

New *Holothuria* species from Australia (Echinodermata: Holothuroidea: Holothuriidae), with comments on the origin of deep and cool holothuriids

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Abstract

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Two aspidochirotid species, new to science, from the continental slope of southern Australia are described: *Holothuria (Panningothuria) austrinabassa* O'Loughlin sp. nov. and *Holothuria (Halodeima) nigralutea* O'Loughlin sp. nov. The first represents the southernmost documented holothuriid, and is the sister species of the northernmost holothuriid species *Holothuria (Panningothuria) forskali* Delle Chiaje. The second is a very recent offshoot of the wide-ranging Indo-west Pacific *Holothuria (Halodeima) edulis* Lesson. Morphological and molecular genetic differences between these species pairs are detailed. *Holothuria (Halodeima) signata* Ludwig is raised out of synonymy with *H. edulis*. A lectotype for *Holothuria (Halodeima) signata* Ludwig is designated, The status of the subgenera *Panningothuria* Rowe and *Halodeima* Pearson is discussed. The occurrence of multiple madreporites in *Halodeima* is discussed.

Keywords

Echinodermata, Holothuroidea, Holothuriidae, *Holothuria*, taxonomy, new species, new lectotypes.

Introduction

The Holothuriidae is one of the most diverse families of sea cucumbers, with the bulk of this diversity in shallow, tropical waters. Of the more than 185 species (Samyn et al., 2005) currently recognized, all but a handful thrive in the tropics, predominantly on coral reefs, at less than 50 m depths. It is therefore noteworthy that recent surveys in Australia revealed two new deepwater species from subtropical to warm temperate latitudes. Specimens of the two new *Holothuria* species were collected from the continental slope off western and south-western Australia during the survey SS10/2005 by Australia's national science agency, the Commonwealth Scientific and Industrial Research Organization (CSIRO), that is aiming "to characterize benthic ecosystems off Western Australia". This was commenced through the Marine National Facility by the RV *Southern Surveyor* in the last months of 2005. Additional specimens were discovered in the collections of Museum Victoria. To ascertain the subgenera to which the two new species belong, comparative morphological and molecular studies were undertaken.

Methods

Genetic characterization was pursued by sequencing portions of the mitochondrial 16S (large subunit) RNA and cytochrome oxidase I (COI) genes. Ethanol-fixed tissues of the new taxa,

related species, and outgroup taxa (see Table 1 for voucher information) were macerated, digested in DNAzol[®] and proteinase K overnight, and genomic DNA isolated using standard procedures (Meyer, 2003). Genomic DNA of most samples was cleaned using the Qiagen polymerase chain reaction (PCR) cleanup kit, following manufacturer's protocols, except that cleaned DNA was resuspended in TE buffer. Qiagen cleanup helped eliminate problems with inhibition prevalent in holothurian samples.

An approximately 1120 bp long (1119 bp in *H. nigralutea* G255) section of the large subunit of the mitochondrial ribosome RNA gene (16S) was amplified with a pair of overlapping primers. 16Sc1 (TACCTT[T/G]TGAT[T/A]ATGG[T/A]TTAAC) and 16Sc2 (TGATTATGCTACCTTNGCAC) (designed new) amplified 678 bp, and 16SAR (CGCCTGTTTATCAAAAACAT) and 16SBR (GCCGGTCTGAACTCAGATCAGT) (Palumbi, 1996), amplified 510 bp in *H. nigralutea* (G255). A 651 bp length of the mitochondrial cytochrome oxidase subunit I gene was amplified with primers COIeF (ATAATGATAGGAGGRTTGG) COIeR (GCTCGTGTRTCTACRTCCAT) (Arndt et al., 1996). PCR products were sequenced at the University of Florida's ICBR center. Electropherograms were edited in Sequencher, aligned with Clustal X, and adjusted by eye. Sequences are deposited in GenBank (see Table 1 for GenBank and voucher information). Sequence data from the two gene regions were analyzed as a

single concatenated dataset. Parsimony trees were generated by PAUP (version 4, Swofford, 2003), with 100 bootstrap replicates. Bayesian analyses were run using Mr. Bayes (version 3.1.2, Ronquist and Huelsenbeck, 2003), with MC³, GTR-I-Gamma, an uninformative prior, for 10 million generations. GTR-I-Gamma was chosen as the simplest model of evolution that fitted the data, using the Akaike Information Criterion as implemented by the program Modeltest 3.6 (Posada and Crandall, 1998), for each gene region as well as for the combined sequences. Indels were included in the analysis. There was no evidence for pseudogene sequences in any of several hundred specimens of *Holothuria* sequenced to date; all reads were clean and unambiguous.

For scanning electron microscopy (SEM), ossicles were

cleared of associated soft tissues in commercial bleach. They were then air-dried, mounted on aluminium stubs, coated with gold, and observed with a JEOL JSM-6480LV scanning electron microscope.

Abbreviations for institutions are: MNHN—Musée national d'Histoire naturelle, Paris; NMV—Museum Victoria, Australia; RBINS—Royal Belgian Institute of Natural Sciences; UF—Florida Museum of Natural History; UH—Zoologisches Museum, Universität Hamburg; UM—University of Murcia, Spain; USNM—United States National Museum of Natural History, Smithsonian Institution, Washington.

Specimen registration number prefixes are: MNHN EChh; NMV F; RBINS IG; UF E; UH E; UM HO; USNM E.

Table 1. Specimens sequenced. GenBank accession numbers given for gene regions.

Voucher	Extraction	Species	Locality	16Sc	16SAR	COIe
NMV F94742	N10	<i>Stichopus ocellatus</i>	Papua New Guinea	EU220793	EU220793	EU220814
UF E4834	G188	<i>Actinopyga obesa</i>	Hawaii	EU220794	EU220794	EU220815
UF E4901	N82	<i>Bohadrschia</i> sp. nov.	Hawaii	EU220795	EU220795	EU220816
UF E1595	G80	<i>H. excellens</i>	Palau	EU220796	EU220796	EU220817
NMV F110524	G257	<i>H. austrinabassa</i>	W Australia	EU220797	EU220797	EU220818
UF E4480	G200	<i>H. forskali</i>	Portugal	EU220798	EU220798	EU220819
UF E4831	G186	<i>H. atra</i>	Hawaii	EU220799	EU220799	EU220820
UF E4460	G175	<i>H. grisea</i>	Florida	EU220800	EU220800	no
UF E3359	G247	<i>H. kefersteini</i>	Panama	EU220801	EU220801	no
UF E4877	G259	<i>H. mexicana</i>	Belize	EU220802	EU220802	EU220821
UF E4822	G230	<i>H. floridana</i>	Florida	EU220803	EU220803	EU220822
NMV F120437	N120	<i>H. nigralutea</i>	W Australia	EU220804	no	EU220823
NMV F111290	G255	<i>H. nigralutea</i>	W Australia	EU220805	EU220805	EU220824
UF E3644	N3	<i>H. edulis</i> "brown" form	Cocos-Keeling	EU220806	EU220806	EU220825
UF E2065	J292	<i>H. edulis</i> typical form	Oman	EU220807	EU220807	EU220826
UF E4987	K140	<i>H. edulis</i> fuschia form	Philippines	EU220808	no	EU220827
UF E4746	G104	<i>H. edulis</i> typical form	Guam	EU220809	EU220809	EU220828
UF E3884	J282	<i>H. edulis</i> grey form	Okinawa	EU220810	EU220810	EU220829
UF E3882	J296	<i>H. edulis</i> grey form	Okinawa	EU220811	EU220811	EU220830
UF E325	G50	<i>H. signata</i>	Rangiroa	EU220812	EU220812	EU220831
UF E329	G55	<i>H. signata</i>	Rangiroa	EU220813	EU220813	EU220832

Table 2. Characters distinguishing *H. (Panningothuria) austrinabassa* O'Loughlin sp. nov. and *H. (Panningothuria) forskali* Delle Chiaje

Characters	<i>H. austrinabassa</i>	<i>H. forskali</i>
Body colour	Grey-brown, small brown spots	Black to dark brown
Papilla tubercles	Distinct, ocellate, off-white	Same colour as body wall
Tables in body wall	Abundant, fully developed	Sparse to absent, reduced form
Dorsal table discs	> 50 µm wide	< 50 µm wide
Spire of tables	Always fully developed	Rarely fully developed
Papillae rods	Unbranched rods absent	Unbranched rods present
Tentacle tables	Present, reduced form	Absent
Tube feet	Spinous rods present	Spinous rods absent
Distribution	W and S Australia	NE Atlantic, Mediterranean Sea

