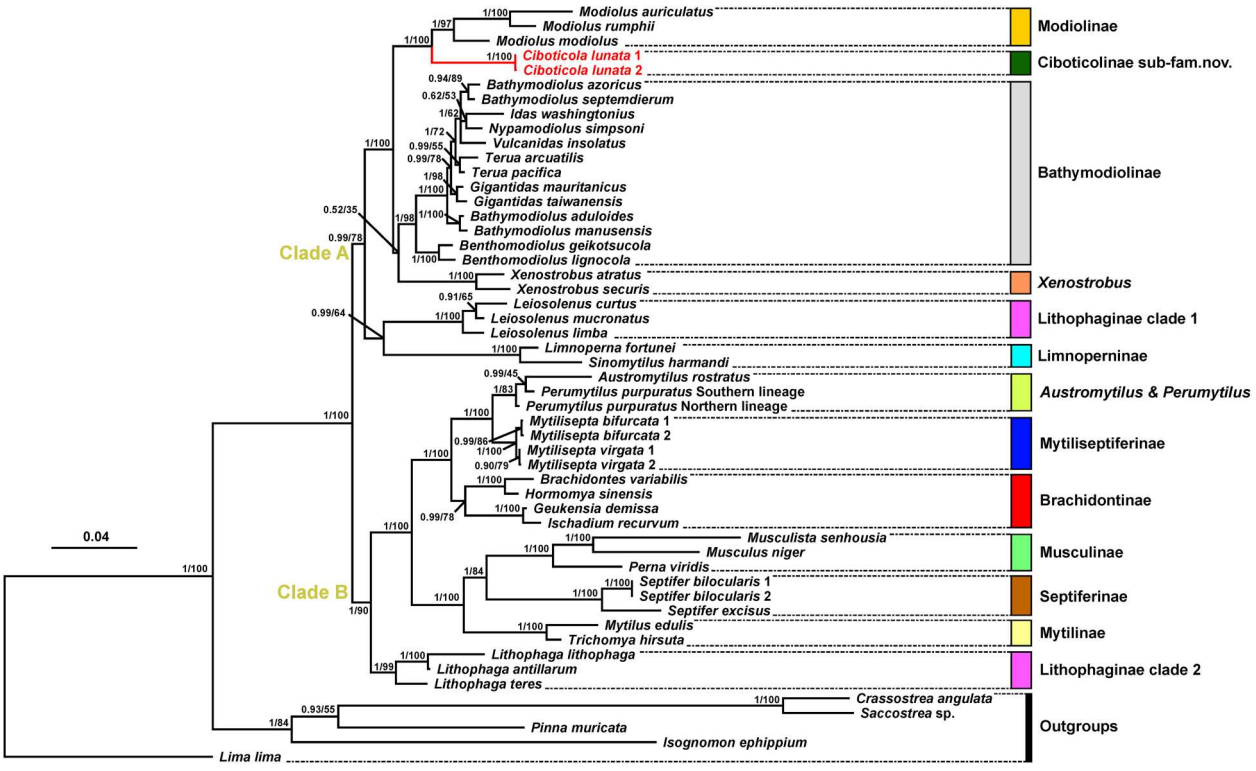

Figure 13. Phylogenetic relationships (Bayesian inference tree) of members of the Mytilidae based on concatenated sequences of COI, 18S rRNA, 28S rRNA and H3 genes. Posterior probabilities (former) and bootstrap values (latter) of nodes are shown. Note the position of the Ciboticolinae subfam. nov. The tree is rooted by *Lima lima* (Linnaeus, 1758). Annotations related to the taxa and clades are modified after Morton et al. (2020a, 2020b).

