Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2018) **84**: 1–11. doi:10.1093/mollus/eyx037
Advance Access publication date: 12 October 2017

The first helicoid sea slug: 3D microanatomy of *Helicohedyle dikiki* n. gen., n. sp. (Panpulmonata: Acochlidiida) from Guam

Konstantina Drainas[1], Clayton H. Carlson[2], Katharina M. Jörger[1], Michael Schrödl[1,3] and Timea P. Neusser[1]

[1]*LMU Munich, Biocenter, Department Biology II, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany;*
[2]*Marine Laboratory, University of Guam, Mangilao, GU 96923, USA; and*
[3]*SNSB, Bavarian State Collection of Zoology, Münchhausenstr. 21, 81247 München, Germany and GeoBio-Center at LMU, Richard-Wagner-Str. 10, 80333 München, Germany*

Correspondence: T.P. Neusser; e-mail: neusser@bio.lmu.de

(Received 19 January 2017; editorial decision 11 August 2017)

ABSTRACT

Large parts of the discovered diversity of sea slugs are still undescribed, despite their potential contribution to a better understanding of the evolution of the group. Here, we describe an enigmatic tiny sea slug that was discovered in the 1970s on subtidal reef flats on Guam (Mariana Islands, Indo-West Pacific). The slug presents a long, coiled visceral sac, unique within the highly diverse body plans of sea slugs, which are usually characterized by different degrees of external body symmetry, linked to the reduction of the shell. Its initial assignment to the order Acochlidiida is confirmed by our 3D-microanatomical study based on a histological section series. *Helicohedyle dikiki* n. gen., n. sp. shows a number of putative plesiomorphic characters such as a simple excretory system—including a sac-like kidney combined with a short nephroduct—and an androdiaulic, hermaphroditic reproductive system, which bears an unarmed copulatory organ. Based on external morphology and microanatomical data it can be assigned to the Hedylopsacea and shows affinities both with marine and brackish Pseudunelidae, and also with limnic Tantulidae. We discuss the different potential scenarios of the evolution of a helicoid visceral sac as a secondary adaptation to the benthic marine lifestyle *vs* its interpretation as a plesiomorphic character from a coiled and shell-bearing ancestor, predating the transition to the interstices of sandy habitats associated with (progenetic) modifications in body plans.

INTRODUCTION

The tropical Indo-West Pacific harbours the highest diversity of marine life, with the 'Coral Triangle' in the Indo-Malayan region recognized as the world's marine diversity hotspot (e.g., Hoeksema, 2007; Carpenter *et al.*, 2011). The dimensions and location of this hotspot varies slightly between marine clades (Kay, 1995; Hoeksema, 2007; Carpenter *et al.*, 2011). Nevertheless, there is a general trend for decreasing species diversity eastwards, so that the oceanic Pacific Islands house an impoverished Indo-Malayan marine fauna combined with variable proportions of endemics (Kay, 1995; Hoeksema, 2007; Carpenter *et al.*, 2011). The island of Guam is the southernmost island of the volcanic Mariana Islands, located east of the Philippines and northeast of Palau. Guam and the Marianas have the best documented marine biotas in Micronesia and, despite their isolated location east of the 'Coral Triangle', the Marianas marine fauna currently comprises over 5,600 recorded species, outnumbering any other area of comparable size in species diversity (Paulay, 2003). Taxon-based comparison between Indo-West Pacific islands indicates, however, that this leading position in overall species diversity of Guam is likely due to the comparatively lower sampling effort in the diversity hotspot of the 'Coral Triangle' (Paulay, 2003). Unfortunately, due to a lack of exploratory and descriptive work, there is still too little data for an evaluation of regional endemism.

Based on the most recent overall species counts of heterobranch sea slugs in the Indo-West Pacific, there are currently 3,000 species recorded, with large percentages of up to 56% of the regional sea slug fauna undescribed (Gosliner, Valdés & Behrens, 2015). In Guam and the Marianas, Carlson & Hoff (2003) listed 485 species of heterobranch sea slugs and, despite a series of descriptions (Marcus, 1965; Carlson & Hoff, 1974, 1993; Hoff & Carlson, 1990; Avila *et al.*, 1998), nearly 50% of the discovered Marianas heterobranch fauna still remains undescribed (Gosliner & Draheim, 1996; Carlson & Hoff, 2003). New lineages need to be at least briefly described, because only named and published species really exist for scientific purposes, i.e. are included in biodiversity assessments and conservation approaches (Jörger *et al.*, 2012; Jörger & Schrödl, 2013). Some new findings, however, are excitingly different from anything else; these are new lineages suggesting a special evolutionary history and merit a more profound investigation and a 'deep description' (Schrödl, 2016).