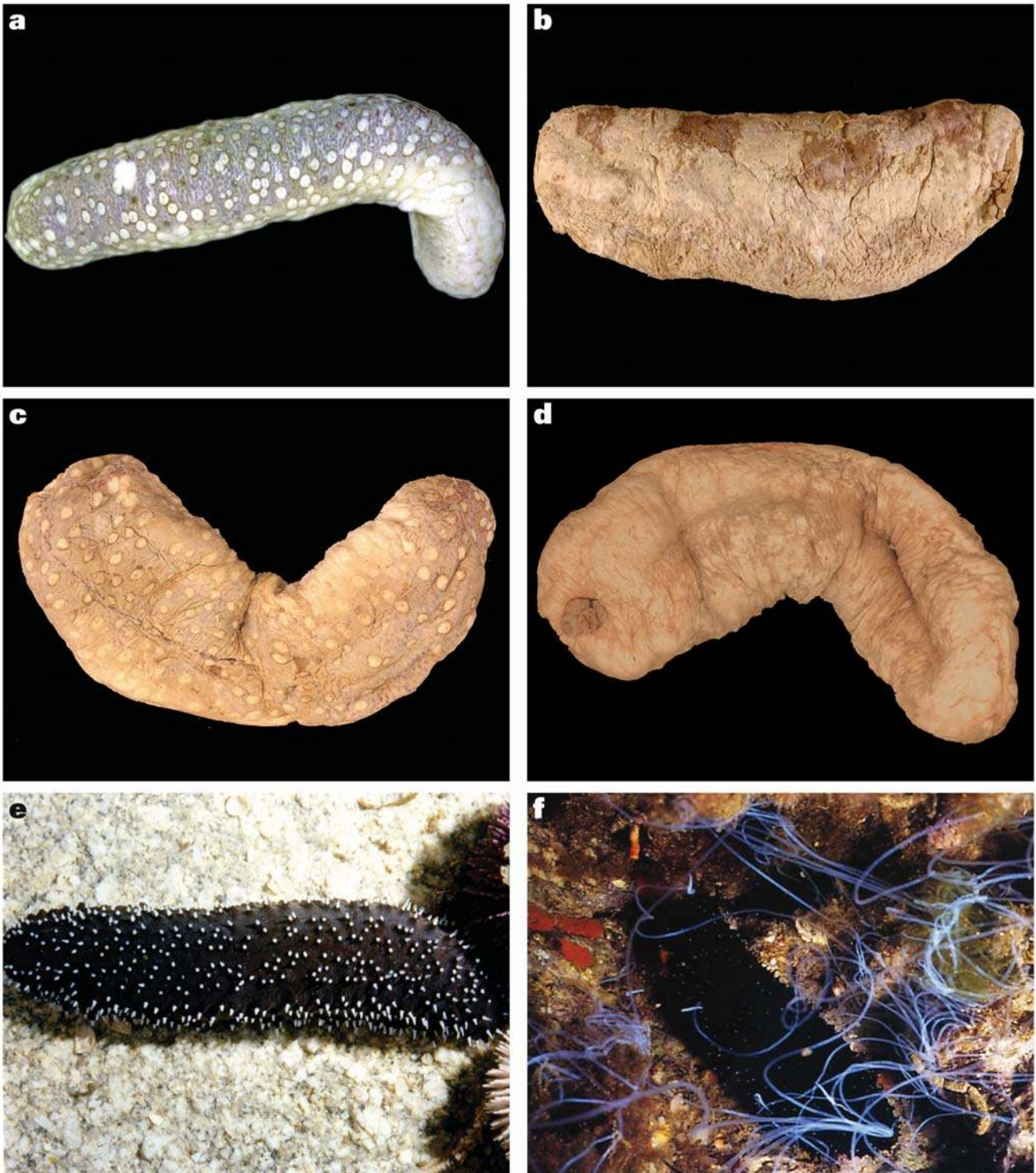


Figure 1. a, live *Holothuria (Panningothuria) austrinabassa* O'Loughlin sp. nov, from Western Australia, off Perth (380 mm long; NMV F110523; photo by Karen Gowlett-Holmes). b, preserved *H. (Panningothuria) austrinabassa*, from Western Australia, off Albany (250 mm long; tentacles at right; NMV F120438; photo by David Staples). c, d, preserved holotype of *H. (Panningothuria) austrinabassa*, from Victoria, off Portland (170 mm long; oral end left; NMV F120447; photos by David Staples): c, dorsal view; d, ventral view. e, live *H. (Panningothuria) forskali* Delle Chiaje, in aquarium in Mons, Belgium (130 mm long; photo by Didier VandenSpiegel). f, live *H. (Panningothuria) forskali*, from south of France, off Banyuls, showing expulsion of cuvierian organ tubules (photo by Didier VandenSpiegel).

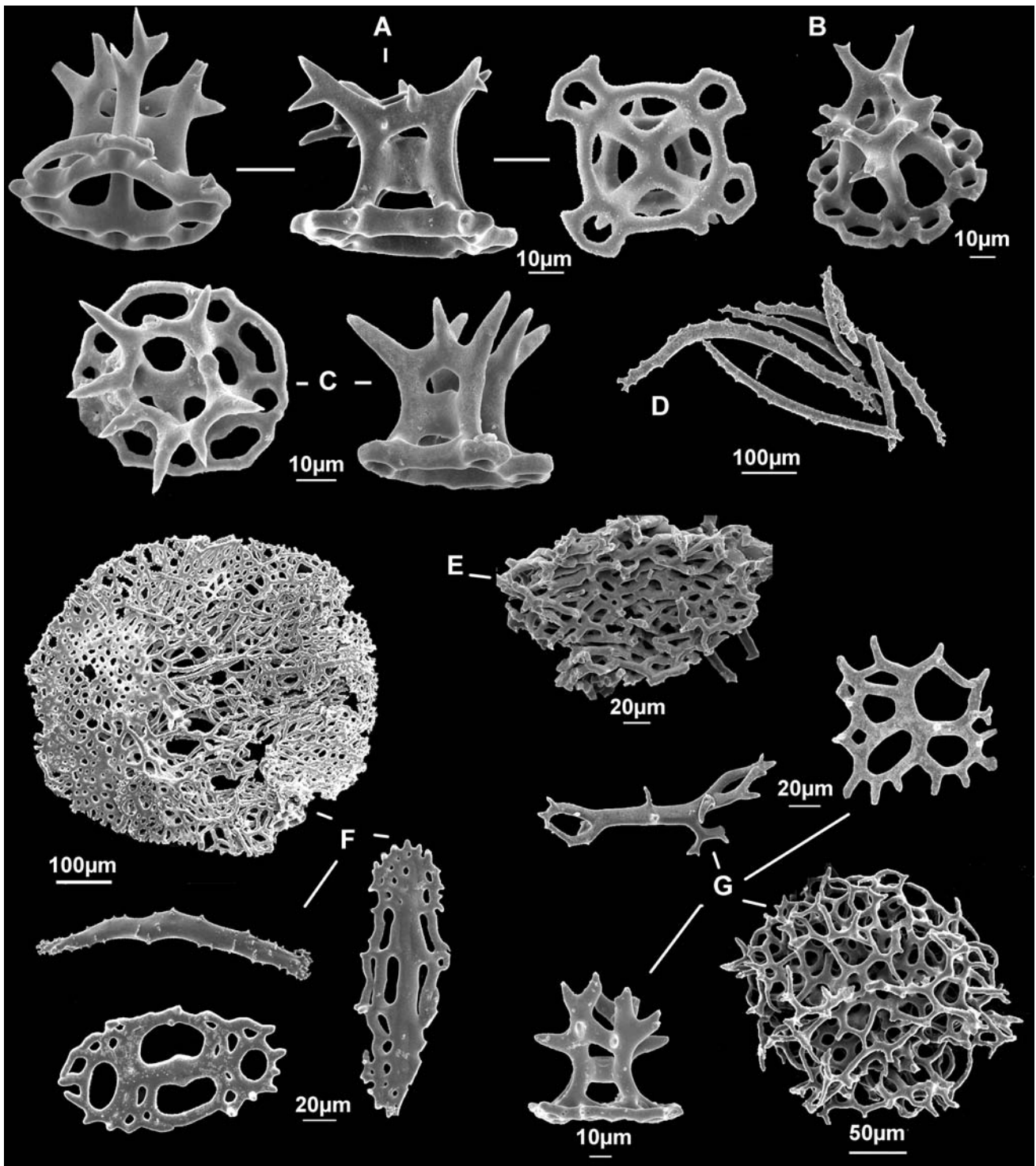


Figure 2. *Holothuria (Panningothuria) austrinabassa* sp. nov. (SEM of ossicles from NMV F120447 and NMV F120438). A, dorsal body wall; B, anal body wall; C, ventral body wall; D, tentacles; E, madreporite; F, tube feet; G, papillae.