Lithophaginae (*sensu stricto,* including species of *Lithophaga* Röding, 1798) and species of *Austromytilus* Laseron, 1956, *Perumytilus* Olsson, 1961, *Perna* Philipsson, 1788 and *Trichomya* Ihering, 1900. The two individuals of *C. lunata* were well supported (Posterior Probability [PP] = 1; Bootstrap [BS] value = 100) as a sister clade to the subfamily Modiolinae and placed within clade A. This clade is herein recognised as a new subfamily (see formal description below). *Sinomytilus harmandi* and *L. fortunei* were well supported (P*P* = 1; BS = 100) as sister species and together formed a sister clade, Limnoperninae, with *Leiosolenus* P.P. carpenter, 1957 in Clade A (Morton et al. 2020a). *Septifer bilocularis* (Linnaeus, 1758) and *S. excisus* (Wiegmann, 1837) (Septiferinae) were resolved as representatives of a sister clade of Arcuatulinae, Musculinae and *Perna*, being supported by a PP value of 1 and a BS value of 84. *Mytilisepta virgata* and *M. bifurcata* were well supported (P*P* = 1; BS = 100) as a sister clade, Mytiliseptinae, of a monophyletic clade (P*P* = 1, BS = 83) formed by species of *Austromytilus* and *Perumytilus*.

Systematics of Ciboticolinae subfam. nov.

A new subfamily, the Ciboticolinae, is described, based upon the morphological data obtained for the type species *C. lunata* and the phylogenetic arrangement of the Mytiloidea proposed in this study, both of which provide sufficient information to justify this decision. Below is the description of this new mytiloid subfamily.

Superfamily Mytiloidea Rafinesque, 1815
Subfamily Ciboticolinae Morton et Dinesen, subfam. nov.

Type genus: *Ciboticola* Iredale, 1939.

Composition

The new subfamily includes the genus *Ciboticola* and its species, inhabiting shallow subtidal waters in bays of Queensland, Australia.

Diagnosis

Representatives of the Ciboticolinae can be separated from other mytiloidean species by the two pairs of exceptionally long labial palps relative to their body size (in preserved specimens the labial palps are approximately half the length of the shell) that are supported along half their length posteriorly from the anterior mouth by a membrane attaching to the mantle in relation to the outer demibranch, and to the dorsal line of the visceral mass in relation to the inner demibranch. Moreover, the Ciboticolinae can be separated from a species of *Sinomytilus* (i.e., *S. harmandi*, which it superficially resembles, in terms of its lifestyle and overall shell form), by the presence of posterior byssal setae, and a modiolid arrangement of the juvenile shell including a nepioconch. As described herein they can also be genetically separated from other mytiloidean genera.

Redescription of the shell of Ciboticola lunata

Shell equivalve, anteriorly sharply pointed, deeply concave ventrally with a distinct byssal notch, roundly inflated posteriorly, thereby trigonal and generally heteromyarian in overall form. Shell with thick periostracum adorned with byssal setae posteriorly. Umbones located antero-ventrally, with nepioconch. Hinge plate in the form of a small septum located anteriorly. Ligament opisthodetic, sunken between weak resilifers. Secondary ligament of fused periostracum connecting the two valves anterior and posterior to the ligament. Scar of pallial line scar thin. Pallial sinus absent. Scars of adductor muscles visible with the posterior larger and the anterior vestigial (if present, redundant), located between the two apical septa. Posterior pedal retractor muscles relatively large. Anterior pedal retractor muscles small, inserted dorsally away from umbonal septum on each valve.

Internal morphology

Siphons short, sensory. Filibranch ctenidia with ciliation of Type B(I) (Atkins 1937), non-plicate, food grooves situated dorsally and ventrally on both dorso-ventrally aligned demibranchs. Labial palps very long, with simple lips and mouth. Mantle margins fused left and right by inner folds only, being type A (Yonge 1982). Foot small, with a ventral and vertically-aligned byssal groove, byssal glands producing stout array of byssal threads. Dioecious. As in many other mytiloideans, outer demibranchs some three or four filaments shorter at anterior ends than inner ones (Fankboner 1971), ctenidial-labial palp junctions all Category I (Stasek 1963).

