Title:

Morphological evidence shows that not all Velesunioninae have smooth umbos

Authors:

ALEXANDRA ZIERITZ[1], ANDRÉ F. SARTORI[2], MICHAEL W. KLUNZINGER[3]

Affiliations:

[1]*University of Cambridge, Department of Zoology, Downing Street, CB2 3EJ Cambridge, UK*

[2]*Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605, U.S.A.*

[3]*Murdoch University, School of Veterinary & Life Sciences, Freshwater Fish Group & Fish Health Unit, Murdoch, 6150 Australia*

Short running head:

Sculpture in Velesunioninae

Corresponding author:

Alexandra Zieritz; University of Cambridge, Department of Zoology, Downing Street, CB2 3EJ Cambridge, UK; email: Alexandra.zieritz@cantab.net

Most species in the freshwater bivalve order Unionida (*sensu* Carter *et al.*, 2011) display some form of shell sculpture during the early post-larval stage. These so-called 'umbonal sculptures', 'beak sculptures' or 'rugae' range from more or less regularly formed V- and zigzag-shapes to pseudoradial, pseudoconcentric and double-looped bars, and single-standing nodules (Modell, 1942, 1964; Watters, 1994; Zieritz, 2010). In some Unionida, this ornamentation may extend to mature ontogenetic stages (e.g. Hyriidae; Haas, 1969a, b; Watters, 1994). Others, however, are regarded as lacking sculptured umbos altogether. These include (1) the Mycetopodidae (according to descriptions by Modell, 1942, 1964; Haas, 1969a, b; Zieritz, 2010); (2) most Iridininae including *Mutela rostrata* (Rang, 1835), *Pleiodon ovata* (Swainson, 1823) and *Pleiodon spekii* (Woodward, 1859) (according to descriptions by Pilsbry & Bequaert, 1927; Zieritz, 2010); and (3) some Unionidae such as *Actinonaias pectorosa* (Conrad, 1834), *Delphinonaias delphinulus* (Morelet, 1849) and *Pseudospatha tanganyicensis* (Smith, 1880) (Zieritz, 2010). Finally, various authors, including Cotton & Gabriel (1932), Iredale (1934), Modell (1942, 1964), McMichael & Hiscock (1958) and Haas (1969a) stated that smooth umbos are also characteristic of all members of (4) the Velesunioninae, a subfamily of the Hyriidae endemic to the Australasian region (Walker *et al.*, 2001; Walker, Jones & Klunzinger, in review). Haas (1969b), on the other hand, described beak sculpture in the subgenus "*Velesunio*" – comprising four of the five currently recognised velesunionine genera (except *Lortiella*) – as "not strong, consisting of broken, nodulose ridges curving toward each other below, generally with smooth space between". Unfortunately, no illustration of these sculptures has been made available by this or any other author to date.

Despite the difficulties imposed by frequent abrasion and rare preservation of umbos (Ortmann, 1912; McMichael & Hiscock, 1958; Good, 1998), interspecific differences in umbonal sculpture morphology have long played an important role in species identification

and reconstruction of phylogenetic relationships within extant and fossil Unionida (e.g. Modell, 1942, 1964; Graf, 2000; Hoeh, Bogan & Heard, 2001; Graf & Cummings, 2006). Disregarding Haas (1969b), in all phylogenetic datasets of the Unionida published to date, velesunionine taxa were coded as smooth (Table 1). This reflects the prevailing opinion that Velesunioninae lack umbonal sculpture. Graf & Cummings' (2006) analysis, for example, recovered smooth umbos as the plesiomorphic condition for the Palaeoheterodonta (= Unionida + Trigonioida). Against this background, the non-sculptured umbos displayed by members of the family Mycetopodidae, the Iridininae (subfamily of Iridinidae) and the Velesunioninae (subfamily of Hyriidae) would represent the ancestral character state. The presence of (V-shaped or "radial") beak sculpture in the remaining Hyriidae, on the other hand, was recovered as the single morphological synapomorphy characterising the second hyriid subfamily Hyriinae.