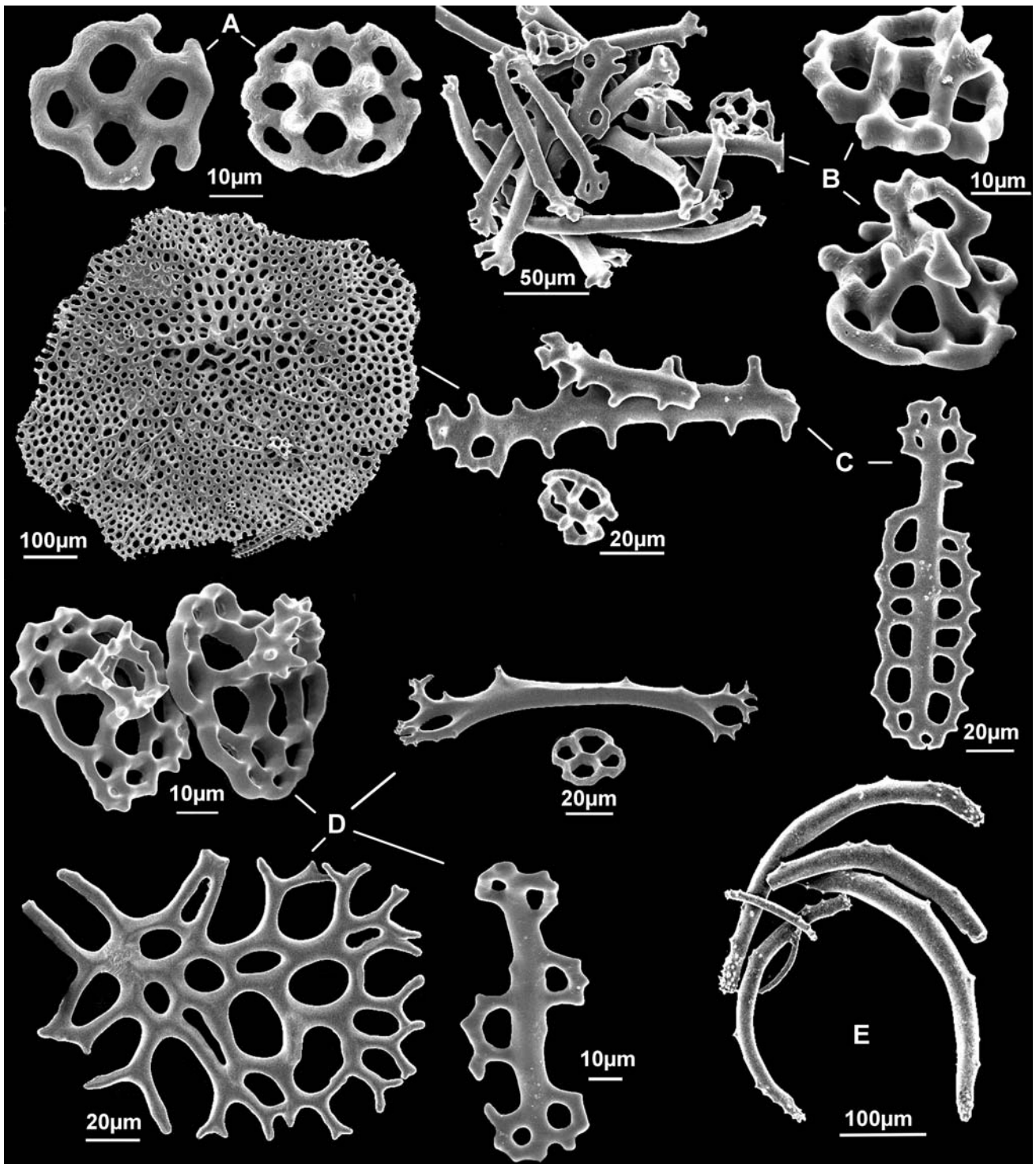


Figure 3. *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823 (SEM of ossicles from HO-1854). A, oral body wall; B, anal body wall; C, tube feet; D, dorsal papillae; E, tentacles.

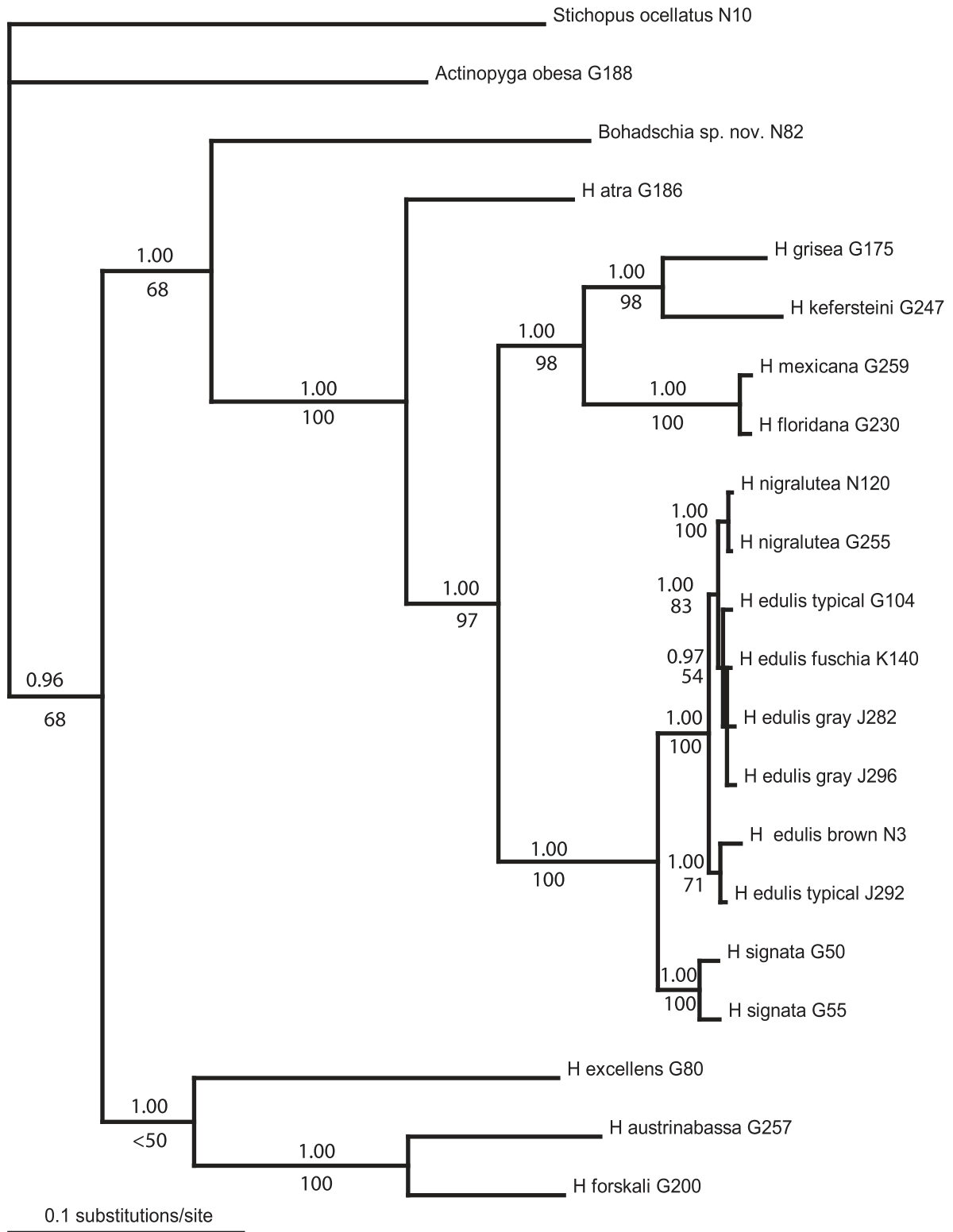


Figure 4. Bayesian phylogram of species studied together with selected outgroup taxa, with posterior probability values (10 million generations, GTR-I-Gamma, and uninformative prior) above branches, and parsimony bootstrap values (100 replicates) below.

Holothuria (Panningothuria) austrinabassa O'Loughlin sp. nov.

Figures 1–4, Tables 1, 2.

Material examined. Holotype: Australia, Victoria, 27 miles SW of Portland, approx. 39°S, 141°E, 293–329 m, *Aquarius*, M. Gomon and R. Plant, May 1979, NMV F120447.

Paratypes: Type locality and date, F109370 (2).

Other material. Western Australia, *Southern Surveyor*, Nov/Dec 2005, SS10/2005 stn 90, off Abrolhos Is, 389–407 m, F110525 (1); SS10/2005 stn 78, off Jurien Bay, 414–401 m, F110524 (3); SS10/2005 stn 6, off Two Rocks (Perth), 329–370 m, F110523 (2); SS10/2005 stn 32, off Bald I. (Albany), 728–710 m, F111301 (1); SS10/2005 stn 34, off Bald I., 431–408 m, F111286 (2); F110526 (2); SS10/2005 stn 39, off Bald I., 97–99 m, F120438 (1); off Cervantes, 30°16' S, 114°30' E, 600–800 m, 8 Feb 1991, F120441 (2); Great Australian Bight, 33°19' S, 127°24' E, 300–310 m, 27 Feb 1976, F120442 (1); South Australia, SW of Beachport, 37°49' S, 139°45' E, 24 Dec 1981, F120439 (1); Victoria, 20.5 miles S of Cape Nelson, 403 m, 10 Mar 1977, F120440 (1).

Comparative material examined. *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823. NE Atlantic Ocean, Portugal, Algarve, Estrajada, 20 m, between rocks, UM HO-1854 (1).

Description (preserved specimens). Body up to 250 mm long, up to 70 mm wide (F120438); elongate, not tapering from mid-body, rounded anteriorly and posteriorly, oval in transverse section, longitudinal, deep, mid-ventral furrow frequently present. Body wall firm-leathery, up to 20 mm thick (F120438). Dorsal and lateral body surface pustulose, wrinkled; tubercles scattered irregularly dorsally and laterally, flat, ocellate, “wart-like”, oval to round, variable size, up to 2–10 mm across, sometimes contiguous, with papillae extending as small nipple-like projections, 1 mm high 0.5 mm wide, 3–12 mm apart, lacking ampullae. Ventral surface soft, pustulose, wrinkled, tube feet hard to discern, arranged in very irregular, scattered, paired series along ventral radii, about 5 mm apart (F110524), tube feet lacking ampullae. Mouth ventral, surrounded by irregular collar of about 50 inconspicuous oral papillae evident only in largest specimen (F120438); tentacles 20, peltate, with long, thin, tubular tentacle ampullae extending off calcareous ring plates, subequal, up to 25 mm (F109370) long. Anus terminal, lacking anal teeth. Left dorsolateral radial plate of calcareous ring 7 mm wide 5 mm high, with 4 anterior points, posterior margin with shallow rounded indentation. Left dorsolateral interradiial plate 3 mm high, 3 mm wide, anterior spire, posterior margin with rounded indentation (F110526). With single dorsal stone canal/madreporite, stone canal 1 mm long with attached madreporite 2 mm long (F109370), to stone canal 2 mm long with attached madreporite 3 mm long (F120441, F120442, F120438). With 1 or 2 sac-like polian vesicles, 12 mm (F110526) to 33 mm (F120441) long, narrowed distally; 2 polian vesicles in holotype, 30 and 15 mm long. Longitudinal muscles flat, broad, thin median groove, dorsal bands up to 12 mm wide, ventral bands up to 30 mm wide (F120438). Gonadal tubules long, thin, multiple branching, extending to mid-body. Respiratory tree extending to anterior end. Cuvierian organ present, tubules up to 25 mm long, 1.5 mm in diameter, not branched. Gut contents calcareous detritus, fragments up to 10 mm long.