Remarks

The Ciboticolinae is represented principally by the Australian endemic species *Ciboticola lunata*. Huber (2010) assigned a second species to the genus *Ciboticola*, that is *C. andersoni* (Martens, 1887) recorded from the Andaman Sea. Huber (2010, p. 549) described *C. andersoni* as being '… broader, higher and less inflated than *lunata*' based on his examination of the syntypes of Martens' taxon. There are no existing molecular data available for this species and it needs reinvestigation.

The systematic separation of the Ciboticolinae from other representatives of the Mytiloidea and its morphological (presence of a nepioconch, byssal setae posteriorly) and genetic (COI, 18S rRNA, 28S rRNA,

Figure 14. Composite plate of eight different sized individuals of the commensal epizoan *Ciboticola lunata* byssally attached to each other and their host individual, *Anadara pilula*. QuestaGame Sighting ID no. 533650, photograph taken and submitted by Jim Mclean, recorded from Brampton Beach (17°21.600′S, 146°01.813′E), Queensland, Australia.

H3) association with the Modiolinae is herein proposed as a distinct subfamily.

Discussion

Ciboticula lunata is considered endemic to Queensland, Australia (Lamprell and Healy 1998), and records are few and far between. The Global Biodiversity Information Facility (GBIF) (www.gbif.org) holds 39 geo-positioned records from Australia and one record (likely mislocalised/misidentified) from the Polynesian country of Tuvalu. Only a single GBIF record (sourced from iNaturalist) from Queensland, comprises photographs of an adult *C. lunata* individual with its posterior end oriented towards the posterior of its *Anadara pilula* host (www.inaturalist.org/observations/90971309). Most fortuitously, upon finalising the revision of this manuscript, a new sighting was brought to our attention by Dr Richard Willan via his role as an expert identifier for QuestaGame (https://questagame.com/). Here, excellent photographs documenting the sighting of a live (or freshly dead) *Anadara pilula* hosting eight individuals of *C. lunata* were reported upon from Brampton Beach (17°21.600′S, 146°01.813′E), northern Queensland, by Mr Jim Mclean (QuestaGame Sighting ID no. 533650, 23 September 2022). The eight *C. lunata* individuals were all byssally attached and of different sizes, and all were oriented with their posterior end towards the posterior end of their host (Figure 14). Four of the five larger *C. lunata* individuals were attached to the right valve and one to the left valve of the host. The three smallest *C. lunata* individuals were attached to the posterior end of three of their larger conspecifics (two to the right and one to the left). These images provide important new scientific insights into this highly specialised bivalve association, thereby emphasising the great value of interactive open access citizen science platforms for improving our knowledge base for sustainable management and protection of global biodiversity and potential vulnerable species.

Given the small size of the larval shells of *C. lunata* (prodissoconchs I and II, 150 and 750 µm, respectively) in the NTM material studied here, it seems highly likely that the species reproduces by planktotrophic larvae

(Ockelmann 1995). In such a case, the larvae may be attracted to a host *A. pilula* individual by its exhalant stream or chemical cues therein. The larvae may, however, also be attracted to pheromones of congeneric adults, as has been identified for *Modiolus modiolus* (Linnaeus, 1758) (Dinesen and Morton 2014). The occurrence of two epibionts on one of the *A. pilula* hosts may suggest an adaptation to assist in the successful reproduction in such a dioecious broadcast spawning species. This hypothesis is strongly supported by the new QuestaGame sighting of eight different-sized *C. lunata* individuals attached to a single *A. pilula* host (Figure 14), and by the attachment of the three smallest individuals to the shells of adult conspecifics rather than to that of the host (Figure 14).

The byssal threads of two *C. lunata* individuals on their ark shell host in the NTM material strongly suggests that these individuals orientated towards and benefited from the incurrent feeding stream created by the host, as depicted in Figure 3. The orientation of all eight *C. lunata* individuals on their host and conspecific adults in the QuestaGame sighting (Figure 14) confirms our interpretation that *C. lunata* individuals optimise their orientation towards the feeding stream of their host, as well as towards each other. This raises the question of which type of relationship exists between this epizoic species and its host.

