

A NEW PHOTOSYMBIOTIC MARINE BIVALVE WITH WINDOW SHELL MICROSTRUCTURE (CARDIIDAE: FRAGINAE)

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ABSTRACT: Species of *Fragum* were collected from shallow waters in the Funafuti Atoll in Tuvalu in 2004, with one species being new. We describe *Fragum funafutiense* n. sp., which in addition to its occurrence at Funafuti, has now been found in five additional countries in the Indo-West Pacific. It can be differentiated from closely similar species only by consideration of a suite of characters that include shell shape, nature of the umbonal keel, rib number, rib sculpture, constitution of the hinge and nature of the shell window formation. Previously published molecular sequence data, including COI and 16S mtDNA, support this species as distinct from other tested fragines as well. Like all species in the genera *Fragum*, *Corculum*, and *Lunulicardia*, this new species is also photosymbiotic. In contrast to many other cardiiids, however, *Fragum funafutiense* n. sp. exhibits deep penetration of fibrous prismatic microstructure on the posterior shell surface, consistent with window shell microstructure. Window shell microstructure is an adaptation that enhances light penetration through the shell to photosymbionts housed in soft tissue such as mantle, foot, and siphonal tentacles. A lectotype of its congener *F. mundum* (Reeve, 1845) is herein designated.

Keywords: cardiiids, *Fragum*, new species, tropical reefs, biodiversity, photosymbiosis, window shell microstructure

INTRODUCTION

Cardiids or cockles are a well-studied family of marine bivalves and its photosymbiotic members are some of the most enigmatic molluscs living today. There are two photosymbiotic lineages within the cardiiids, the giant clams, or Tridacninae and heart cockles and relatives, or Fraginae. In contrast to earlier work (Schneider 1998), a recent transcriptomic study supported these two lineages as sisters, and ancestral state reconstructions supported a dual origin of photosymbiosis, once in each subfamily (Li *et al.* 2020). While giant clams are well studied, heart cockles are not as well known (Kirkendale and Paulay 2017). However, the capacity for insight into the evolution of photosymbiosis with fragines is unparalleled given a wider suite of adaptations for photosymbiosis and higher species diversity than in giant clams.

All tested species in the genera *Corculum*, *Lunulicardia* and *Fragum* are photosymbiotic (Kirkendale 2009) and presently 22 species level taxa are listed as valid (after WoRMS accessed

27/09/2020). Within this group, the members of the paraphyletic genus *Fragum sensu lato* are the most species-rich and least characterized assemblage, exhibiting a wide range of putative morphologies for a photosymbiotic lifestyle. *Fragum* s.l. is in need of systematic revision but currently includes eleven valid species (after WoRMS accessed 27/09/2020) that display a diversity of form. Some species are not specialized in shell form (*F. sueziense* (Issel, 1869)), others are robust and highly specialized for a photosymbiotic lifestyle in the manner of giant clams (valve gaping, soft anatomy with leaf-like extensions packed with photosymbionts in *F. unedo* (Linnaeus, 1758)) while others exhibit sophisticated window shell microstructure (*F. mundum* (Reeve, 1845)) (Persselin, 1998).

One of the most enigmatic characters in fragines is the occurrence of windows in the shell of some species (Carter and Schneider 1997). These appear as a series of translucencies in the shell that are visible as dark patches. They are concentrated on the posterior slope of the shell, the surface incident to light, and are therefore considered adaptations to

enhance light penetration through the shell (Carter and Schneider 1997). Microstructural windows are characterized by the incursion of fibrous prismatic microstructure from the outer shell layer inward through the shell (Schneider and Carter 2001). In *Corculum*, which has the most sophisticated window formation, windows are underlain by convexities that function as a lens (Carter and Schneider 1997). The entire form of the individual has taken on a solar panel morphology. Other species are known to possess windows, including incursion of fibrous prismatic microstructure and convexities, just fibrous prismatic microstructure or suspected intermediate stages such as variation in pigmentation (Persselin 1998). This wide degree of window shell features from sophisticated window shell microstructure (*F. mundum*) through to intermediate window shell microstructure (*F. fragum* (Linnaeus, 1758)) (Persselin 1998) is most prevalent in the speciose genus *Fragum*.

The smaller-bodied species in the genus *Fragum* are the most challenging to identify because of the many gross-morphological similarities and high intraspecific variability with regards to shape, rib number, and rib sculpture. Contrary to the larger *F. fragum* and *F. unedo*, these taxa share a maximum size generally well below 25 mm, a rib number that is commonly well below 30 (hence informally termed the ‘25-rib group’), a more or less quadrate outline, and have a predominantly overall whitish colouration. Species boundaries within this group are poorly resolved and the number of species is growing, with remote areas of the Indo-West Pacific yielding two new species in the last two decades (ter Poorten 2009; 2015); additional undescribed species are expected from this region. The addition of molecular data over the last decade has helped to clarify species boundaries (Kirkendale 2009; Herrera *et al.* 2015; Li *et al.* 2020) and will continue to be important in taxonomic work of the group.

One of the earliest and most extensive malacological surveys of the remote western Pacific island of Funafuti was conducted by Hedley (1899a; b). He published his survey as part of a larger volume on natural and human history of the atoll. Three fragines were reported (“*Cardium cardissa* var. *dionaeum*, Sowerby” = *Corculum cardissa* (Linnaeus, 1758), “*Cardium fragum*, Linne” (sic.) =

Fragum fragum, “*Cardium fragum* var. *sueziense*, Issel” (sic.) = ?*Fragum sueziense*) and although no new fragines were described during the expedition, Hedley (1899a: 504) did make an interesting note under his records of *C. fragum* var. *sueziense*, Issel stating that “Separate valves were abundant on the lagoon beach, and one was obtained outside the atoll at a depth of eighty to forty fathoms. The four dozen odd valves before me exhibit much variation in contour, and they appear to pass by gradual transition into typical *C. fragum* (sic.)”. It is possible that the new species described herein fits into the morphological spectrum that concerned Hedley. In 2004, over 100 years after the visit by Hedley, fragines were hand collected on Funafuti atoll during the course of fieldwork by the first author. *F. funafutiense* n. sp., *F. whiteleyi* Iredale, 1929, *F. mundum*, *F. fragum* and *C. dionaeum* (Broderip and Sowerby, 1829) were found. Much like Hedley, the variety of small white fragines was exciting but also challenging, given the presence of intermediate forms, including a species of *Fragum*, similar in form to *Fragum mundum* but slightly larger (Kirkendale 2009). That species (then referred to as *F. aff. mundum*) is now described here as *F. funafutiense* n. sp.