Remarkable among the heterobranch discoveries from Guam was the finding of a sand-dwelling helicoid slug, which was preliminary assigned to the order Acochlidiida and whose photograph

© *The Author 2017. Published by Oxford University Press on behalf of The Malacological Society of London, all rights reserved.*
For Permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

was posted on the 'Sea Slug Forum' nearly 20 years ago (Carlson & Hoff, 1998, 2003). The Acochlidiida are a comparatively small clade of Panpulmonata, which encompass an extraordinary morphological and ecological diversity. While most acochlidians are minute and inhabit the marine mesopsammon (Schrödl & Neusser, 2010; Jörger *et al.*, 2010), this clade also includes one recently discovered benthic species in the deep-sea (Neusser *et al.*, 2016), one brackish species (Neusser & Schrödl, 2009), several lineages of slugs that have invaded freshwater systems (Wawra, 1974, 1979, 1980; Haynes & Kenchington, 1991; Neusser & Schrödl, 2007; Brenzinger *et al.*, 2011a, 2011b) or even colonized land (Swennen & Buatip, 2009; Neusser *et al.*, 2011a, 2015; Kano *et al.*, 2015). All known aquatic acochlidian species are characterized by the subdivision into a head-foot complex and a shell-less, short, elongated, free and straight visceral sac, inspiring Strubell's (1892) original generic name *Acochlidium*. Recently discovered (semi-) terrestrial Aitengidae deviate from this typical acochlidian bauplan, but their compact body was interpreted as a secondary adaption to the amphibious lifestyle, achieving better stability and a smaller body surface (Neusser *et al.*, 2011a). The acochlidian discovered in Guam appears to be a unique lineage in this already highly diverse slug clade, because it is the first representative with a very long and coiled visceral sac. To our knowledge it is, moreover, the only sea slug with a coiled visceral sac; usually the loss of the shell is combined with various degrees of detorsion and, typically, with the formation of a compact slug shape (e.g. Gosliner, 1994; Wägele *et al.*, 2008).

In the present study we investigate this enigmatic and externally unique slug (Carlson & Hoff, 1998, 2003) using modern 3D-imaging techniques. Based on a series of histological semithin sections, we prepared 3D reconstructions (Amira®) of all organ systems and describe this novel lineage in microanatomical detail. Preliminary results were presented at the World Congress of Malacology 2016 (Drainas *et al.*, 2016). Based on our microanatomical data, we discuss the phylogenetic affinities of the novel lineage and different scenarios for the evolution of the first helicoid acochlidian.

MATERIAL AND METHODS

Sampling and embedding

Originally, five specimens were collected from two localities from the subtidal reef flat and shallow-water sandy areas in southwestern Guam between 1972 and 1981 by C.H. Carlson. Three specimens are unfortunately lost, but two specimens were available for detailed analyses (Table 1). They were documented by photographs while alive and subsequently relaxed in $MgCl_2$ solution, prior to fixation in 70% ethanol. Recently, specimen no. 5 (see Table 1) was decalcified in Bouin's solution overnight and dehydrated in a graded series of acetone in distilled water (30, 50, 70, 3 ×100%). This specimen was embedded in Spurr's low viscosity resin (Spurr, 1969).

Sectioning and 3D reconstruction with Amira®

We prepared a complete series of ribboned semithin sections (thickness 1.5 µm) using a RCM MT 700 ultramicrotome with a diamond knife (Histo Jumbo, Diatome, Biel, Switzerland) and contact cement at the lower cutting edge, according to Ruthensteiner (2008). The sections were stained with methylene blue-azure II (Richardson, Jarett & Finke, 1960) and are deposited at the State Collection of Zoology, Munich (ZSM Mol 20160232). The microscope slides were scanned using an Olympus® dotSlide microscope with 20× magnification and saved as .vsi files. Digital images of every section were prepared using the image-viewer software OlyVia®. Images were converted to 8-bit greyscale format, then contrast-enhanced and unsharp-masked with standard image-editing software. All organ systems were three-dimensionally reconstructed with Amira®, largely following Ruthensteiner (2008).