Is the relationship between Ciboticola lunata *and* Anadara pilula *commensalism?*

There are 41 species of shallow-water patelliform gastropod, all members of the Lottiidae, Calyptraeidae and Hipponicidae, plus other more fixed epibionts, which are specialised for life on other gastropod shells (Vermeij 2020). Other epizoan patelliform gastropods are facultative commensals kleptofeeding on the inhalant current of their bivalve hosts, such as *Crepidula fornicata* (Linnaeus, 1758) on *Ostrea edulis* Linnaeus, 1758, and *Mytilus edulis* Linnaeus, 1758 (Thieltges et al. 2003) and *Capulus ungaricus* (Linnaeus, 1758) on *Modiolus modiolus* (Sharman 1956).

Many pteriomorphian bivalves are gregarious and habitat-forming (e.g., some Mytiloidea, Pectinidae and Ostreidae), while others, such as members of the Anomiidae, may attach to the shell exterior of larger species. Amongst the gregarious Mytiloidea, the small modioline *Modiolula phaseolina* (Philippi, 1844) lives byssally attached to adults of the larger *Modiolus modiolus* (Dinesen and Ockelmann 2005) but also forms independent aggregations (Mutlu 1994). Such symbiotic relations are considered facultative commensalism, in which the smaller individuals and species may benefit from the substrate, shelter and feeding streams provided by the larger.

Among the non-gregarious mytiloideans, the musculine *Musculus subpicta* (Cantraine, 1835) lives epizoically symbiotic with *Ascidiella aspersa* (Müller, 1776) (Urochordata) (Morton and Dinesen 2011). The bathymodioline *Idas argenteus* Jeffreys, 1876 inhabits pholadoidean burrows, feeding there on their settling larvae and other organisms brought in by the host streams (Ockelmann and Dinesen 2011). Species of *Lithophaga* are borers of hermatypic corals, sometimes exhibiting host specificity (Morton 1990), whereas *Leiosolenus patagonicus* (d'Orbigny, 1842) bores into live oyster shells (Mauna et al. 2005). There are few other bivalves, except for borers such as *Rocellaria dubia* (Pennant, 1777) (Gastrochaenidae) (Morton et al. 2011), that are adapted to an obligatory symbiotic life on the shell externior of other living bivalves.

The kind of symbiotic relationship between *C. lunata* and its *A. pilula* host has been a puzzle. It appears to be species-specific for *C. lunata*, although several other *Anadara* species also occur in similar littoral habitats of sand and shell debris in Queensland. Species of *Anadara* do not possess fused siphons, and thus the exhalant aperture is oriented near perpendicular to the sediment surface, whereas the dorsal end of the inhalant aperture is situated just at the sediment surface and the remainder of its wide gape is situated within the sediment (Morton 1996). The larger and heavier shell of *A. pilula* would provide a more stable substrate for *C. lunata* than the surrounding sediment, and this may have initiated the development into an obligatory symbiosis for *C. lunata*. It is, however, certain that from a very early age individual *C. lunata* orient towards and tap into the inhalant feeding stream of their host as kleptofeeding commensals. With age, the fan-shaped juveniles attain their adult moon-like shape and aggregate tightly to each other and to their host. Whether this remarkable symbiosis is to be regarded as mutualistic remains uncertain, as *A. pilula* may benefit from its epizoans for camouflage and better anchoring, or may be rendered more vulnerable to dislodgement during storm events and suffer metabolically.

Symbiotic commensalism involving not only behavioural but also morphological adaptations to kleptofeeding, and possibly ectoparasitism, is rare among mytiloideans in this obligatory form. Besides *C. lunata* living on its *A. pilula* host, the only other known example is *Sinomytilus harmandi* that lives upon the living shells of *Corbicula fluminea* (Figure 15) in the Mekong River (Morton and Dinesen 2010), although we are uncertain as to how species-specific it is (Brandt 1974).