Here we report the discovery and provide the first photographic evidence of umbonal sculpture in two velesunionine genera and species, *Westralunio carteri* Iredale, 1934 and *Alathyria* cf. *pertexta* Iredale, 1934. In contrast, the umbos in two other velesunionine genera examined, i.e. *Lortiella froggatti* Iredale, 1934, and *Velesunio* [*Velesunio wilsonii* (Lea, 1859) and *Velesunio* cf. *wilsonii*], were smooth. Our observations suggest that refinement of current hypotheses of beak sculpture evolution within the Unionida is needed. Umbonal sculpture may have been lost in some Velesunioninae, rather than gained in their sister subfamily Hyriinae.

Sixteen specimens of Velesunioninae with well-preserved beaks (n = 2 *L. froggatti*, 2 *V. wilsonii*, 3 *V.* cf. *wilsonii* and 9 *W. carteri*) were analysed and photographed under a stereomicroscope (Table 2). Taxonomic identification of specimens followed McMichael & Hiscock (1958).

Despite being found in abrasive, coarse sand substrates, three juvenile *W. carteri* were recovered with little periostracal wear. These displayed, in addition to fine commarginal growth lines, elaborate oblique sculpture on the umbonal region (Fig. 1A-F). At the anterior and posterior thirds of the ornamented umbonal surface, sculpture is represented by discontinuous low ridges punctuated by a few higher nodules, running along a quasi-radial path and divaricating slightly (Fig. 1). The middle third of the ornamented surface features more prominent nodulose ridges, which merge to form V-shaped and/or W-shaped patterns. Intraspecific variation was observed mainly in the prominence of the sculptural elements and in the morphological onset and offset of ornamentation (compare Figs 1A-F).

Umbonal sculpture was also found in an adult specimen of *A.* cf. *pertexta* (*University Museum of Zoology Cambridge*, UK; CUMZ 103519; Supplementary Fig. 1). Beak sculpture in this *Alathyria* specimen differs considerably from that of *W. carteri* in that it does not consist of connected ridges but is rather composed of two radiating lines of nodules (Fig. 2). A similar beak sculpture pattern occurs, for example, in the European *Unio pictorum* (Linnaeus, 1758) and *Pseudanodonta complanata* (Rossmässler, 1835), the North American *Pleurobema sintoxia* (Rafinesque, 1820) (all species of Unionidae), and in some African Iridinidae including *Aspatharia rugifera* (Dunker, 1858) and *Chambardia nyassaensis* (Lea, 1864) (see Zieritz, 2010).

In contrast to *Westralunio* and *Alathyria*, no umbonal sculpture could be detected in the perfectly preserved specimens of *L. froggatti*, *V. wilsonii* and *V.* cf. *wilsonii* examined (Fig. 3). These taxa must thus remain to be considered as exhibiting smooth umbos.

The assumption that all Velesunioninae have smooth umbos as a rule can no longer be maintained. However, considering the difficulties involved, it is not particularly surprising that most previous authors have overlooked velesunionine beak sculptures. Most umbonal sculptures described in the present paper are rather faint and poorly developed. As such,

detection necessitates well preserved umbonal regions and, in some cases, the use of a microscope. Umbo wear is typical in adult Unionida, so that sculpturing as seen in the juvenile specimens presented here is not usually visible in older individuals of the same population. Small juvenile unionoids from wild populations, on the other hand, are notoriously difficult to locate (Neves & Widlak, 1987; Strayer, 2008), and rarely represented in museum collections. As a consequence, almost all velesunionine specimens depicted in previous publications (e.g. Cotton & Gabriel, 1932; Iredale, 1934; Modell, 1942; McMichael & Hiscock, 1958; Modell, 1964) and used in phylogenetic studies (Table 1) have abraded umbos, thus rendering accurate determination of their ornamentation impossible.

Our observation of the presence of beak sculpture in two velesunionine species and genera may provide an impetus to refine current hypotheses on the evolution of umbonal sculptures within the Hyriidae. As mentioned above, Graf & Cummings' (2006) analysis retrieved the V-shaped/nodulous umbonal sculpture as the single morphological synapomorphy of the hyriid subfamily Hyriinae, discriminating it from the smooth Velesunioninae. However, beak sculptures in *Alathyria* and *Westralunio* correspond closely to those of hyriines not only in topology, but also in their mode of formation, with the generative zone of sculpture migrating along the mantle margin with growth. This complex morphogenetic pattern, which results in oblique ribs on the shell surface, has evolved only a few times within the Bivalvia (Checa & Jiménez-Jiménez, 2003), being hence strongly suggestive of homology. Rather than having evolved independently in Hyriinae and in some Velesunioninae, we gather it more likely that oblique beak sculpture is synapomorphic for a more inclusive clade than either subfamily, having been subsequently lost, perhaps iteratively, in those Velesunioninae now characterized by smooth umbos. Testing these hypotheses will require phylogenetic trees with denser taxonomic sampling than is currently available.