Fragum funafutiense n. sp. was included in a previous molecular phylogenetic study by Kirkendale (2009). At that time, it was provisionally identified as *F. aff. mundum* and recovered as a distinct, reciprocally monophyletic and well supported sister to a clade of 25-rib group members in a four gene Bayesian tree (fig. 3 of Kirkendale 2009). This position was also captured in the Maximum likelihood phylogram of the same concatenated data set (Kirkendale 2009, fig. 4), although support for *F. funafutiense* n. sp. as sister to the clade of other 25 rib group members was low. While the position of *F. funafutiense* n. sp. relative to other members of the genus *Fragum* is not fully resolved, it is well supported as a distinct lineage from all other tested fragines.

Here we describe this new species from the Funafuti collection, present new morphological data, and review previous molecular evidence to place these findings in the context of other 25-rib group members. We also demonstrate the presence of windows in the new species and designate a lectotype for *F. mundum*, another windowed *Fragum*.

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MATERIALS AND METHODS

Institutional Abbreviations: MNHN = Muséum national d'Histoire naturelle, Paris, France; NHMUK = Natural History Museum, London, United Kingdom; NTM = Museum and Art Gallery of the Northern Territory, Darwin, Australia; TP = Coll. J.J. ter Poorten, Hilversum, the Netherlands; UF = Florida Museum of Natural History, University of Florida, Gainesville, United States.

Abbreviations: A = found alive; H = height; L = length; p.v. = paired valves; s.v. = single valve; LV = left valve(s); RV = right valve(s); W = width.

Collecting methodology

Fragines were collected by snorkeling at various sites around Funafuti atoll (see Material Examined section for specific locality details) in 2004 by the first author. A simple kitchen sieve was used to screen clean coarse sand and rubble for small specimens in the field. Live specimens were preserved in 100% ethanol. All samples are registered and stored at Florida Museum of Natural History in Gainesville, Florida, USA, with collection data available online. Fisheries staff from Funafuti provided the boat, as well as staff assistance in the field. Other material was collected through large scale expedition work led by MNHN (see Material Examined section for specific campaign details).

Morphology

Shell length was measured as the greatest distance between the anterior and posterior ends, parallel to the hinge line formed by the tip of the lateral teeth; shell height as the maximum dimension measured along an axis perpendicular to the hinge line. The anterior zone is the anterior and median part of the shell up to the radial umbonal keel; the posterior zone is the remaining posterior part of the shell.

Window Shell Microstructure

The presence of translucencies concentrated in the posterior shell region suggested that window shell microstructure may be present in *Fragum funafutiense* n. sp. A two-tiered approach was taken to determine whether observed translucencies were areas of window shell microstructure. The first step was to examine microstructure via SEM or Scanning Electron Microscopy. Given the small size of the

shells (<1 cm in length), the entire shell was fractured radially (anti-marginally) in order to achieve a vertical break through one of the windows. Single valves of two individuals were embedded in separate dry erase mounts and fractured with forceps. Once fractured, shell fragments were cleaned and stored in 95% EtOH. All fractured portions of each valve were mounted on SEM stubs using double-sided tape. Silver conducting paint was used to draw a line of conductivity from the shell fragment down to the exposed metal and the stub and shell were gold-coated via sputtering (Carter and Ambrose 1989). SEM of shell microstructure was conducted at the University of Victoria Electron Microscopy Facility using a Hitachi SM3500N variable pressure scanning electron microscope with an energy dispersive X-ray micro-analyser. Digital images were taken to document the extent of penetration of fibrous prismatic microstructure into the shell interior in *Fragum funafutiense* n. sp. The region surrounding enhanced fibrous prismatic microstructure was also photographed, as were shell areas exhibiting other types of microstructure (e.g. crossed lamellar) to facilitate comparison. Magnification was increased in areas of fibrous prismatic microstructure to examine the structure of individual fibers and third-order lamellae.

The second tier of analyses to test for the presence of window shell microstructure in *F. funafutiense* n. sp. involved examination of light penetration through areas of putative window compared to non-window shell regions. Shells were wetted with ethanol and oriented via mounting on a pin, held in place with putty, to allow fiber optic light (Intralux 4000) to come from the exterior of a single valve through to the shell interior. Nikon Digital Camera was mounted on a Wild M8 scope and images were taken with and without fiber optic backlighting.

SYSTEMATICS

Family Cardiidae Lamarck, 1809

Subfamily Fraginae Keen, 1951

Fragum Röding, 1798

Fragum Röding, 1798: 189. Type species by absolute tautonymy *Fragum flavum* Röding, 1798 (= *Cardium fragum* Linnaeus, 1758); Recent, 'O. Asiatico, Americano' (Indo-West Pacific, restricted to Ambon, Indonesia by Wilson and Stevenson, 1977: 37).

Hemicardium Swainson, 1840: 373 (non Spengler, 1799; nec Schweigger, 1820). Type species by subsequent designation (Gray, 1847: 185): *Cardium unedo* Linnaeus, 1758; Recent (type locality not mentioned).