In this context, *C. lunata* joins *S. harmandi* in terms of a commensal epizoic lifestyle, which raises the question of whether or not the two taxa have a phylogenetic relationship. And, if so, whether or not the freshwater *S. harmandi* has evolved from a common ancestor with the marine *C. lunata*? The anatomical and genetic evidence, the former summarised in Table 2,

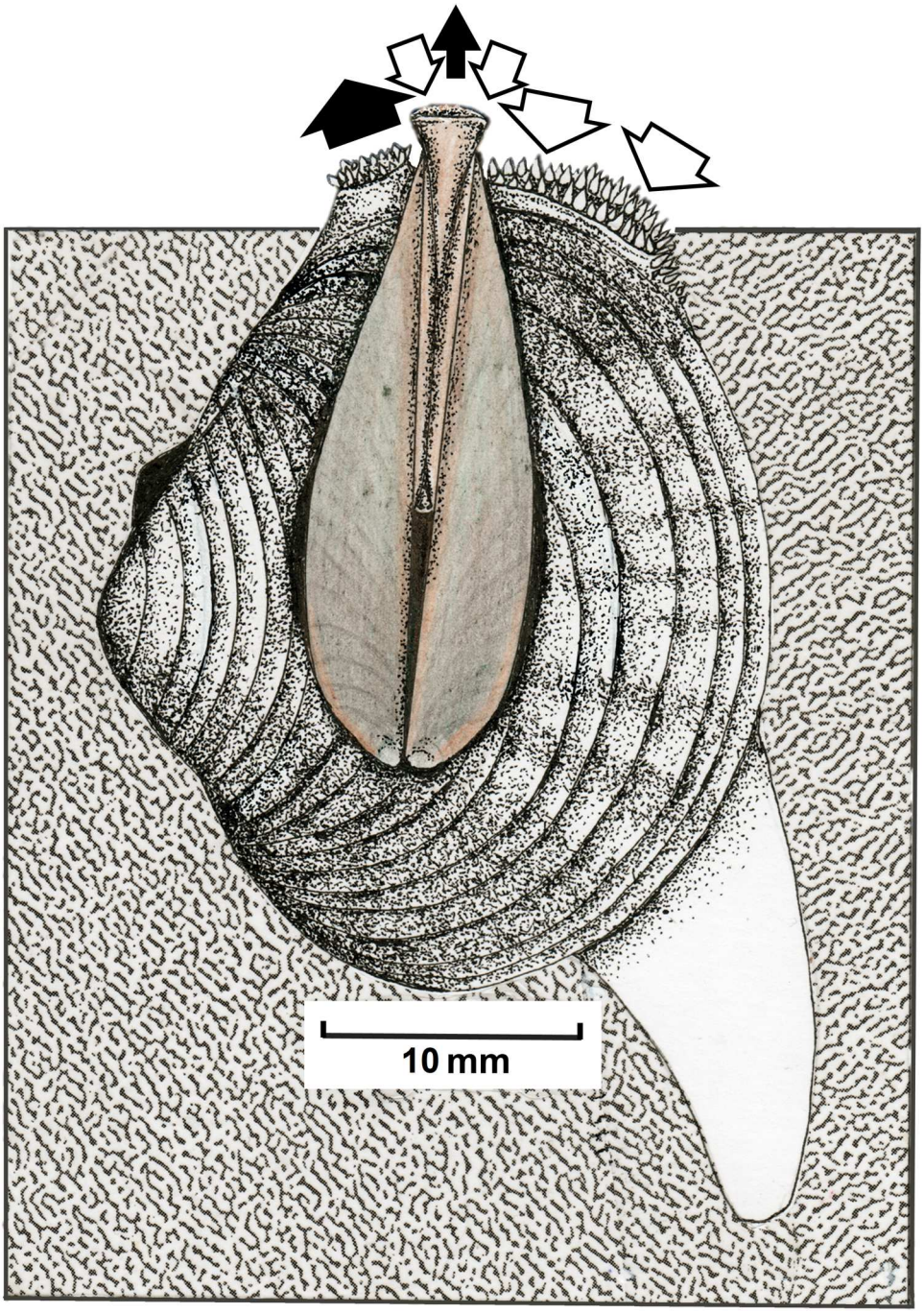

Figure 15. An illustration of a *Corbicula fluminea* shell in its postulated position in the sediment and colonised by an individual of *Sinomytilus harmandi*. Large open and closed arrows indicate the respective inhalant and exhalant currents of *C. fluminea*. Smaller arrows indicate those of *C. lunata*.

dismisses this notion. That is, *S. harmandi* joins *L. fortunei* within the subfamily Limnoperninae, whereas *C. lunata* is allied with representatives of the Modiolinae (albeit in its own subfamily, Ciboticolinae herein).