Molecular data

Unfortunately, the specimen no. 4 (see Table 1) was dried up. We nevertheless attempted to generate molecular data and extracted DNA with the NucleoSpin Tissue Kit (Macherey and Nagel), following the manufacturer's protocol and the elution procedure for high DNA yield. We tried to amplify mitochondrial cytochrome *c* oxidase subunit I (COI) and 16 S rRNA genes, as well as nuclear 28 S rRNA and 18 S rRNA, using a variety of primers and protocols established for Acochlidiida and related heterobranch gastropods (see e.g. Klussmann-Kolb *et al.*, 2008; Jörger *et al.*, 2010, 2014a). Unfortunately, we obtained no or only contaminated PCR products for all markers, thus it is likely that the DNA is too degenerated for direct PCR-based approaches.

SYSTEMATIC DESCRIPTION

Heterobranchia *sensu* Haszprunar, 1985
Panpulmonata *sensu* Jörger *et al.*, 2010
Acochlidiida *sensu* Bouchet *et al.*, in press
Hedylopsacea *sensu* Wawra, 1987
Family incertae sedis

***Helicohedyle* new genus**

Type species: *Helicohedyle dikiki* n. sp., here designated.

ZooBank registration: urn:lsid:zoobank.org:act: F3800B4F-D45E-43B7-9329-A9A1421DBEBF

Table 1. Collection data and body length of living specimens of *Helicohedyle dikiki* n. sp. from Guam.

No	Locality	Collection date	GPS data	Body length (mm)	Status of specimen	ZSM registration no.
1	Anae Island	12 April 1972	13°21.429′N 144°38.290′E	5.5	Lost	
2	Anae Island	15 April 1972	13°21.429′N 144°38.290′E	5.5	Deteriorated in the fridge after sampling	
3	Anae Island	16 April 1972	13°21.429′N 144°38.290′E	5.5	Lost	
4	Bile Bay	8 May 1977	13°16.487′N 144°39.763′E	4.0	DNA extraction	ZSM Mol 20170072
5	Bile Bay	1 June 1981	13°16.487′N 144°39.763′E	6.0	Section series	Holotype ZSM Mol 20160232

Abbreviation: ZSM, Bavarian State Collection of Zoology.