The fact that umbonal sculptures in *Alathyria* and *Westralunio* eluded detection for more than a century may hint at a wider problem regarding our current understanding and use of this character. In particular, we suspect that other putatively smooth, but comparatively poorly studied taxa such as the South American Mycetopodidae, African Iridininae and other Australasian hyriids, may be found to display umbonal sculptures. Field efforts and the re-examination of museum collections may be fruitful in this respect.

Acknowledgements

K.F. Walker and H.A. Jones assisted in identification of the *Alathyria* cf. *pertexta* specimen. *Velesunio* specimens were identified by W.F. Ponder.

References

CARTER, J.G., ALTABA, C.R., ANDERSON, L.C., ARAUJO, R., BIAKOV, A.S., BOGAN, A.E., CAMPBELL, D.C., CAMPBELL, M., JIN-HUA, C., COPE, J.C.W., DELVENE, G., DIJKSTRA, H.H., ZONG-JIE, F., GARDNER, R.N., GAVRILOVA, V.A., GONCHAROVA, I.A., HARRIES, P.J., HARTMAN, J.H., HAUTMANN, M., HOEH, W.R., HYLLEBERG, J., BAO-YU, J., JOHNSTON, P., KIRKENDALE, L., KLEEMANN, K., KOPPKA, J., KŘÍŽ, J., MACHADO, D., MALCHUS, N., MÁRQUEZ-ALIAGA, A., MASSE, J.-P., MCROBERTS, C.A., MIDDELFART, P.U., MITCHELL, S., NEVESSKAJA, L.A., ÖZER, S., JR., J.P., POLUBOTKO, I.V., PONS, J.M., POPOV, S., SÁNCHEZ, T., SARTORI, A.F., SCOTT, R.W., SEY, I.I., SIGNORELLI, J.H., SILANTIEV, V.V., SKELTON, P.W., STEUBER, T., WATERHOUSE, J.B., WINGARD, G.L. & YANCEY, T. 2011. A synoptical classification of the Bivalvia (Mollusca). *Palaeontological Contributions,* **4**: 1-47.

CHECA, A.G. & JIMÉNEZ-JIMÉNEZ, A.P. 2003. Evolutionary morphology of oblique ribs of bivalves. *Palaeontology,* **46**: 709-724.

COTTON, B.C. & GABRIEL, C.J. 1932. Australian Unionidae. *Proceedings of the Royal Society of Victoria,* **44**: 155-161.

GOOD, S.C. 1998. Freshwater bivalve fauna of the Late Triassic (Carnian-Norian) Chinle, Dockum, and Dolores Formations of the Southwest United States. In: *Bivalves: an eon of evolution* (P.A. Johnston & J.W. Haggart, ed.), pp. 223-249. University of Calgary Press, Calgary.

GRAF, D.L. 2000. The Etherioidea revisited: A phylogenetic analysis of hyriid relationships (Mollusca: Bivalvia: Paleoheterodonta: Unionoida). *Occasional Papers of the Museum of Zoology University of Michigan*: 1-21.

GRAF, D.L. & CUMMINGS, K.S. 2002-2012. The MUSSEL Project — Home Page. 2012. Available at http://mussel-project.ua.edu.

GRAF, D.L. & CUMMINGS, K.S. 2006. Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zoological Journal of the Linnean Society*, **148**: 343-394.

GRAF, D.L. & CUMMINGS, K.S. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies,* **73**: 291-314.

HAAS, F. 1969a. Superfamilia Unionacea. In: *Das Tierreich (Berlin)* (R. Mertens & W. Henning, ed.), pp. 1-663. de Gruyter & Co., Berlin.

HAAS, F. 1969b. Superfamily Unionacea. In: *Treatise on invertebrate paleontology* (R.C. Moore, ed.), pp. N411-N470. Geological Society of America & University of Kansas Press, Lawrence, Kansas.