Molecular support for phylogenetic affiliations of mytiloidean genera and subfamilies

The superfamily Mytiloidea is currently considered monophyletic and basal within the infraclass Pteriomorphia (Carter et al. 2000; Bieler et al. 2010). Although general consensus exists about higher clade taxonomy, the phylogenetic delineation of Mytiloidea and the affiliation of its members at the lower taxonomic levels remain unsettled (Bieler et al. 2010; Morton 2015a; Liu et al. 2018; Morton et al. 2020a, 2020b; Tan et al. 2022). The topology of the molecularly-based phylogenetic tree presented herein (Figure 13) is almost identical to the one described in Morton et al. (2020a), wherein the Mytilidae was further divided into two major clades (Clades A and B) which were well supported by high posterior probabilities and bootstrap values (Clade A, $PP = 0.99$, $BS = 78$;

Table 2. Anatomical comparison of *Ciboticola lunata* (this study) with *Sinomytilus harmandi* (after Morton and Dinesen 2010; Morton et al. 2020b).

Character	*Ciboticola lunata*	*Sinomytilus harmandi*
Dissoconch (adult shell) shape	Deeply convex antero-ventrally	Deeply convex antero-ventrally
Anterior shell septum	Present	Present
Anterior adductor muscle	Vestigial	Absent
Posterior byssal retractor muscles in relation to the heart	Category 2	Category 2
Byssal setae	Present	Absent
Hinge teeth	Shell crenulations along the antero-ventral rim, possibly arising from either c- or d-series teeth	Absent
Nepioconch	Present, as a juvenile shell	Absent
Dissoconch growth direction	Dissoconch growth axis angled at 90° to the growth axis of the PI, PII and N stages	Dissoconch growth axis angled at almost 180° to the growth axis of the PI and PII stage(s)

Clade B, P*P* = 1, BS = 90; Hillis and Bull 1993; Alfaro and Holder 2006). The presence of two major clades within the Mytiloidea is supported by other recent genetic-based phylogenetic tree topologies (e.g., Liu et al. 2018; Lee et al. 2019; Tan et al. 2022).

The tree topologies indicate polyphyly within several mytiloidean subfamilies, which challenges the systematics of Mytilidae as currently defined by Huber (2010) (MolluscaBase 2022). Phylogenetics based on certain mitochondrial and nuclear genes (i.e., COI, 16S rRNA, 18S rRNA, 28S rRNA and H3) represent the currently popular molecular method to evaluate evolutionary relationships of the Mytiloidea (e.g., Liu et al. 2011, 2018; Audino et al. 2020; Morton et al. 2020a, 2020b; Zhang et al. 2021). However, only 28 of 52 (53.8%) valid genera could be included in the phylogenetic analyses, which limited our ability to evaluate monophyly of the family taxon Mytilidae *sensu lecto* and certain subfamilies (e.g., Modiolinae and Musculinae, a single genus, included; Crenellinae and Dacryiinae not included). It is believed that future taxon sampling and genomic-level data (e.g., ultraconservative elements, target enrichment and whole genome sequencing) can support cross validation and potential reshuffling of the affiliation of subfamilies and thus broaden our understanding of mytiloidean evolution.