Ossicles. Dorsal body wall with numerous tables only; tables variable in size, variable in form of disc and spines; disc

52–72 μm in diameter, with 4–8 perforations, with alternating narrow and wide perforations that give slightly angular, quadrate aspect to disc, sometimes with fine spinelet at edge; spire with 4 pillars, typically 40 μm high (including spines), single cross-beam, crown with conspicuous spines that may extend beyond disc margin, these spines variable in length and form, up to 32 μm long, straight, curved, forked, with side branch. Dorsal papillae with tables, perforated plates, spinous spherical bodies; tables as for body wall, but some larger, with discs to 96 μm across, spires up to 64 μm high; plates irregularly rectangular (up to 144x128 μm) to narrowly oval (184x80 μm), plates formed around thick central rod, with large perforations centrally with angular edges and smaller perforations marginally with angular edges, and bluntly spinous marginal edge; reticulate spinous spherical body at apex of papilla, 320 μm wide. Ventral body wall with tables only, tables similar in form to dorsal ones, but often smaller, discs to 48 μm wide only, spire to 32 μm high only. Tube feet with endplates, support plates, support rods; endplates irregularly oval, up to 600 μm long, of complex form, partly single-layered plate with small perforations or mesh-like, partly with incomplete mesh-like secondary layering; support plates more elongated and more finely perforate than in papillae, up to 200 μm long; support rods rare, thick, curved, with some thick spines on outer edge, up to 120 μm long. Body wall around anus with tables and rods; tables as dorsally, but many larger, disc to 80 μm wide, spire 48 μm long; rods rare, thick, bent, with rugose spinous surface, up to 552 μm long. Tentacles with rods, reduced tables; rods thick to thin, rarely with terminal perforations, rarely branching, with thick spines, up to 652 μm long; tables irregular, mostly lacking a spire, discs 48–80 μm wide, spire up to 24 μm long if present, disc with 4–18 perforations, disc variably with bluntly spinous margin. Stone canal/madreporite with massed irregular rods, some branched, some branches anastomosing to form perforations, some with irregularly perforated mesh.

Tentacle ampullae, polian vesicles, gonad tubules, respiratory trees, longitudinal muscles, circular muscles, wall of cloaca and cuvierian organ devoid of ossicles.

Colour. Colour (live): background colour grey dorsally and dorsolaterally, yellowish laterally, and off-white ventrally. Dorsal and lateral tubercles white “wart-like” flat papillae cones with green margin and small dark central spot. Body with grey-brown spots in addition to dark papillae spots. Colour (preserved): background colour grey-brown dorsally and dorsolaterally, brown to pale brown ventro-laterally and ventrally. Tubercles off-white with small dark brown or off-white central papilla. Body with scattered grey-brown spots in addition to papillae spots. Tube feet similar colour to body surface. Tentacles yellow-brown. Coelomic wall with closely paired series of radial dark spots radially, spots scattered interradially, not associated with papillae or tube feet. An exceptionally large specimen (F120438) has extensive, brown, dorso-lateral patches, and papillae not conspicuously ocellate.

Distribution. Australia, Western Australia, Abrolhos Is (29°S), to Victoria, Portland (39°S, 141°E); southern continental slope, 97–800 m.

Etymology. From the Latin *austrinus* (southern) and *bassus* (deep), referring to the unusually high latitude and deep occurrence for the genus (feminine).

Remarks. The new species is assigned to *Holothuria* Linnaeus, 1767, and provisionally referred to the subgenus *Panningothuria* Rowe, 1969, as diagnosed in Rowe (1969). Rowe (1969) erected the monotypic sub-genus *Panningothuria* for *Holothuria forskali* Delle Chiaje, 1823, the principal diagnostic character being the sparse presence in the body wall of very reduced tables only. Molecular data (discussed below) indicate that *H. (Panningothuria) austrinabassa* sp. nov. and *H. (Panningothuria) forskali* are sister species. Fully developed tables are abundant in the body wall of *H. austrinabassa* sp. nov., but reduced tables, similar to those in *H. forskali*, are present in the tentacles. Both species lack buttons and rosettes in the body wall. Rowe (1969) also noted a collar of oral papillae in *H. forskali*. An inconspicuous irregular collar is evident only in the largest of the *H. austrinabassa* specimens. It is premature to either raise *Panningothuria* to generic status or create a synonymy (discussed below).

Types were not designated for *Holothuria forskali* Delle Chiaje, 1823, and the author of the species referred to the image of an undescribed species illustrated by Forsskål (1776). Koehler (1921) stated that the two characters that distinguish *H. forskali* amongst Mediterranean species are the very dark colour and presence of a cuvierian organ, although other

Mediterranean species also have a cuvierian organ. Koehler (1921) also noted the white papillae, although not all specimens of *H. forskali* have white papillae. All three characters are true of the specimen examined here and judged to be *H. forskali* (UM HO-1854).

H. austrinabassa resembles *H. forskali* in several morphological characters, such as: maximum length of 25 cm (*H. forskali* in Koehler, 1921); well-developed tuberculated papillae dorsally and laterally; collar of inconspicuous oral papillae; single dorsal stone canal and madreporite (pers. comm. for *H. forskali* by Giomar Helena Borrero Perez); tables the only ossicles in body wall; stout cuvierian tubules. VandenSpiegel et al. (1995) noted and illustrated three-dimensional, irregularly spherical, mesh-like, "bud-supporting ossicles" for *H. forskali*. Similar ossicles are present in the papilla apices of *H. austrinabassa*. Both species occur at exceptional depths for holothuriids. Perez-Ruzafa et al. (1987) reported *H. forskali* from the Mediterranean at depths of 0–193 m, and the Canary Is at a depth of 348 m. *H. austrinabassa* has been taken as deep as 800 m. Sequence data indicate significant separation of these sister species (discussed below). Significant morphological differences also are detailed in Table 2.

Table 3. Characters distinguishing *H. (Halodeima) nigralutea* O'Loughlin sp. nov. and *H. (Halodeima) edulis* Lesson

Characters	<i>H. nigralutea</i>	<i>H. edulis</i>
Colour	Discontinuous black over yellow	Dorsal black, ventral fuschia (red); or dorsal "grey", ventral cream
Ventral black stripe	Present	Absent
Dark brown spots	Only at papillae and tube feet	Additional to papillae and tube feet
Depth	100 m, on continental slope	0–20 m (Rowe and Gates, 1995)

Table 4. Characters distinguishing *H. (Halodeima) signata* Ludwig and *H. (Halodeima) edulis* Lesson

Characters	<i>H. signata</i>	<i>H. edulis</i>
Colour	Grey brown with cream spots	Dorsal black, ventral fuschia (red); or dorsal "grey", ventral cream
Length	Mostly 5–15 cm	Mostly 10–25 cm
Tables	Narrower spire (10–15 μ m at narrowest)	Broader spire (15–20 μ m at narrowest)
Rosettes	Mostly simple (mostly 2–5 perforations)	Simple to complex (2–15+ perforations)
Habit	Cryptic in reef during day	Exposed on sand during day

Table 5. Pairwise uncorrected p-distances among specimens of *H. edulis* complex

	1	2	3	4	5	6	7	8	9
1. <i>H. nigralutea</i> N120									
2. <i>H. nigralutea</i> G255	0.002								
3. <i>H. edulis</i> brown N3	0.028	0.028							
4. <i>H. edulis</i> typical J292	0.024	0.024	0.010						
5. <i>H. edulis</i> fuschia K140	0.013	0.011	0.023	0.016					
6. <i>H. edulis</i> typical G104	0.011	0.010	0.024	0.021	0.008				
7. <i>H. edulis</i> grey J282	0.015	0.013	0.024	0.018	0.005	0.010			
8. <i>H. edulis</i> grey J296	0.013	0.011	0.023	0.016	0.003	0.008	0.005		
9. <i>H. signata</i> G50	0.062	0.060	0.058	0.058	0.058	0.057	0.058	0.055	
10. <i>H. signata</i> G55	0.066	0.065	0.066	0.063	0.063	0.062	0.063	0.060	0.023