HOEH, W.R., BOGAN, A.E. & HEARD, W.H. 2001. A phylogenetic perspective on the evolution of morphological and reproductive characteristics in the Unionoida. In: *Ecology and Evolution of the Freshwater Mussels Unionoida* (G. Bauer & K. Wächtler, ed.), pp. 257-280. Springer-Verlag, Berlin.

IREDALE, T. 1934. The freshwater mussels of Australia. *Australian Zoologist,* **8**: 57-78.

KLUNZINGER, M.W. 2013. *Ecology, life history and conservation status of Westralunio carteri Iredale 1934, an endemic freshwater mussel of south-western Australia.* PhD thesis, Murdoch University, Perth, Western Australia.

KLUNZINGER, M.W., BEATTY, S.J., MORGAN, D.L., THOMSON, G.J. & LYMBERY, A.J. 2012. Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for an endemic freshwater mussel of south-western Australia. *Australian Journal of Zoology,* **60**: 26-36.

KLUNZINGER, M.W., JONES, H.A., KELEHER, J. & MORGAN, D.L. in press. A new record of *Lortiella froggatti* Iredale, 1934 (Bivalvia: Unionoida: Hyriidae) from the Pilbara region, Western Australia, with notes on anatomy and geographic range. *Records of the Western Australian Museum*.

MCMICHAEL, D.F. & HISCOCK, I.D. 1958. A monograph of freshwater mussels (Mollusca: Pelecypoda) of the Australian region. *Australian Journal of Marine and Freshwater Research,* **9**: 372-508.

MODELL, H. 1942. Das natürliche System der Najaden. *Archiv für Molluskenkunde,* **74**: 161-191.

MODELL, H. 1964. Das natürliche System der Najaden. 3. *Archiv für Molluskenkunde,* **93**: 71-126.

NEVES, R.J. & WIDLAK, J.C. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. *American Malacological Bulletin,* **5**: 1-7.

ORTMANN, A.E. 1912. Notes upon the families and genera of the Najades. *Annals of the Carnegie Museum,* **8**: 222-364.

PARODIZ, J.J. & BONETTO, A.A. 1963. Taxonomy and zoogeographic relationships of the South American naiades (Pelecypoda: Unionacea and Mutelacea). *Malacologia,* **1**: 179-213.

PILSBRY, H.A. & BEQUAERT, J.C. 1927. The aquatic mollusks of the Belgian Congo: with a geographical and ecological account of Congo malacology. *Bulletin of the American Museum of Natural History,* **53**: 69-602.

PINDER, A.M., HALSE, S.A., SHIEL, R.J. & MCRAE, J.M. 2010. An arid zone awash with diversity: patterns in the distribution of aquatic invertebrates in the Pilbara region of

Western Australia. *Records of the Western Australian Museum, Supplement*, **78**: 205-246.

PONDER, W.E. & BAYER, M. 2004. A new species of *Lortiella* (Mollusca : Bivalvia : Unionoidea : Hyriidae) from northern Australia. *Molluscan Research*, **24**: 89-102.

SMITH, B.J. 1998. Order Unionoida. In: *Mollusca: the southern synthesis. Fauna of Australia* (P.L. Beesley, G.J.B. Ross & A. Wells, ed.), pp. 296–298. CSIRO Publishing, Melbourne.

STRAYER, D.L. 2008. *Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance.* University of California Press, London.

WALKER, K.F., BYRNE, M., HICKEY, C.W. & ROPER, D.S. 2001. Freshwater mussels (Hyriidae) of Australasia. In: *Ecology and Evolution of the Freshwater Mussels Unionoida* (G. Bauer & K. Wächtler, ed.), pp. 5-31. Springer-Verlag, Berlin.

WALKER, K.F., JONES, H.A. & KLUNZINGER, M.W. in review. Bivalves in a bottleneck: Taxonomy, phylogeography and conservation of freshwater mussels (Bivalvia: Unionoida) in Australasia. *Hydrobiologia*.

WATTERS, G.T. 1994. Form and function of unionoidean shell sculpture and shape (Bivalvia). *American Malacological Bulletin*, **11**: 1-20.