The Lithophaginae as defined by Huber (2010) (MolluscaBase 2022) is divided into two subgroups, one in each of the clades A and B. The three *Leiosolenus* representatives were closely linked to Limnoperninae within Clade A, whereas the three representatives of the Lithophaginae type genus *Lithophaga* were resolved as the outermost clade within Clade B. Owada (2007) found molecular (18S rDNA) phylogenetic support for a paraphyletic relation between *Leiosolenus* and *Lithophaga*, which is in line with the polyphyletic relation between these two genera identified herein. Furthermore, Owada (2007) regarded the cylindrical shell form of boring mytiloideans, the so-called 'lithophagiform' shape, as an evolutionary convergence caused by adaptations to habitats, and similar to the molecular polyphyly identified in 'mytiliform' and 'modioliform' taxa by Distel (2000).

Perna viridis Linnaeus, 1758 (Mytilinae, MolluscaBase 2022) was resolved as a sister clade of the Arcuatulinae and Musculinae, whereas the true Mytilinae (represented by the genus *Mytilus*) showed a more distant relationship with the species. However, the phylogenetic affiliation of *Perna* and the abovementioned subfamilies would require a wider inclusion of representative taxa. The current affiliations of the two genera *Austromytilus* and *Perumytilus* with the Brachidontinae (MolluscaBase 2022) are challenged, as together they form a sister clade to the Mytiliseptinae (Morton et al. 2020a; herein). However, these two genera could not be included in the Mytiliseptinae due to their lack of an umbonal septum, which is the diagnostic character for the Mytiliseptinae (Trovant et al. 2015; Morton et al. 2020a). Although this study concluded that the genera *Austromytilus*, *Leiosolenus*, *Perna*, *Perumytilus* and *Trichomya* should be excluded from their currently allotted subfamilies, it is still uncertain if these genera affiliate with existing or new subfamilies.

Further evidence for the convergent evolution of the septum in the Mytiloidea

Molecular and morphological evidence provided (Morton et al. 2020a, 2020b; herein) suggests that the four genera currently assigned to the Septiferinae (by Huber 2010; MolluscaBase 2022) should be re-assigned to different subfamilies. Subfamilies have been assigned for *Ciboticola* (Ciboticolinae herein), *Septifer* (Septiferinae) and *Sinomytilus* (Limnoperninae) (Morton et al. 2020b), whereas a subfamily designation for the aseptate septiferine genus *Trichomya*, is as yet unavailable.

This study of *C. lunata* presents further evidence for the convergent evolution of an internal anterior septum in the Mytiloidea. Our phylogenetic tree clearly indicates a distant separation among the four known septate mytiloid genera, that is, *Mytilisepta* (Mytiliseptinae), *Sinomytilus* (Limnoperninae), *Septifer* (Septiferinae), and *Ciboticola* (Ciboticolinae) reported upon in this study. This suggests that their septa have each evolved independently instead of being an inherited character from a common septate ancestor. The septum in the four genera thus represents a case of grossly convergent evolution (Stayton 2015) within the Mytiloidea.

In a more general comparison, Combosch et al. (2017) carried out molecular analyses indicating no relationship between the septate Dreissenidae and Mytiloidea (Karatayev et al. 2007). Our study confirms that *C. lunata* should be the sister group to the Modiolinae in the Mytiloidea, even though most of its genera are aseptate (Huber 2010). Additionally, most of the available mytilid fossils are either aseptate or only weakly septate, implying that the ancestor of the family was probably also aseptate, thereby providing evidence that the septum might have developed independently on several separate occasions in the Mytiloidea (infraclass Pteriomorphia) but also in the unrelated Dreissenidae (infraclass Heteroconchia). An umbonal septum might, therefore, have little phylogenetic significance and is thus a poor systematic character for the Bivalvia in general and, more particularly, the Mytiloidea.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

CRediT authorship contribution statement

[The late] Brian Morton: Conceptualisation, Visualisation – original anatomical and life habits drawings, Writing – original draft. Jiehong Wei: Formal analyses – molecular phylogeny, Visualisation, Data curation, Writing – original draft, Writing – review and editing. Pricilla T.Y. Leung: Supervision, Methodology – molecular phylogeny, Data curation, Writing – original draft, Writing – review and editing. Sharon S. W. Chan: Formal analyses – molecular phylogeny. Grete E. Dinesen: Visualisation, Writing – original draft, Writing – review and editing.

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