Diagnosis. Shell height to 82 mm, triangular, trapezoidal or ovoid, inflated, with longitudinal medio-posterior angulation (umbonal keel of variable strength). Posterior margins strongly serrated. Ribs ornamented with scales or tubercles. Lunule and escutcheon poorly defined. Hinge not parallel to the ventral margin, often short and rather angled. Cardinal teeth about equal and separate in left valve, unequal and joined in right valve by a dorsal saddle.

Distribution. Miocene to Recent, Indo-Pacific, South Africa, Japan. Living in shallow water, infaunally or partly epifaunal and photosymbiotic, harboring dinoflagellate zooxanthellae in soft tissues.

***Fragum funafutiense* n. sp. Kirkendale, ter Poorten and Middelfart**
(Figs.1, 3A)

Fragum aff. *mundum* (Reeve, 1845) - Kirkendale, 2009: 455–457, 461, 465 (UF 374156, 374157); - Herrera *et al.*, 2015: 99–100, figs. 2–3 (UF 374156).

Fragum mundum (Reeve, 1845) - ter Poorten, 2009: 37 (part), pl. 7 fig. 3 (NOT fig. 4); - ter Poorten *et al.*, 2017: 135–136 (pars), fig. 10A–D, 10K–L.

Fragum whitleyi Iredale, 1929 - ter Poorten *et al.*, 2017: 139 (part), figs. 10P–S, 18F.

Description. Shell small for the genus (H up to 11 mm), solid, semi-glossy, moderately inflated, sub-quadrate to sub-rhomboidal and rather elongate (L/H 0.75–0.92, average 0.82, n = 22), inaequilateral with umbo in front of midline and strongly prosogyrous. Anterior margin rounded, ventral margin straight with slight indentation just before strongly angular postero-ventral margin, posterior margin weakly rounded. Strongly angulate and rather curved umbonal keel present, coinciding with 1–2 often slightly more pronounced radial ribs and 1–2 relatively wide interstices, dividing the shell in two distinct zones. In total 24–30 rounded radial

ribs of which 14–18 are much flattened ribs on the anterior zone and generally 10–12 are more rounded ribs on the posterior zone. Ribs carrying densely placed, straight or slightly curved knobby scales, almost covering the full rib in the anterior zone, becoming more nodular anteriorly, but much tinier, more erect and placed slightly oblique posteriorly. Interstices minutely pitted and narrow, about a $\frac{1}{4}$ of the rib width in both shell zones. Rib impressions only visible in the shell interior close to the margins, anterior and ventral margins crenulate, posterior margin more or less digitate. Lunule small, demarcated by a depressed area just anterior of the umbo. Pallial line entire, adductor muscle scars subequal in size, anterior muscle scar rounded-trigonal, slightly deepened, posterior muscle scar slightly larger, high oval.

Hinge plate broad, hinge typical for the genus with large, triangular ventral cardinal in both valves, similar sized dorsal cardinal in LV, slightly smaller peg-like dorsal cardinal in RV. Cardinal teeth joined in RV by a dorsal saddle, laterals in both valves approximately equidistant from cardinals. Nymph plate short.

Exterior coloration white, pale yellow, or cream, interstices on the posterior slope sometimes yellow or orange, interior coloration white. Translucent patches present, most numerous on the posterior shell slope, often coinciding with the width of a rib and giving a grey appearance. Periostracum not observed.

Exhalant siphon covered in tentacles at tip and at base. Mantle overlying ctenidia at the dorsal edge covered in iridophores. Outer demibranch about $\frac{1}{3}$ to $\frac{1}{4}$ size of inner demibranch on both sides. Outer demibranch consisting of about 12 plications and inner demibranch with about 18 plications. Labial palps small, clasping the inner demibranchs only. Foot hatchet shaped, as typical for cardiids, with pronounced byssal groove running right to the tip of the foot. No pigmentation of the animal observed in preserved specimens, besides iridescence in inner areas of the mantle. Mantle edge beside exhalant siphon smooth.

Distribution. At present known from the Philippines, W. Indonesia, N. Australia, Papua New Guinea, Tuvalu and Fiji (Fig. 3). Bathymetric range: 0–2 m (alive); 0–21 m (shells only).

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Etymology. This species is named after the type locality, Funafuti Island in the country of Tuvalu. The name was chosen to honour the people of Funafuti.

Remarks. *Fragum funafutiense* n. sp. is a small *Fragum* species that at first sight much resembles several other ‘25 rib group’ members - similar sized congeners that roughly share a rib number between 20 and 30. Moreover, the L/H ratio is below 1, the umbonal radial keel is sharp and the rib sculpture comparable. It differs from *F. mundum* and *F.*

loochooanum Kira, 1959 by a higher number of ribs on the posterior slope. Whereas *F. scruposum* (Deshayes, 1855) has a strongly saw-toothed posterior margin, in *F. funafutiense* n. sp. it is more crenulate. The fourth related species, *F. whitleyi*, has a more quadrate shape with a less sharp postero-ventral angle, attains a larger adult size and has wider interstices. Additional morphometric data have been gathered and statistical analyses conducted for selected members of the “*Fragum* group” (Kirkendale 2009) that support distinction of *Fragum funafutiense* n. sp. (Kirkendale unpublished).