ZIERITZ, A. 2010. *Variability, function and phylogenetic significance of unionoid shell characters.* PhD thesis, University of Cambridge.

Tables

Table 1. Velesunioninae specimens, accession numbers and literature references utilised in previous morphological phylogenetic analyses and concerning beak sculpture morphology. Note that according to Graf & Cummings (2007), the Velesunioninae comprises 16-17 species from five genera (i.e. *Velesunio*, *Alathyria*, *Lortiella*, *Microdontia* and *Westralunio*).

Publication	Velesunioninae specimens analysed	Notes on umbonal erosion status of specimen(s)	Supporting references
Graf (2000)	*Velesunio ambiguus* UMMZ_111839	Unknown	McMichael & Hiscock (1958), Parodiz & Bonetto (1963), Haas (1969a, b), Smith (1998)
Hoeh *et al.* (2001)	*Lortiella rugata* *Velesunio angasi*	Unknown	Modell (1942, 1964)
Graf & Cummings (2006)	*Lortiella froggatti* INHS_16213 *Velesunio ambiguus* ANSP_41802 *Velesunio angasi* ANSP_71739	All specimens display highly eroded umbos (see Graf & Cummings, 2002-2012)	McMichael & Hiscock (1958), Parodiz & Bonetto (1963), Haas (1969a, b), Smith (1998), Ponder & Bayer (2004)

Table 2. Specimens with intact umbos examined for sculpturing. Abbreviations: CUMZ = University Museum of Zoology Cambridge, UK; DEC = Department of Environment and Conservation, Government of Western Australia, Woodvale, Australia; WA = Western Australia; WAM = Western Australian Museum (Perth).

Lot ID	Species	*N*	Name of water body	Site	Latitude	Longitude	Collection date	Source
CUMZ_103519	*Alathyria* cf. *pertexta* Iredale, 1934	1	Unknown	'New South Wales'	Unknown	Unknown	1873	MacAndrew Collection
CUMZ[1]	*Lortiella froggatti* Iredale, 1934	2	Snake Creek	Durack Pool, West Kimberleys, WA	17°34'60"S	124°09'14"E	13 Nov 2009	Klunzinger *et al.* (in press)
DEC_PSW044	*Velesunio* cf. *wilsonii* (Lea, 1859)	1	Tunnel Creek	Yandabiddy Pool	23°54'11"S	118°42'36"E	4 June 2004	Pinder *et al.* (2010)
DEC_PSW067	"	1	Brumby Creek	Wannagunna Spring Pool	24°18'04"S	118°52'26"E	20 April 2005	Pinder *et al.* (2010)
DEC_PSW046	"	3	Red Hill Creek	Red Hill Creek Pool	21°57'47"S	116°02'56"E	26 Aug 2004	Pinder *et al.* (2010)
CUMZ[1]	*Westralunio carteri* Iredale, 1934	3	Collie River	100 m downstream from Southwest Hwy, WA	32°18'08"S	115°49'03"E	18 Feb 2010; 26 Jan 2010; 3 Nov 2010; 17 Oct 2011	Klunzinger *et al.* (2012)
CUMZ[1], WAM[1]	"	3	Yeagarup Lake	Warren State Forest, WA	34°32'35"S	115°52'25"E	14 March 2011	Klunzinger (2013)
CUMZ[1]	"	3	Yule Brook	Beckenham, WA	32°01'58"S	115°57'25"E	22 March 2012	Klunzinger (2013)

[1]Accession number will be provided after acceptance of manuscript

Figure captions

Figure 1. Left (A,C,E) and right (B,D,F) umbos of *Westralunio carteri* from Yule Brook (A,B), Yeagarup Lake (C,D) and Collie River (E,F). Scale bars: A,B,E,F = 2 mm; C,D = 1 mm.

Figure 2. Left (A) and right (B) umbos of *Alathyria* cf. *pertexta* from New South Wales, Australia (CUMZ_103519). Scale bar: 1 mm.

Figure 3. Velesunioninae with smooth umbos (left valves towards the top). A, *Lortiella froggatti* from Snake Creek, Western Australia; B, *Velesunio wilsonii* from Tunnel Creek; C, *Velesunio* cf. *wilsonii* from Red Hill Creek. Scale bars: A,C = 2 mm; B = 0.5 mm.