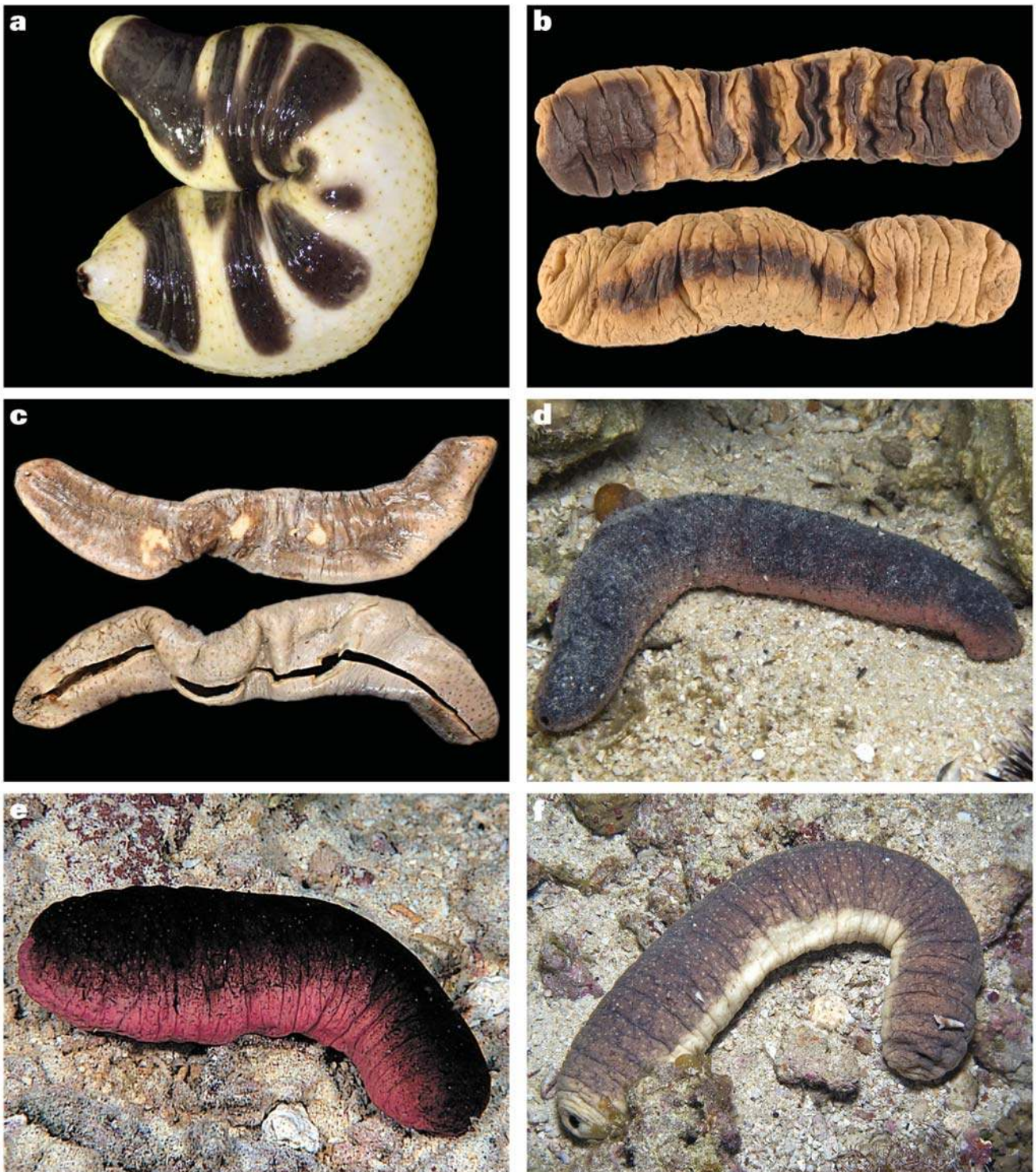


Figure 5. a, live paratype of *Holothuria (Halodeima) nigralutea* O’Loughlin sp. nov, from Western Australia, off Point Cloates (220 mm long; NMV F111290; photo by Karen Gowlett-Holmes). b, preserved holotype of *H. (Halodeima) nigralutea*, from Western Australia, off Point Cloates (148 mm long; upper dorsal, lower ventral; oral end right; NMV F120437; photos by David Staples). c, lectotype of *Holothuria edulis* Lesson, 1830 from Indonesia, Moluccan Is (160 mm long; MNHN EcHh 543; upper dorsal, lower ventral; photos by Yves Samyn). d, live *H. (Halodeima) edulis*, from Japan, Okinawa (not collected, photo by Gustav Paulay). e, live *H. (Halodeima) edulis*, from northern Australia (not collected, photo by Neville Coleman). f, live atypical “grey” form of *H. (Halodeima) edulis*, from Japan, Okinawa (UF E3882, photo by Gustav Paulay).

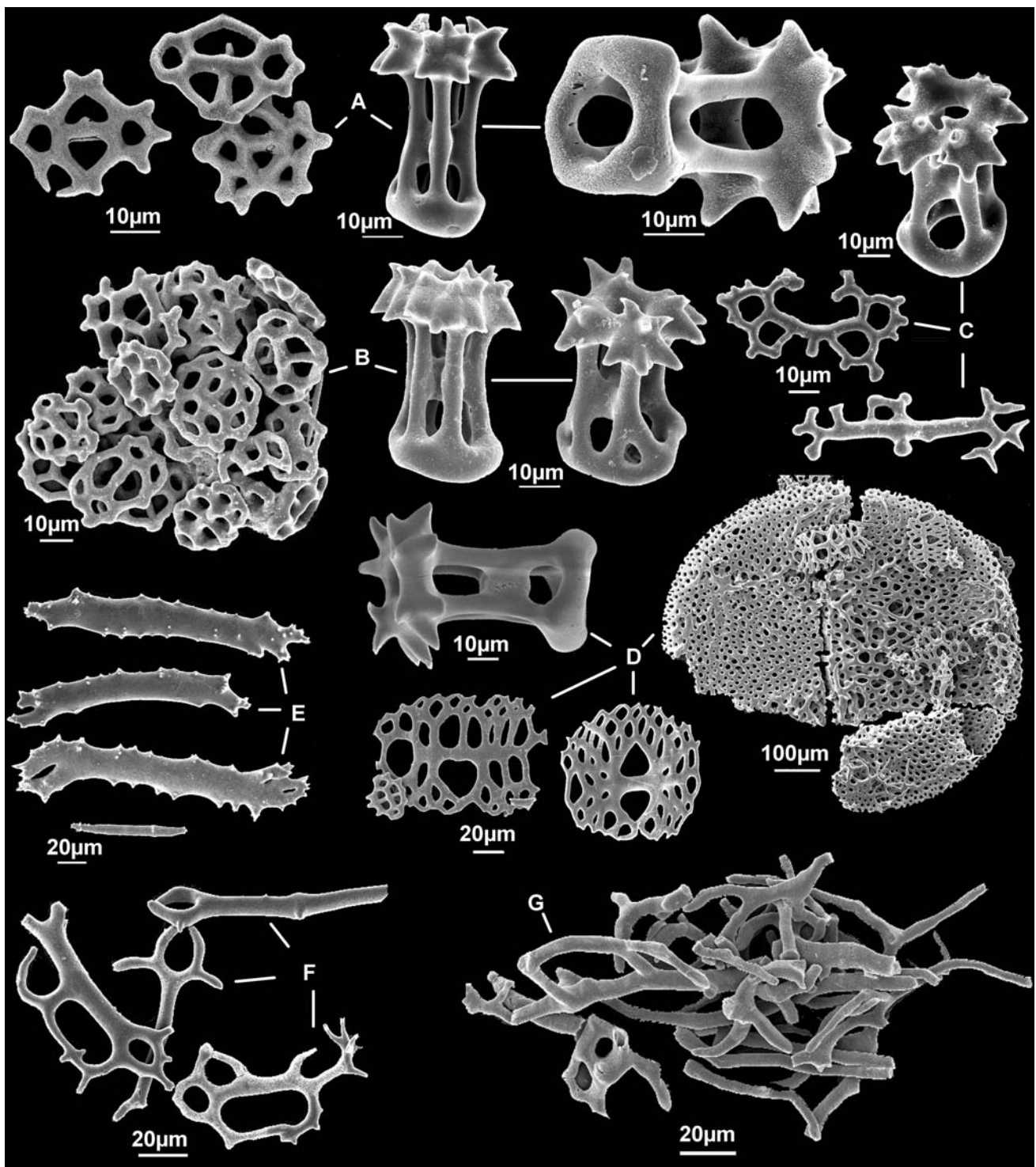


Figure 6. *Holothuria (Halodeima) nigralutea* O'Loughlin sp. nov. (SEM of ossicles from NMV F111290). A, dorsal body wall; B, ventral body wall; C, anal body wall; D, tube feet; E, tentacles; F, respiratory trees; G, madreporite.

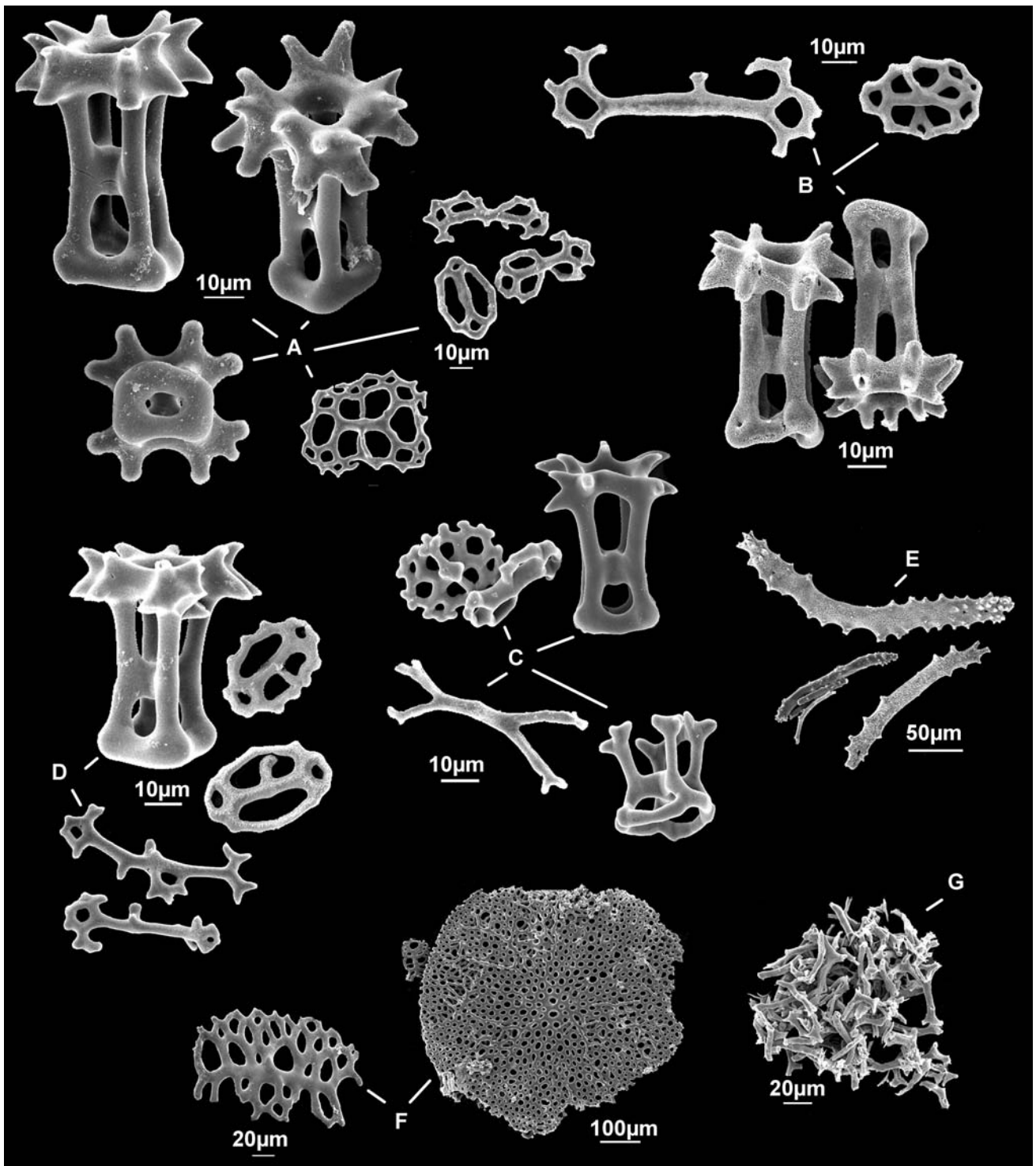


Figure 7. *Holothuria (Halodeima) edulis* Lesson, 1830 (SEM of ossicles from NMV F113599). A, dorsal body wall; B, anal body wall; C, oral body wall; D, ventral body wall; E, tentacles; F, tube feet; G, madreporite.