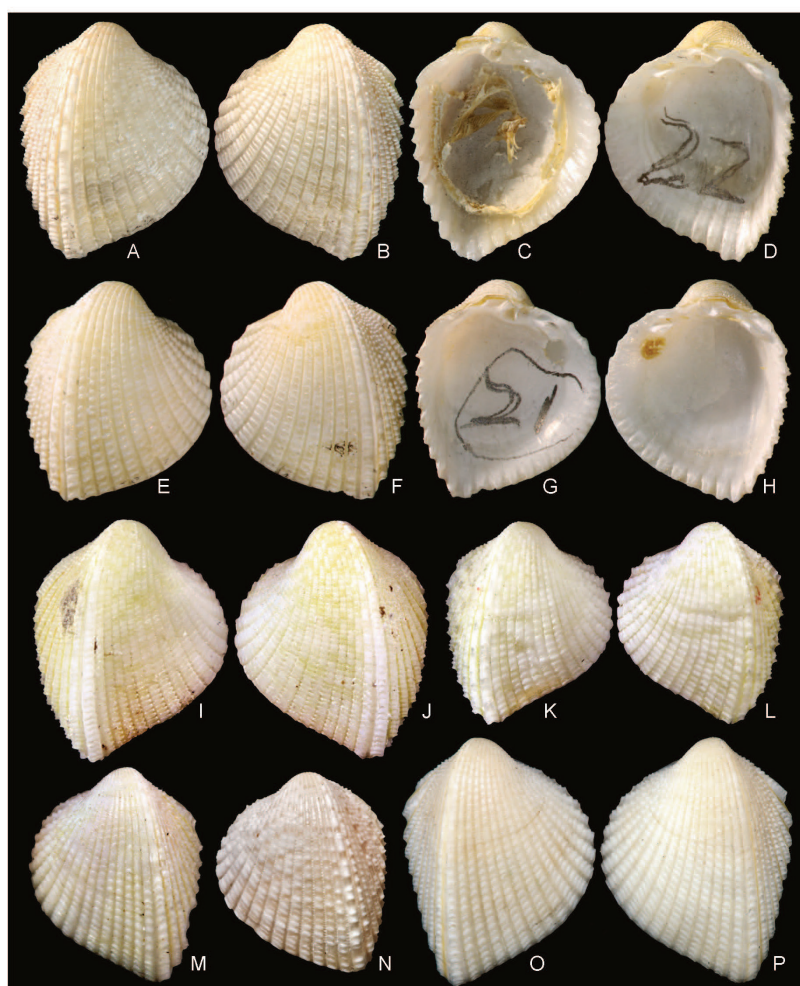


Figure 1. *Fragum funafutiense* n. sp., holotype (A–D) and paratypes (E–P). A–D. Funafuti Atoll (UF 374156, holotype). H 8.4 mm. E–H. Same locality (UF 351685, paratype). H 6.4 mm. I–J. Australia, Western Australia, Ashmore Reef (WAM S91571, paratype). H 10.5 mm. K–L. Same locality (WAM S75212, paratype). H 7.6 mm. M. Australia, Western Australia, Cartier Reef (NTM P.54210, paratype). H 8.3 mm. N. Philippines, Panglao Island, Looc (MNHN-IM-2010-1202, paratype). H 7.5 mm. O–P. Fiji, Viti Levu, Yanula Island (TP 5321, paratype).

Fragum mundum (Fig. 2A–D) differs by a slightly higher rib number (26–32 radial ribs of which 8–9 on the posterior slope, Table 1), by a sharper umbonal keel that is generally less curved when adult, by a more sub-rhomboidal outline and a more elongate shell and by a rib sculpture on the posterior slope that is more lamellate or spinose. Moreover, the anterior ventral lateral tooth of both valves usually contains a well-marked hook-shaped extension, of which the base overhangs the anterior adductor scar. The interior posterior margin and the interstices on the posterior slope can be deep orange. It has a much wider distribution (Fig. 3B), also occurring in the western Indian

Ocean and in the tropical Central Pacific.

Western Australian material has been confused with *Fragum whitleyi* (Fig. 2E–H); see ter Poorten *et al.* 2017) but that species differs by a more sub-quadrate shape with a less pointed postero-ventral angle, by a less sharp umbonal keel, by a rib sculpture on the posterior slope that predominantly consists of semi-circular nodules, by having wider interstices ($\frac{1}{3}$ to $\frac{1}{2}$ the rib width) and by a larger size (H up to 18 mm). This species also has a much wider distribution (Fig. 3C), also occurring in the western Indian Ocean and in the tropical Central Pacific.



Figure 2. Exterior and interior shell morphology of closely related *Fragum* species. A–D. *Fragum mundum*. Lord Hood's Island (NHMUK 1978138, lectotype, herein selected). E–H. *Fragum whitleyi*. Australia, Queensland, Lizard Island (UF 374114). H 9.5 mm. I–L. *Fragum loochooanum*. Japan, Kagoshima Prefecture, Amami Oshima, Tsuchihama (colln M. Chino, Japan, no reg. no.). M–P. *Fragum scruposum*. Malacca (NHMUK 1974140, largest of the three syntypes). H 7.8 mm.