Supplementary Figure 1. External and internal views of the left (A,C) and right (B,D) valves of *Alathyria* cf. *pertexta* (CUMZ_103519). Scale bar: 1 cm.

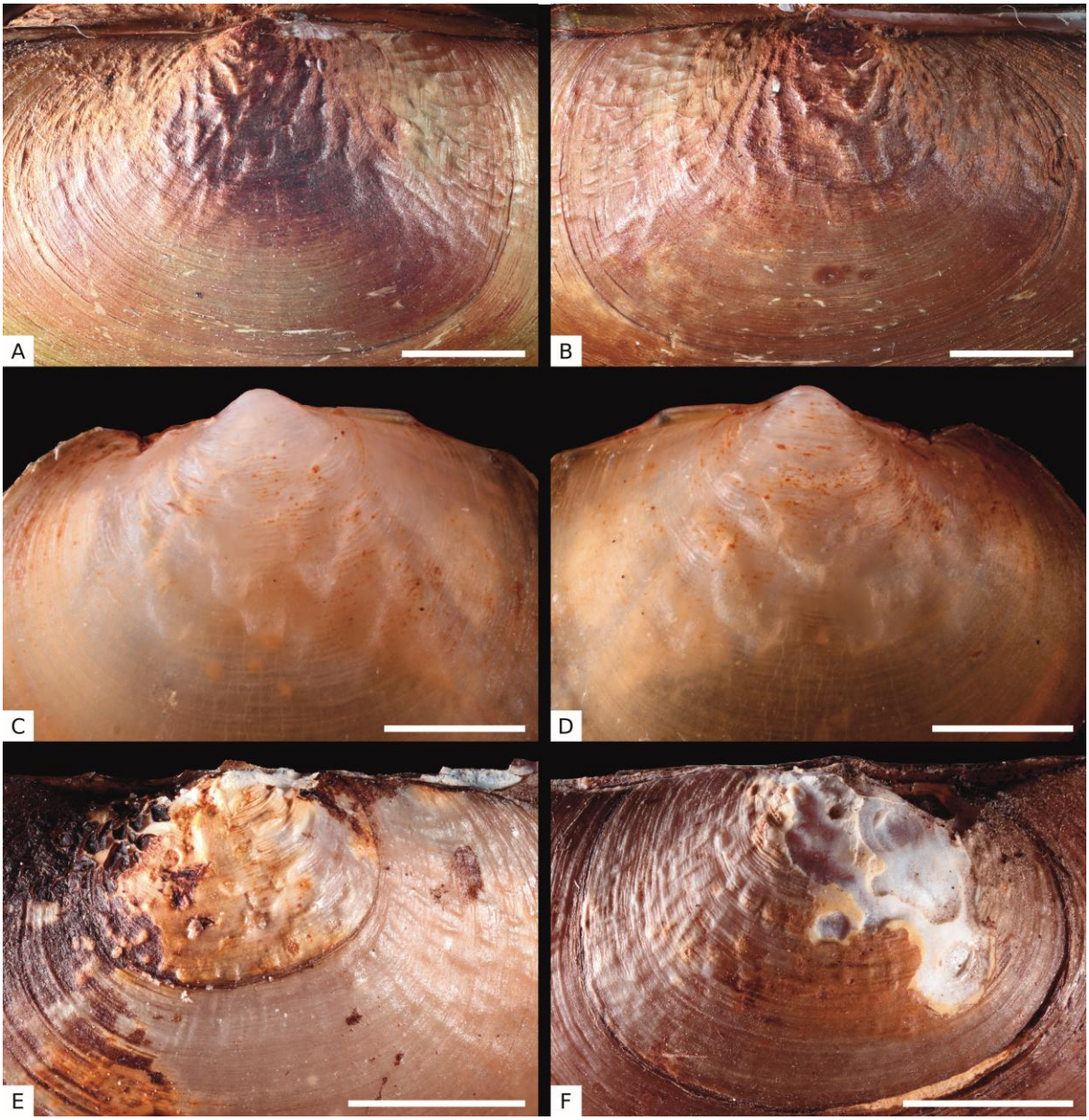


Figure 1. Left (A,C,E) and right (B,D,F) umbos of *Westralunio carteri* from Yule Brook (A,B), Yeagarup Lake (C,D) and Collie River (E,F). Scale bars: A,B,E,F = 2 mm; C,D = 1 mm.