Holothuria (Halodeima) nigralutea O'Loughlin sp. nov.

Figures 4–7, Tables 1, 3–5.

Material examined. Holotype: Western Australia, off Point Cloates, 22.86° S, 113.51° E, 100 m, *Southern Surveyor*, SS10/2005 stn 135, 9 Dec 2005, NMV F120437.

Paratypes: Type locality and date, F111290 (1); Dampier, 95–90 m, 19.79°S, 115.47°E, SS05/2007 stn 29, 12 Jun 2007, F146582 (1).

Comparative material examined. *H. (Halodeima) atra* Jäger, 1833. Fiji, F113579 (1); New Caledonia, Noumea, F95939 (1); N Australia, Gulf of Carpentaria, E Bremer I., F112194 (1).

H. (Halodeima) edulis Lesson, 1830. Lectotype, Indonesia, Moluccan Is, Lesson and Garnot, 1825, MNHN EcHh 543; N Australia, Gulf of Carpentaria, Bremer I., F95094 (1); Great Barrier Reef, Heron I., F95093 (1); F95095 (1); F113599 (1); Pacific Ocean, Wake Atoll, UF E4670.

Description (preserved specimens). Holotype 155 mm long, up to 35 mm high, up to 40 mm wide; paratype 145 mm long, up to 30 mm high, up to 45 mm wide; body length/width ratio less than 4; oval in transverse section, not tapering from mid-body, rounded anteriorly and posteriorly; live body form short, squat, narrow anterior neck, narrow posterior tail (see photo of paratype). Body wall thick, soft-leathery, 2–5 mm thick, wrinkled, surface smooth to slightly rugose. Mouth ventral, surrounded by an irregular collar of about 60 inconspicuous papillae. Tentacles 20, peltate, with long thin tubular ampullae extending off calcareous ring plates, subequal, up to 20 mm long. Anus terminal, lacking anal teeth, with few anal papillae dorsally, with paired anal tube feet ventrally. Dorsal and lateral papillae inconspicuous in size but conspicuous in colour, flat or nipple-like, about 0.5 mm diameter, scattered irregularly, 2–10 mm apart (holotype), lacking ampullae. Tube feet scattered irregularly over ventrum, 1–5 mm apart (holotype), retracted or slightly exposed, about 0.4 mm diameter, lacking ampullae. Left dorsolateral radial plate of calcareous ring 12 mm wide and 6 mm high, with 4 anterior points, posterior margin with shallow rounded indentation. Left dorso-lateral interradiial plate 4 mm high and wide, anterior margin with spire, posterior margin with rounded indentation. Tuft of small stone canals/madreporites on each side of dorsal mesentery, extending freely in coelom, up to 25 per tuft, each up to 3 mm long, some stone canals branched. Holotype with 4 sac-like polian vesicles, up to 5 mm long, 1 branched; paratype with 4 tubular, thin, polian vesicles, 3, 8, 10, 25 mm long, 2 branched from common base. Longitudinal muscles flat, broadly attached, with narrowly free edges, up to 5 mm wide dorsally, up to 15 mm wide ventrally. Gonad tubules long, thin, multiple branching, extending half of body length. Respiratory trees extending to anterior end. Cuvierian organ absent. Gut contents calcareous detritus, with fragments up to 6 mm long.

Ossicles. Dorsal body wall with numerous rosettes, few tables; tables variable in form and size, 48–64 μm , commonly 56 μm long; disc reduced, typically 28 μm wide, smooth with a single central perforation; spire typically 20 μm wide, with 4 pillars united by a single cross-beam; crown widely spinous, typically 40 μm wide, with 16–20 large spines; rosettes plate-like, variable in form, with obtusely angular branches arising from primary rod, 24–48 μm long, frequently 2 small

terminal and 2 large, lateral perforations (frequently with transverse bridging connection), but with up to 8 perforations. Papillae with rods, some mesh-like ossicles; rods up to 160 μm long, variably bluntly spinous, curved, with some distal perforations; papilla apex with irregular small rods resembling those in madreporite, some anastomosing to form an irregular open mesh. Ventral body wall with numerous rosettes, fewer tables; tables same as dorsal; rosettes larger than dorsally, up to 10 perforations, up to 40 μm long. Tube feet with endplates, perforated plates, tables, rosettes; endplates multilayered, up to 480 μm wide; perforated plates smooth, thin, subrectangular, formed from primary rod with perpendicular lateral branches, up to 128x104 μm , typically with 2 large lateral perforations mid-rod; tables and rosettes as in ventral body wall. Oral body wall with rosettes, tables, rods; tables similar to dorsal; rosettes frequently larger than dorsal ones, up to 56 μm long; rods same as in tentacles, up to 184 μm long. Anal body wall with rosettes, few tables, some rods; tables and rosettes as in ventral body wall; rods frequently with lateral branches, branches frequently joined to form lateral and terminal perforations, rods up to 88 μm long, intergrade with rosettes. Tentacle rods up to 344 μm long, frequently curved, thick to thin, coarsely or finely spinous, spines close or sparse, rare branches, with rare, mostly terminal perforations. Stone canal/madreporite ossicles massed irregular rods, some branched, some anastomosing to create perforations, some with an irregular, perforated mesh, up to 134 μm long. Respiratory tree with numerous irregular rods, up to 160 μm long, frequently with small node in middle of rod, and with branches at ends and node, variable in length and form, some branches joined to create terminal or lateral perforations.

Ossicles absent from tentacle ampullae, polian vesicles, gonad tubules, longitudinal muscles, circular muscles, and wall of cloaca.

Colour. Live colour (paratype): black on pale yellow; with scattered, small, brown spots at papillae. Preserved colour: variable pattern of black over pale yellow; with small red-brown spots around papillae and tube feet, spots irregularly distributed all over body, such spots always associated with papillae or tube feet; interior body wall with scattered, superficial, irregular black spots, that are not associated with papillae or tube feet.

Distribution. Off Point Cloates, Western Australia; 100 m.

Etymology. From the Latin *niger* (black) and *luteus* (yellow), referring to the black and yellow live colour (feminine).

Remarks. This species is assigned to *Holothuria* Linnaeus, 1767, and provisionally referred to the subgenus *Halodeima* Pearson, 1914, as diagnosed in Rowe (1969). Samyn et al. (2005) suggested that *Halodeima* might need to be raised to generic rank, but added that “revision of Holothuriidae will depend on further comparative taxonomic studies as well as on more detailed phylogenetic analyses before any of the changes proposed can be solidified into a new classification”. This work is progressing, and it remains premature to raise *Halodeima* to generic status or erect a new genus (see below).

The type species of *Halodeima* Pearson, 1914 is *Holothuria atra* Jäger, 1833 (by original designation). Rowe (1969) considered the following species to constitute *Halodeima*: *H. chilensis* Semper, 1868; *H. edulis* Lesson, 1830; *H. floridana* Pourtalés, 1851; *H. grisea* Selenka, 1867; *Stichopus kefersteini* Selenka, 1867; *H. mexicana* Ludwig, 1875; *H. pulla* Selenka, 1867; *Halodeima stocki* Cherbonnier, 1964. Pawson (1978) added *H. manningi*. Samyn (2003), Pawson (1995) and Paulay (1989, 2003) also listed *H. signata* Ludwig, 1875 as a valid species of *Halodeima*.

Molecular data indicate that *H. (Halodeima) nigralutea* is most closely related to *H. (Halodeima) edulis* Lesson (see below), and the morphology of these species is closely similar. Distinguishing characters are listed in Table 3. In describing his new species Lesson (1830) referred principally to its widespread commercial use, but he noted: cylindrical rounded

thin slightly rugose sinuous form; ventral cover of irregularly distributed papillae; upper body deep sooty black colour; under body and sides pleasant red colour, speckled with black spots. Cherbonnier (1951) gave a more detailed description and illustrated the ossicles of the lectotype of *H. edulis*. He noted it had 6 polian vesicles, ranging in size from large to very small. The specimens of *H. edulis* examined in this study are in accord with these features. Féral and Cherbonnier (1986) illustrated live colour (p. 82 only).

Both specimens of *H. nigralutea* have ossicles in the respiratory trees. No ossicles were encountered in the respiratory tree of the lectotype of *H. edulis*. Ossicles were noted in only 1 of 7 specimens of *H. edulis* from northern Australia (NMV F95095), as they were in a specimen from Wake Atoll (UF E4670) (GP). Presence or absence of respiratory tree ossicles in *H. edulis* appears to be a variable character.



Figure 8. a, preserved lectotype of *Holothuria signata* Ludwig, 1875 from Tahiti, French Polynesia (100 mm long in Ludwig, 1875; UH E2638; photo by Peter Stiewe). b, live *H. (Halodeima) signata*, from Moorea, French Polynesia (UF E4986; photo by Gustav Paulay). c, close-up of preserved lectotype of *H. signata* (UH E2638). d, close-up of live *H. (Halodeima) signata* (UF E4986).