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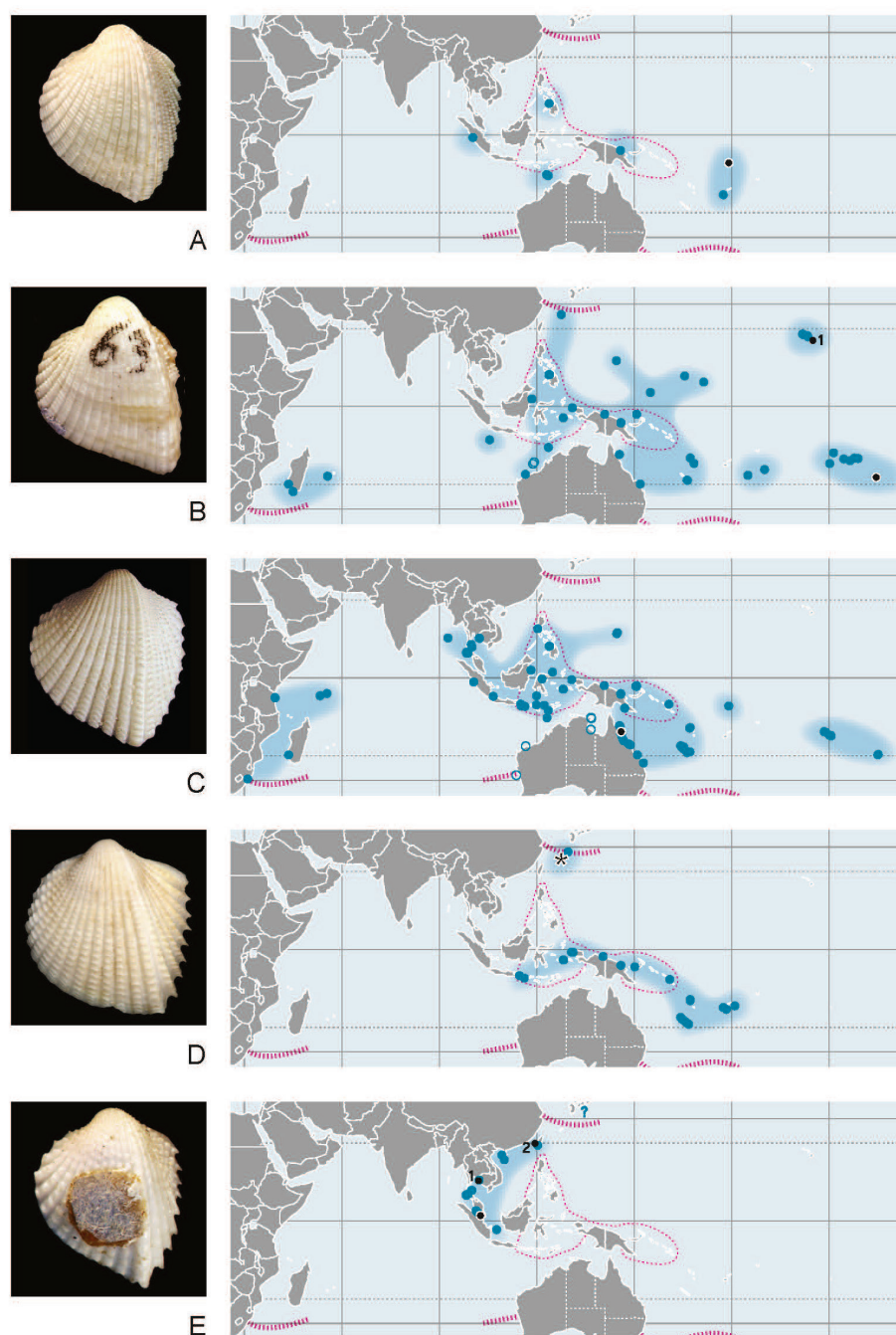


Figure 3. Distributions of closely related *Fragum* species as currently known, with number of examined samples in brackets. A. *Fragum funafutiense* n. sp. (n = 17). B. *Fragum mundum* (n = 108). C. *Fragum whitleyi* (n = 245). Photo: A. Miller, copyright Australian Museum. D. *Fragum loochooanum* (n = 43). E. *Fragum scruposum* (n = 27). Borders of fauna provinces with red striped lines; blue circles: verified samples; open blue circles: samples in need of verification; black circles and asterisk: type localities. B-1 circle: type locality of *Fragum thurstoni* Dall, Bartsch and Rehder, 1938. E-1 circle: type locality of *Hemicardium (Fragum) fragum* var. *carinata* Lyngø, 1909; E-2 circle: type locality of *Corculum (Fragum) bannoii* Otuka, 1937.

A third related species is the poorly known *Fragum loochooanum* (Fig. 2I–L) which previously has been confused with *F. scruposum* and *F. whitleyi*. It can be differentiated from *F. funafutiense* n. sp. by a more sub-quadrate outline, by an umbonal keel that is poorly demarcated, by a lower rib number (21–24, nearly all of which possess 9 high-

triangular ribs on the posterior slope), by having a strongly serrate posterior margin and by having wider interstices (up to $\frac{1}{2}$ the rib width). Its distribution (Fig. 3D) is incompletely known, but appears to largely overlap with that of *F. funafutiense* n. sp. (Fig. 3A).

Table 1. Comparative characters of all currently accepted *Fragum* species (MolluscaBase 2020) and *F. funafutiense* n. sp. Sculpture and outline based on the predominant condition in adult material; L/H based on the majority of the investigated specimens. Rib number in brackets refers to the ribs on the posterior slope. Modified and extended after ter Poorten (2015).

<i>Fragum</i> species	L/H (adult)	Rib number	Rib sculpture on posterior part	Max. size (L or H) mm	Umbonal keel	Outline
<i>F. fragum</i> (Linnaeus, 1758)	L < H	31–37 (9–11)	Oblique scales	H 30–45	Very sharp	Sub-rhomboidal–trigonal
<i>F. nivale</i> (Reeve, 1845)	L < H	28–33 (10–12)	Oblique scales	H 15–30	Very sharp	Sub-rhomboidal–trigonal
<i>F. grasi</i> ter Poorten, 2009	L $\approx$ H	15–21	Weakly expressed knobs	H 5–8	Very weak	Sub-circular–obliquely oval
<i>F. funafutiense</i> n. sp.	L < H	24–30 (10–12)	Projecting oblique scales	H 7–11	Sharp	Sub-quadrate–Sub- rhomboidal
<i>F. mundum</i> (Reeve, 1845)	L < H	26–32 (8–9)	Projecting imbricating scales	H 10–12	Very sharp	Sub-rhomboidal
<i>F. loochooanum</i> Kira, 1959	L $\leq$ H	21–24 (9)	Nodular scales	H 10–14	Sharp	Sub-quadrate
<i>F. scruposum</i> (Deshayes, 1855)	L < H	19–25 (9–10)	Nodular scales	H 10–14	Very sharp	Sub-rhomboidal
<i>F. sueziense</i> (Issel, 1869)	L $\approx$ H	22–28	Oblique curved scales	L 10–12	Weak	Roundly sub-quadrate
<i>F. unedo</i> (Linnaeus, 1758)	L < H	25–32 (9–11)	Oblique curved scales	H 50–81	Rather sharp	Sub-rhomboidal–trigonal
<i>F. whitleyi</i> Iredale, 1929	L < H	24–29 (10–12)	Semi-circular nodules	H 15–18	Sharp	Sub-quadrate
<i>F. vanuatuense</i> ter Poorten, 2015	L < H	19–26	Nodular scales	H 5–7	Very weak	Ovoid
<i>F. erugatum</i> (Tate, 1889)	variable	22–28	Weak rugae or lacking	H 10–20	Weak	Roundly sub-quadrate