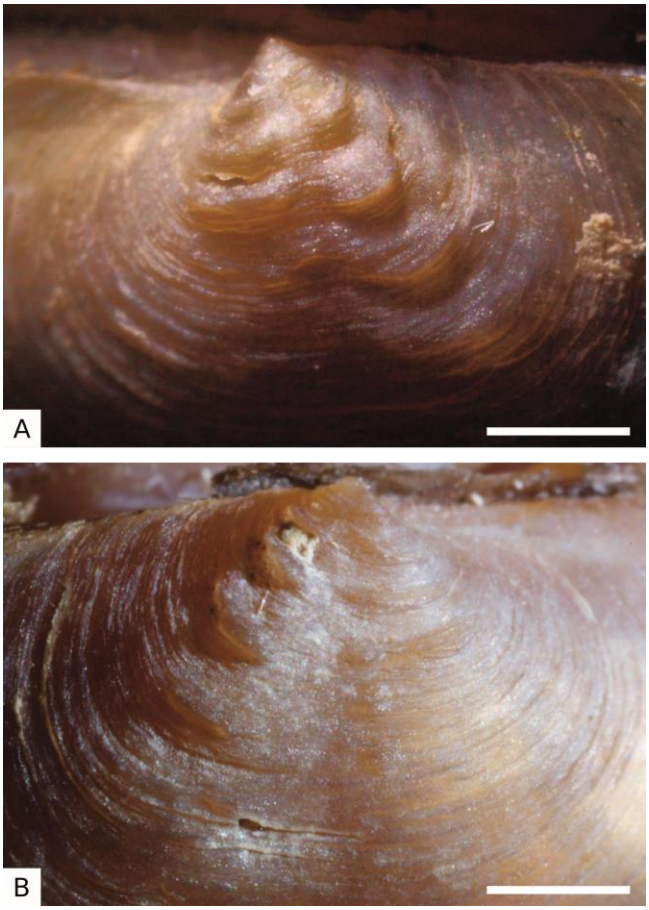


Figure 2. Left (A) and right (B) umbos of *Alathyria* cf. *pertexta* from New South Wales, Australia (CUMZ_103519). Scale bar: 1 mm.