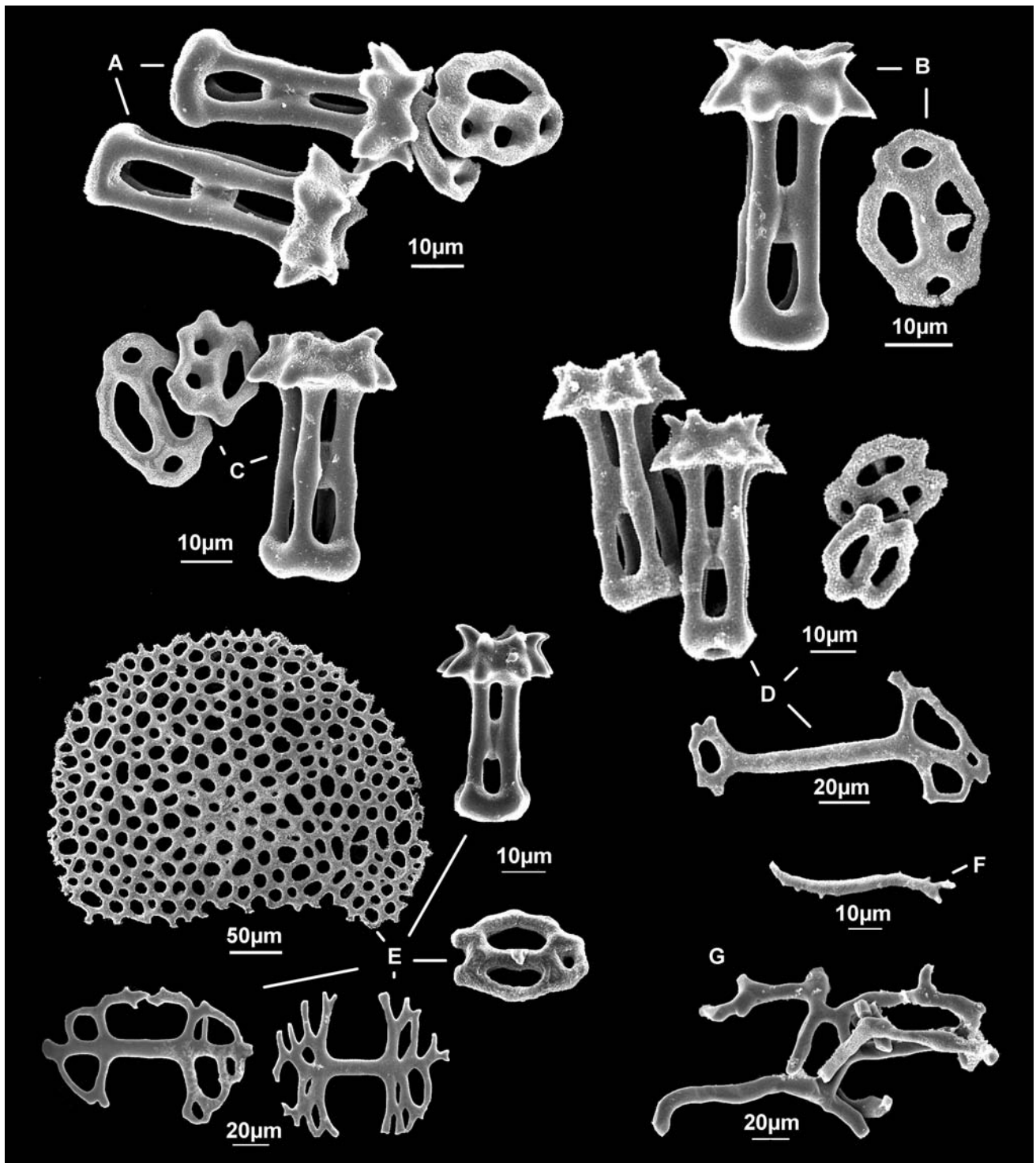


Figure 9. *Holothuria (Halodeima) signata* Ludwig, 1875 (SEM of ossicles from UF 173). A, dorsal body wall; B, ventral body wall; C, anal body wall; D, papillae; E, tube feet; F, tentacles; G, madreporite.

Holothuria (Halodeima) signata Ludwig, 1875

Figures 4, 8, 9, Tables 1, 4, 5.

Holothuria signata Ludwig, 1875: 99, pl. V7, fig. 36.—Lampert, 1885: 64.—Théel, 1886: 222–223.—Ludwig, 1889–92: 330.—Lampert, 1896: 53.—Ekman, 1926: 438, fig. D1.

Holothuria edulis.—Ludwig, 1899: 559–560.—Domantay, 1933: 63 (part, *H. signata* treated as a junior synonym of *H. edulis*).

Holothuria (Halodeima) signata.—Paulay, 1989: 10, 27.—Paulay, 2003: 577.—Pawson, 1995: 188.—Samyn 2003: 35.

Holothuria sp. (?) *signata*.—Erhardt and Baensch, 1998: 1088.

Material examined. Lectotype (UH E2638 here designated): Pacific Ocean, Tahiti, UH E2638.

Other material. Mariana Is, Guam I., Asan, reef slope, in crevice on sand, night, 22 Jul 1992, RBINS IG30817; Orote Peninsula, south side, under rubble, 15–25 m, 22 Aug 1994, UF E173; Piti Bombholes, reef flat, moat, or lagoon, 5–10 m, 19 Jul 2003, UF E4713; Saipan I., outside Managaha Survey Site, forereef, under rubble, 8–12 m, 5 Jan 2003, UF E3447; Tinian I., Unai Babui, forereef, under rocks on sand, 15 m, 12 May 2005, UF E4640.

Niue I., off Alofi wharf, outer reef slope, on reef rock, 14 m, 20 May 1986, UF E1333; Namukulu, Limu Reef flat, pools, undersides of rocks, 0–5 m, 7 Oct 1991, UF E1406; reef flat at Tuapa, <10 m from shore, 27 Aug 1986, UF E1886; off Alofi wharf, outer reef slope, 12–15 m, 9 Mar 1986, UF E1663.

Cook Is, Rarotonga I., Nikao, outer fringing reef, exposed in shallow pools, 20 May 1984, USNM E37966; Mauke I., Taunganui Harbor, 15–20 m, 3 Dec 1984, USNM E37968; Mauke I., Taunganui Harbor, 12 Dec 1984, UF E1831.

Society Is, Tahiti I., Tautira, in coral rubble zone, under dead coral blocks, 0.5–1.5 m, 3 Sep 1984, UF E4999; Moorea I., NE corner of Moorea, Aroa, 200–300 m E of channel, 6–15 m, 7 May 2006, UF E5015; Moorea I., barrier reef between Cook's and Opunohu Bays (Vaipahu), outer part of barrier reef, within 1–60m of reef crest, 0–2 m, 3 Jul 2006, UF E4986.

Tuamotu Is, Rangiroa Atoll, off Hotua Ura Motu, ca. 1 km W. of Avatoru Pass, outer reef slope, under rocks, 15–21 m, 10 Oct 2001, UF E325; Rangiroa Atoll, ca. 1 km S of NW point of atoll, off Motu Maeherehona, outer reef slope, under rocks, 6–12 m, 10 Nov 2001, UF E329; Rangiroa Atoll, ca. 2 km S of NW point of atoll, at southernmost storm lock zone, off Motu Maeherehona, outer reef slope, exposed, 3–12 m, 26 Oct 2001, UF E591.

Pitcairn Is, Henderson I., outer reef slope off North Beach, 15 m, 17 May 1987, USNM E50251; outer reef slope off Northwest Beach, 10–14 m, 15 May 1987, USNM E50252; Oeno Atoll, lagoon near south shore of main island, 2.5 m, 28 May 1987, USNM E50253; Pitcairn I., 8–20 m, May 1987, USNM E50254.

Description (anatomy based on UF E173 only). Cylindrical, >5 times as long as wide (14.5 cm x 2.5 cm in UF E173), with rounded anterior and posterior; anus terminal, mouth ventral; body wall smooth, with velvety texture provided by dense layer of table crowns arranged right beneath the surface, 0.5–2 mm thick, thicker dorsally than ventrally. Interior of body wall off-white, with conspicuous, large, scattered, black spots that do not positionally correspond to location of tube feet. Ventral and dorsal tube feet in rough rows, but spread out, all small, not elevated on tubercles. Pedicels with well developed terminal disc; dorsal tube feet also with terminal discs, but narrow, reduced. 2 stone canals and madreporites on left side, 4 on right side; single, ampulliform, 11 mm long, polian vesicle ventrally. Gonad on left side. Ring canal 9 mm posterior to calcareous

ring, 18–21 peltate oral tentacles (UF E173 - 21, UF E325 - 18). No specialized perianal tube feet. No cuvierian tubules. Longitudinal muscles narrow, bifid, attached only medially, with broad free margins.

Dorsal body wall with abundant tables and sparser rosettes. Tables with well developed crown of maltese cross with double ring of 8 spines typical of species group; spire elongate, comprised of 4 pillars joined at ends and by mid-level cross beam; base of table with smooth knob, lacking disk. Tables 1.75+/-0.10 times as long as wide, 51.5+/-2.3 um (N=10, range: 47.5–55 um) long, 29.5+/-2.7 um (N=10, range: 25–35 um) in diameter (at crown). Rosettes usually simple, with two parallel perforations, one of these subdivided in some, with 1 or 2 additional, terminal perforations developed in some, rarely more complex. Respiratory tree with abundant, thin, spiny rods. Longitudinal muscles, circular muscles, polian vesicles, and tentacle ampullae without ossicles.

Colour. In life: greyish-brown, somewhat lighter ventrally than dorsally; with small, round, cream to light tan spots surrounding each pedicel and papilla, both dorsally and ventrally. Tentacles yellowish to cream. Pedicels light tan basally, like the spot from which they arise, rapidly darkening to black-brown terminally, but with light tan terminal disc. Papillae same.

Distribution. Oceania, at least from the Mariana Is and Niue I. in the west, to the Pitcairn Is in the east (Paulay, 1989, 2003, herein).