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Fragum scruposum differs by a lower rib number (19–25 radial ribs of which 9–10 on the posterior slope, Table 1), by a sharper umbonal keel that is generally less curved and that coincides with 1 (LV) or 2 (RV) much more pronounced radial ribs terminating in a bulged postero-ventral margin, by a more sub-rhomboidal outline, by a more distantly placed rib sculpture, by a serrate and saw-toothed posterior margin and by pointed triangular posterior ribs. This short-range species is confined to Thailand, Malaysia, China and Taiwan (Fig. 3E).

The type material of *Cardium mundum* Reeve, 1845 (NHMUK 1978138) consists of three syntypes, all complete specimens, and all figured by ter Poorten (2015: figs. 9–11). The first one (L 6.9; H 8.8; W 6.4 mm; 29 ribs) is marked '63' and agrees with the specimen figured by Reeve (1845: pl. 22 fig. 125). The second one (L 6.8; H 8.8; W 6.0 mm; 28 ribs) is rather atypical and could involve *F. funafutiense* n. sp. The poor state of preservation does not allow for a positive identification. The third one typically agrees with *mundum* and is a juvenile (L 4.8; H 6.0; W 3.85 mm; 30 ribs). In order to settle the identity unambiguously, the first one (Fig. 2A–D) is hereby selected as lectotype of *Cardium mundum* Reeve, 1845.

Holotype. Tuvalu, Funafuti Atoll, on offshore sand bank next to channel (te Ava Fuagea) adjacent to Fualopa Islet, 08°32.9'S, 179°3.4330'E, windrow accumulation of *Halimeda*; under small rocks and sand, 0 m, 22.06.2004. Leg. L. Kirkendale, stn LAK-246 (UF 374156, holotype, 1 p.v., A). H 8.4 mm. Paratypes as listed below.

Zoobank registration. urn:lsid:zoobank.org:act:D1A1AD55-4AA8-4456-906B-3E8E117E129D

Other material examined

Philippines. Panglao Island, Looc, 9°35.7'N, 123°44.4'E, subtidal platform, mixed bottoms, 0–2 m, 18.06.2004, PANGLAO 2004, stn S18 (MNHN-IM-2010-1202, paratype, 1 p.v., A); Panglao Island, Looc, 9°35.8'N, 123°44.6'E, hard plateau with sand covering rocks, 2–3 m, 28.06.2004, PANGLAO 2004, stn S32 (MNHN-IM-2010-1207, paratype, 1 s.v.); Bohol Isl., Ubajan, 9°41.5'N, 123°51.0'E, mud, 21 m, 23.06.2004, PANGLAO 2004, st. S25 (TP 3844, 4 s.v.); Pamilacan Island,

9°29.4'N, 123°56.0'E, hard ground covered with sand, 15–20 m, 21.06.2004, PANGLAO 2004, st. S22 (TP 3845, 4 s.v.).

Indonesia. Indonesia, Sumatra, West Sumatra Province, Sungai Beremas, 01°01'18.6"S, 100°23'16.0"E, beach with rocks and stones. Leg. B. Gras, 22.01.2008 (TP 3578, paratype, 1 s.v.).

Australia. Western Australia, Oceanic Shoals, Ashmore Reef; Lower East Side, 12°16'38.389"S, 123°08'09.935"E, 1 m, 28.09.2013. Leg. L. Kirkendale & C. Bryce, Woodside Kimberley Survey 2013, stn 129/K13 (WAM S75212, paratype, 1 p.v.; WAM S91688, 1 s.v.); Ashmore Reef; S. end, 12°15'44.388"S, 122°59'05.425"E, 1 m, 02.10.2013. Leg. L. Kirkendale and C. Bryce, Woodside Kimberley Survey 2013, stn 137/K13 (WAM S91571, paratype, 1 p.v., A); Cartier Reef, N.E. end of cay, large shallow pool adjacent to reef flat, 12°31.7'S, 123°33.5'E, on the undersurface of dead coral slabs, substrate of coarse coral sand with small pieces of unconsolidated coral rubble, 2.5–6 m, 05.05.1992. Leg. R.C. Willan (NTM P.54210, paratype, 1 s.v.).

Papua New Guinea. Wonad Island, 05°08.2'S, 145°49.4'E, 10–20 m, 26.11.2012, PAPUA NIUGINI, stn PD48 (MNHN-IM-2014-7078, 1 s.v.); Lauhamug Island, 04°59.4'S, 145°47.6'E, 4 m, 21.11.2012., PAPUA NIUGINI, stn PS21 (MNHN-IM-2014-7079, 1 s.v.).

Fiji. Viti Levu, off SW coast, Yanula Island, unknown depth. 28.07.1975. (JJTP 532, paratype, 1 p.v.).