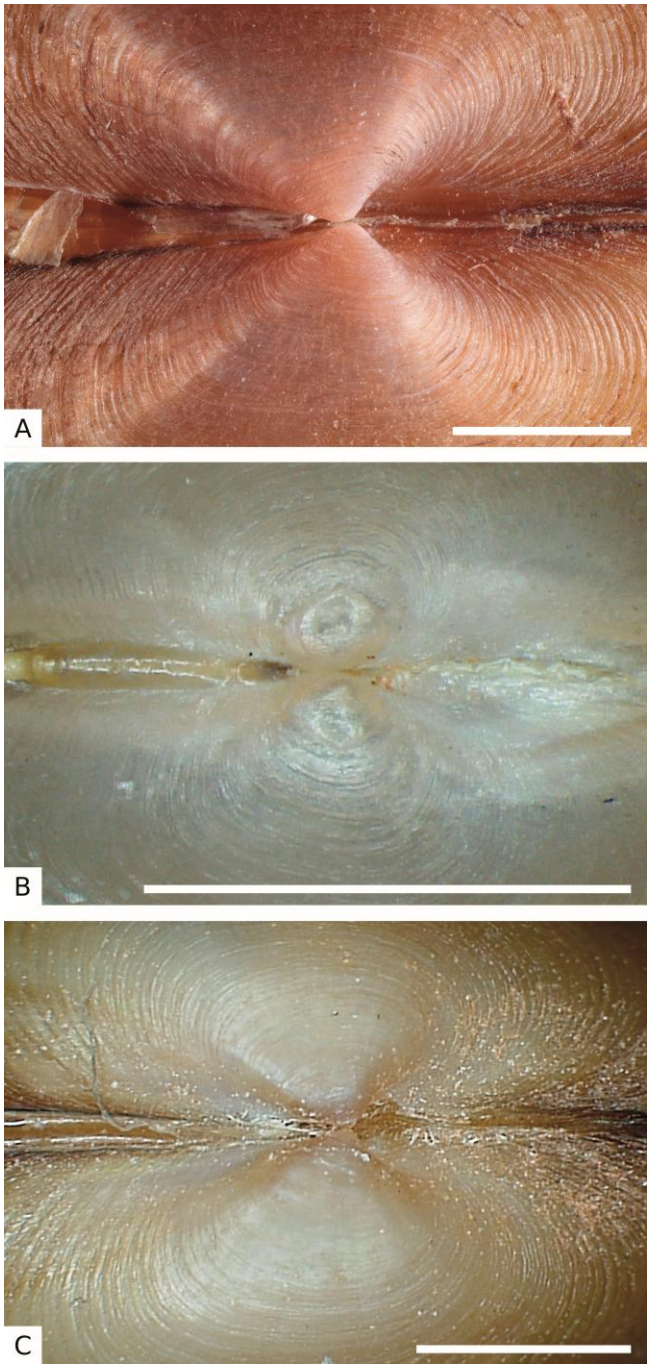
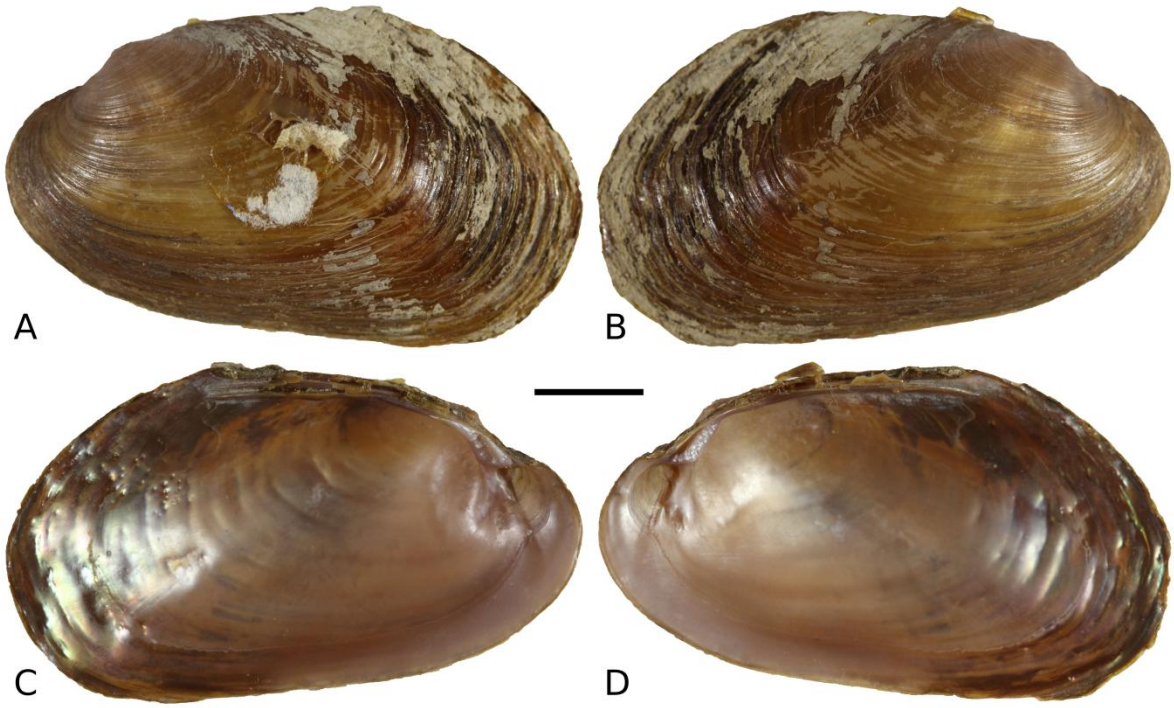


Figure 3. Velesunioninae with smooth umbos (left valves towards the top). A, *Lortiella froggatti* from Snake Creek, Western Australia; B, *Velesunio wilsonii* from Tunnel Creek; C, *Velesunio* cf. *wilsonii* from Red Hill Creek. Scale bars: A,C = 2 mm; B = 0.5 mm.



Supplementary Figure 1. External and internal views of the left (A,C) and right (B,D) valves of *Alathyria* cf. *pertexta* (CUMZ_103519). Scale bar: 1 cm.