Remarks. *Holothuria signata* was relegated into the synonymy of *H. edulis* soon after its description. Ludwig himself later (1899) considered his species synonymous with *H. edulis*, based in part on Lampert's (1896) suggestion that they may be conspecific. Most literature records subsequent to the original description (such as Lampert, 1896) are secondary citations, or records of specimens that, on the basis of their description, are referable to *Holothuria edulis*. The only records of additional specimens of *H. signata* are Paulay's (1989, 2003) records from the Pitcairn and Mariana Is, although little information was provided in those papers, and Erhardt and Baensch's (1998) record.

During the preparation of this paper, we re-examined the description and, remotely, the type specimen of *H. signata* and were able to confirm its identity, as well as its distinctiveness from *H. edulis*. Ludwig (1875) clearly describes the unusual and unique colour pattern of this species, a pattern that, albeit faded, is still discernible in the lectotype today. Ludwig also illustrates the body wall tables, which are distinctly narrower than those of *H. edulis*. Finally, the identity of the species is also suggested on biogeographical grounds. Only two species of *Holothuria (Halodeima)* are known from French Polynesia, the type locality of *H. signata*: *H. signata* and the quite different *H. atra*. As far as we know *H. edulis* does not reach this area. One of us (GP) has studied the holothurians of French Polynesia on several occasions over the past 25 years, including a 2-month survey in 2006 of Moorea I. (just 17 km from Tahiti), and has never seen *H. edulis* in the area. In contrast *H. signata* is fairly common there.

Holothuria signata is a relatively small species that conceals itself during the day within the reef matrix (including under rocks), emerging at night to feed on the reef surface. Its

habit thus contrasts markedly with that of *H. edulis*, a day-active, exposed animal that prefers pockets of soft sediments within the reef, often in a lagoonal setting. The 2 species are immediately distinguishable on colour pattern, the shape of table ossicles, as well as genetically. *Holothuria signata* also does not grow as large as *H. edulis*.

Discussion

The discovery of these two new holothuriid species is noteworthy for several reasons. It shows that holothuriids are better represented at moderately high latitudes and in deep water than heretofore suspected. Rowe and Gates (1995) reported numerous holothuriid species in the Tasman Sea as far south as Lord Howe I. (32°S), *Holothuria integra* Koehler and Vaney as far south on the east coast of Australia as Botany Bay (34°S), *Actinopyga echinites* (Jaeger) and *H. atra* Jäger as far south on the west coast of Australia as Fremantle (32°S), and *H. hartmeyeri* Erwe as far south as Port Lincoln on the South Australia coast (35°S). Ludwig (1898) (see also Samyn and Massin, 2003 for a redescription) described *H. platei* from the Juan Fernandez Is (33°S). Marsh and Pawson (1993) reported *H. cinerascens* (Brandt), *H. arenicola* Semper and *H. macropersona* H.L. Clark from Western Australia, Rottneest I. (32°S). Similarly on the east coast of Africa several holothuriids have been reported at high latitudes. For instance Deichmann (1948) reported *H. parva* from Port Edward (31°S) and *H. cinerascens* from Umtwalumi (31°S). Samyn (2003, dataset as annex in Samyn and Tallon, 2005) gives accurate distribution maps of the species reported in the Western Indian Ocean. *H. austrinabassa* occurs as far as 39°S. Rowe and Gates (1995) reported the deepest occurrence of a holothuriid in Australasian waters as *H. uncia* Rowe at Norfolk I. in the Tasman Sea at 342–360 m. *H. austrinabassa* occurs to a depth of 800 m.

Ongoing investigation into the phylogenetic relationships of holothuriid sea cucumbers (Paulay and others, unpublished), now covering more than 100 species in the family, identifies *H. (Panningothuria) forskali* as the closest sequenced relative of *H. (Panningothuria) austrinabassa*, and *H. (Halodeima) edulis* as the closest sequenced relative of *H. (Halodeima) nigralutea*. Both relationships are well supported (100/1.0 parsimony bootstrap and Bayesian posterior probability). The evolutionary origins of the two new species described here are markedly different: *H. austrinabassa* represents an old lineage, the only other known member of which is the northernmost holothuriid *H. forskali*. In contrast, *H. nigralutea* is a very recent offshoot of the shallow, tropical *H. edulis* complex.

Holothuria forskali, the type and only species of *Holothuria (Panningothuria)* Rowe, 1969, and *H. austrinabassa*, together form an isolated, deep branch in the family, suggesting that *Panningothuria* may warrant generic recognition. However, additional sampling and analysis are necessary to resolve the deep branching order in the Holothuriidae, before we are prepared to revise the genus level classification of the family. The two species differ at 13% of base pairs in the sequenced portion of 16S-COI, a level of differentiation typical of widely divergent sister species in this family. The

relationship of these two species is intriguing, as they are the northernmost and southernmost species of Holothuriidae, demonstrating extreme temperate, cool water invasion, and bipolar distribution and dispersal. Holothuriids are predominantly tropical, shallow water forms, and only a handful of species invade warm temperate environments. *Holothuria forskali* reaches by far the highest latitude among holothuriids, extending to at least 57°N (Global Biodiversity Information Facility <gbif.org>) in the northeast Atlantic. *Holothuria austrinabassa* is known south to 39°S. It is also unusual in occupying the only known deep, cold water habitat. It thus represents the southernmost, and most cold-tolerant southern hemisphere holothuriid.

Additional morphological and genetic work is needed to resolve whether *Halodeima* is monophyletic. Our preliminary work indicates that *Halodeima* clusters with the subgenera *Vaneyothuria*, *Holothuria*, *Selenkothuria*, *Semperothuria*, and some *Thymiosycia*. Three well supported clades of *Halodeima* are recognizable based on sequence data: *H. atra*, *H. mexicana-floridana-grisea-kefersteini*, and *H. signata-edulis-nigralutea* (fig. 4). In addition to their unusual ossicle complement (reduced discs on tables, and rosettes), most investigated *Halodeima* spp. (*nigralutea*, *edulis*, *atra*, *signata*, *floridana* (Edwards, 1908, with illustration), and *mexicana* (Hyman, 1955, with illustration)) have multiple madreporites, providing further morphological evidence of their potential relationships. On the other hand, the tables in the *H. signata-edulis-nigralutea* clade have a single central disc perforation, while the tables in *H. atra* (see Rowe, 1969) and *H. floridana-grisea-mexicana* (see Hendlar et al., 1995) have additional disc perforations.

H. (Halodeima) nigralutea is morphologically and genetically closest to *H. (Halodeima) edulis*. Species in the clade *signata-edulis-nigralutea* are very similar genetically, as well as morphologically, with maximum 16S-COI sequence divergence of 5.5–6.5 % between the basal *H. signata* and other forms (Table 5). While *H. signata* is clearly differentiated, specimens assigned to *H. edulis* and *H. nigralutea* show very limited divergence and a more complex pattern (fig. 4). Thus three forms are reciprocally monophyletic based on the sequence data on hand: *H. edulis* from the Pacific basin (Philippines, Okinawa, Guam), *H. edulis* from the Indian Ocean (J292, N3, Oman and Cocos Keeling), and *H. nigralutea*, with *H. nigralutea* sister to the Pacific *edulis* clade. Thus this species complex appears to have undergone rapid, recent differentiation into three forms: *H. edulis* in the Western Pacific, *H. edulis* in the Indian Ocean, and *H. nigralutea*. While Pacific and Indian Ocean populations of *H. edulis* look similar, *H. nigralutea* has a distinct colour pattern and also differs in other details (see above). Similar rapid speciation has also been documented within the teatfish complex *Holothuria (Microthele)* by Uthicke et al. (2004).

Several colour variants are represented among the sequenced *H. edulis* specimens. G104 and J292 represent typical forms, with a dark dorsum and a fuschia venter. K140 is a specimen that is uniformly fuschia, without the dark dorsum. Although no live colour information is available for it, N3 is represented by a specimen that has a distinctive colour

in pickle: brown both dorsally and ventrally, and tan laterally. While the above represent rare colour variants, a fairly common colour form often assigned to *H. edulis* was also sequenced. This grey form (J282, J296; fig. 5f), also illustrated in Féral and Cherbonnier (1986), is known to us from New Caledonia (Féral and Cherbonnier, 1986), Okinawa and Mauke (Cook Is) (GP), and Nauru (Alex Kerr, pers. comm.). It differs from typical *H. edulis* in its greyer colour, lacking the fuschia pattern of the latter, dark transverse creases, and habit of hiding in the reef matrix during the day (at least in Okinawa, Mauke, and New Caledonia (P. Laboute, pers. comm.)). Although we expected this form to represent a distinct species, there are no fixed nucleotide differences discernible within the sequenced 16S-CO1 region, between it and typical Western Pacific *H. edulis*. Determining the status of this form will require further work, but it may be an ecomorph of *H. edulis*. Potentially the fuschia colour present in typical *H. edulis* could be due to a UV-photo-protective pigment that may be restricted to animals that live exposed to the sun, and is not developed in individuals living in cryptic habitats. The other colour morphs mentioned above were also genetically undifferentiated from typically-coloured individuals of *H. edulis* (fig. 4).

The contrasting evolutionary histories of these two high-latitude holothurians in Australia have close parallels in other invertebrates, most notably in cypraeid gastropods (cowries) (Meyer, 2003). Southern and western Australia are home to a large number of endemics, including endemic cowries. These include radiations of *Umbilia*, *Zoila*, and *Notocypraea*; all old, divergent cowrie genera that must have evolved the ability to live at high latitudes some time ago. The last is sister to *Cypraeovula*, a temperate genus in South Africa, showing biogeographic disjunction within the temperate zone. Extinct Japanese *Zoila* indicate this genus had a bipolar distribution in the past. In contrast the cowrie genus *Cribarula* has given rise to a series of subtropical western Australian endemic forms rapidly and in succession, much like the origin of *H. nigralutea*.

These and other invertebrates show that tropical species can rapidly give rise to western Australian subtropical and temperate endemics, but also that other cool-water elements of this region have specialized to high latitudes a long time ago.

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