Tuvalu. Funafuti Atoll, On offshore sand bank next to channel (te Ava Fuagea) adjacent to Fualopa Islet, 08°32.9'S, 179°3.4330'E, windrow accumulation of *Halimeda*; under small rocks and sand, 0 m, 22.06.2004. Leg. L. Kirkendale, stn LAK-246 (UF 436379, paratypes, 2 s.v.); Funafuti Atoll, southernmost end, Avalau Lagoon, 08°37.6'S, 179°4.7110'E, lagoon, sand on coral pavement, 0–2 m, 25.06.2004. Leg. L. Kirkendale, stn LAK-249 (UF 374157, paratype, 1 p.v., A); Funafuti Atoll, Fualopa Islet, reef flat, sand on coral pavement, no seagrass, sand, 0–1 m, 21.06.2004. Leg. L. Kirkendale, stn LAK-244 (UF 351685, paratype, 1 p.v., A).

RESULTS

Window shell microstructure

The presence of translucencies concentrated in the posterior shell region were macroscopically observed by the first author in 2004 and led to *F. funafutiense* n. sp. provisionally referred to as *F. aff. mundum* (Kirkendale, 2009) because of this feature. Backlighting clearly highlighted darker shell areas or patches where light could be seen to penetrate more than in surrounding shell areas (Fig. 4). However, whether this was indicative of

fibrous prismatic microstructure or pigmentation required SEM examination of shell microstructure. SEM micrographs through a region of fractured posterior shell showed deep penetration of fibrous prismatic microstructure (Fig. 4). No obvious convexities were observed to underlie areas of fibrous prismatic microstructure incursion. Using backlighting, *Fragum whitleyi* exhibits no indication of window formation on the posterior shell slope, while *F. mundum* exhibits evidence of even more pronounced window shell formation than *F. funafutiense* n. sp. (Fig. 4).

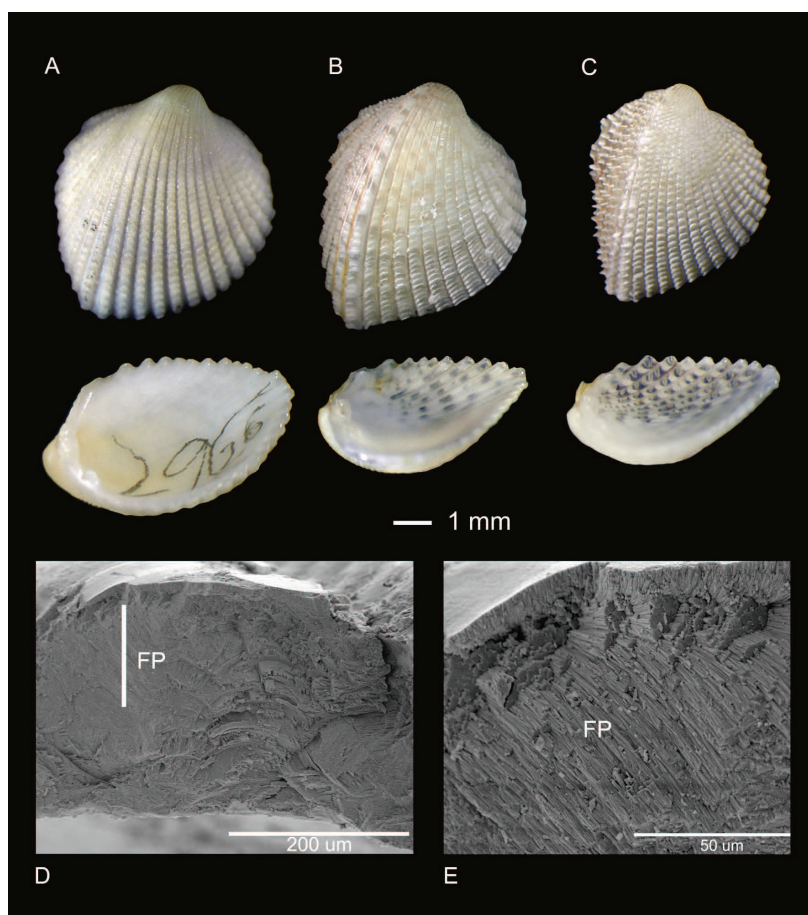


Figure 4. Evidence for window shell formation in *Fragum funafutiense* n. sp. and comparison with closely related species. A. *Fragum whitleyi* (UF 374153), B. *Fragum funafutiense* n. sp. (holotype, UF 374156) and C. *Fragum mundum* (UF 374155) showing features of external shell morphology (top) and backlighting through posterior shell surface (bottom) to display window formation, D. Deep penetration of fibrous prismatic (FP) microstructure, as shown by white vertical bar, in *Fragum funafutiense* n. sp. windowed area on posterior shell surface, E. Close-up of fibrous prismatic (FP) microstructure in *Fragum funafutiense* n. sp. in windowed area on posterior shell surface. Exterior of shell is at top of image and interior of shell is at bottom of image in D and E.

DISCUSSION

Kirkendale (2009) showed that *F. funafutiense* n. sp. (cited as *F. aff. mundum*) was genetically distinct from other tested fragines using a suite of standard molecular markers, including the barcoding gene (COI). It is now shown to be different from other tested species of *Fragum* by several morphological features, including outline, shell shape, size, nature of the umbonal keel, rib number and rib sculpture. The new species is reported from many sites in the Indo west Pacific including the Philippines, W. Indonesia, N. Australia, Papua New Guinea, Tuvalu and Fiji, where it has been live-collected from the shallow intertidal. A limitation of the current molecular phylogenetic studies on cardiids (e.g. Kirkendale 2009; Herrera *et al.* 2015) is that they do not include described small fragines such as *F. grasi* ter Poorten, 2009 and *F. vanuatuense* ter Poorten, 2015. However, morphological analyses indicate *F. grasi* and *F. vanuatuense* are clearly differentiated from *F. funafutiense* n. sp. (Table 1). Suitably preserved tissue for molecular study is available for both *F. grasi* and *F. vanuatuense* and these need to be included in future studies to continue to test species boundaries among fragines using molecular genetic data. These types of data can help resolve the position of *F. funafutiense* n. sp. relative to other 25 rib group members, which is not well understood (Kirkendale 2009). This is a viable area for future research, especially considering the new molecular markers identified by Li *et al.* (2020) for improved resolution among Fraginae.

SEM photos through a region of fractured posterior shell showed deep penetration of fibrous prismatic microstructure that is consistent with interpretation of these areas as exhibiting window shell microstructure in *F. funafutiense* n. sp. (Fig. 4). This is similar to the microstructure type of *F. fragum* reported by Persselin (1998), although complete characterization of all shell layers was not possible as acetate peels were not made in this study (Table 2). These additional steps are necessary to more fully characterize windows in *F. funafutiense* n. sp. No well-developed convexities underlying areas of deep penetration of fibrous prismatic microstructure were observed (Fig. 4). Therefore, these areas are unlikely to focus light as observed in the more

highly developed windows of *Corculum*, *F. mundum*, *Lunulicardia* sp. 1 and *F. nivale* (Reeve, 1845) (Carter and Schneider 1997, Persselin 1998) (Table 2). The functional significance of windows in *F. funafutiense* n. sp. could be to offer increased illumination to tissues where photosymbionts are stored, when the two valves are closed, just as for highly developed windows; this could be important in areas of high predation. Window shell microstructure is the only character that is strictly associated with photosymbiotic bivalve species, extant or extinct (Kirkendale and Paulay 2017). Characterizing windows in all extant photosymbiotic species will help quantify how common this adaptation is and help to understand the early evolution of this significant and unique microstructural feature. Placing this work in a phylogenetic context will illuminate the evolution of window shell microstructure in the Fraginae.

CONCLUSIONS

Cardiids or cockles are one of the most well studied families of marine bivalves (Kirkendale and Paulay 2017; ter Poorten *et al.* 2017) and photosymbiotic members of the family are also becoming better characterized from a functional perspective (Li *et al.* 2018; 2020). However new species continue to be discovered, especially in hyperdiverse remote corners of the world (ter Poorten 2009; 2015; this study). These new species shed light on the tempo and mode of photosymbiotic evolution in the Fraginae. The need for a completely resolved tree that integrates new species is required to understand relationships among taxa. Additionally, systematic revision of paraphyletic *Fragum* is a persistent impediment to ongoing taxonomic and evolutionary work. A completely resolved tree that integrates new species coupled with revisionary work would aid in evolutionary studies, such as determining the evolution of window shell microstructure in the group. Furthermore, continued fieldwork in little explored areas is necessary to more fully understand marine biodiversity in this and other invertebrate groups so effective conservation measures can be developed that take into account smaller bodied species (the so called ‘charismatic microfauna’) (Middelfart *et al.* 2020).

Table 2. Incidence of window shell microstructure (WSM) in photosymbiotic Fraginae (after Kirkendale 2009).

Species	Window shell microstructure
<i>Fragum fragum</i> (Linnaeus, 1758)	Intermediate, Persselin 1998
<i>F. nivale</i> (Reeve, 1845)	Developed, Persselin 1998
<i>F. grasi</i> ter Poorten, 2009	untested
<i>F. funafutiense</i> n. sp.	Developed, this study
<i>F. mundum</i> (Reeve, 1845)	Developed, Persselin 1998
<i>F. loochooanum</i> Kira, 1959	Absent, this study
<i>F. scruposum</i> (Deshayes, 1855)	untested
<i>F. sueziense</i> (Issel, 1869)	untested
<i>F. unedo</i> (Linnaeus, 1758)	Absent, Persselin 1998
<i>F. whitleyi</i> Iredale, 1929	untested
<i>F. vanuatuense</i> ter Poorten, 2015	untested
<i>F. sp. 11</i> Persselin, 1998	Absent, Persselin 1998
<i>F. carinatum</i> of Kirkendale 2009	untested
<i>Corculum cardissa</i> (Linnaeus, 1758)	Highly developed, Carter and Schneider 1997
<i>C. aselae</i> Bartsch, 1947	untested
<i>C. aequale</i> (Deshayes, 1855)	untested
<i>C. impressum</i> (Lightfoot, 1786)	untested
<i>C. lorenzi</i> M. Huber, 2013	untested
<i>C. monstrosus</i> (Gmelin, 1791)	untested
<i>C. roseum</i> (Gmelin, 1791)	untested
<i>Lunulicardia hemicardium</i> (Linnaeus, 1758)	untested
<i>L. orlini</i> Mienis, 2009	untested
<i>L. retusa</i> (Linnaeus, 1767)	untested
<i>L. tumorifera</i> (Lamarck, 1819)	untested
<i>L. sp. 1</i> Persselin, 1998	Developed, Persselin 1998

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sequenced paratype WAM S91571 (COI). This sequence was generated as part of the Gorgon Project's Barrow Island Net Conservation Benefits Fund. Thanks to Niko and the Fisheries Team on Funafuti atoll, Tuvalu that made sampling on a remote island possible, not to mention fun. The new species is dedicated to them and to all Pacific Islanders who continue to assist so many of us to study insular faunas of the western Pacific. We also thank our two reviewers who made suggestions that greatly improved the manuscript.

A new photosymbiotic marine bivalve

Dedication: We dedicate this paper to Jørgen Hylleberg given his long love of tropical marine biology and study of molluscan faunas in the Indo-west Pacific. His contribution to the field is realized in his mentoring of marine biology students from across the world, but particularly Denmark at the University of Aarhus, Thailand, Indonesia, Vietnam, and Cambodia through the Master of Science

Programme and many more countries through the Tropical Marine Mollusc Programme. However, he also had an inordinate love of cockles and his papers on cardiids and the important compendium *Lexical Approach to Cardicea* has been an important reference point for malacologists. He very kindly donated his entire cardiid collection to the second author for which he is extremely grateful.

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