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

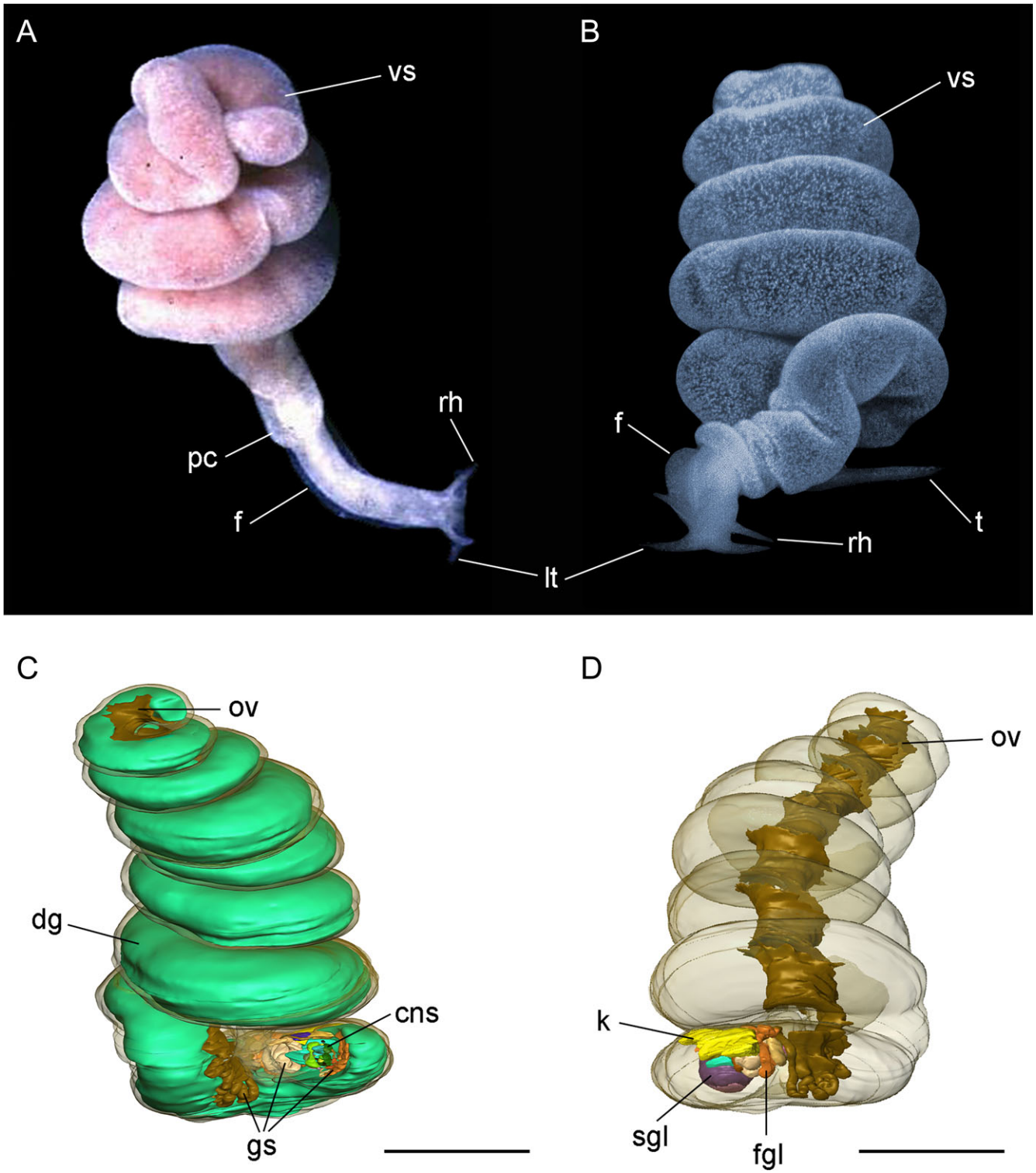

Figure 1. A, B. Living specimens of *Helicohedyle dikiki* n. sp. **A.** Anae Island, Guam; with pinkish-brown digestive gland; length 5.5 mm. **B.** Holotype; Bile Bay, Guam; with grayish digestive gland; length 6 mm. **C, D.** 3D reconstructions of holotype. **C.** Voluminous digestive gland. **D.** Ovotestis and kidney. Abbreviations: cns, central nervous system; dg, digestive gland; f, foot; fgl, female gland; gs, genital system; k, kidney; lt, labial tentacle; ov, ovotestis; pc, pericardial swelling; rh, rhinophore; sgl, salivary gland; t, tail; vs, visceral sack. Scale bars: **C, D** = 1 mm.

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

Etymology: From Latin, *helica* referring to coiled snail shells, and *Hedyle*, one of the first genus names among Acochlidiida.

Diagnosis: Marine sand-dwelling hedylopsacean Acochlidiida with two pairs of cephalic tentacles of equal length, foot slightly broader than body, unpigmented eyes, unarmed copulatory organs, simple sac-like kidney and a unique, highly coiled visceral sac.

Helicohedyle dikiki *new species*

(Figs 1–6)

Type material: Holotype (ZSM Mol 20160232, a series of semithin sections), Bile Bay, southwestern Guam, Mariana Islands

ZooBank registration: urn:lsid:zoobank.org:act: 3766A856-C4BE-44B3-9794-4C22279BBE38

Etymology: The specific epithet *dikiki* refers to the word 'small' or 'minute' in the Chamorro language of the native inhabitants of Guam.

Diagnosis: as for genus.

External morphology and integument: Body divided into anterior head-foot complex and posterior long, highly coiled, visceral sac in which head can be (partially) retracted (Fig. 1B). Head-foot complex with broad connection to free visceral sac. Length of resting and crawling slugs 3.5–6 mm, respectively, measuring from head to tip of visceral sac/end of coiled visceral sac. Body coloration translucent with pinkish-brown (Fig. 1A) or greyish (Fig. 1B) digestive gland showing through epidermis in specimens from Anae Island and Bile Bay, respectively. Head with two pairs of cephalic tentacles of same length. Labial tentacles slightly flattened, broad at base, pointed. Solid rhinophores digitiform, pointed, curved and held forward in crawling specimens. Eyes not visible externally. Anterior part of foot slightly broader than head (Fig. 1A). Tail (i.e. free posterior foot) long, tapered (Fig. 1B). Gills and mantle cavity absent. Epidermis highly glandular (Fig. 1B). Pericardial swelling visible externally (Fig. 1A). Subepidermal calcareous spicules present in head-foot complex and anterior of visceral sac. Visceral sac with thin, subepidermal muscle fibres ventrally.

Central nervous system (CNS): CNS euthyneurous and prepharyngeal with paired cerebral, optic, rhinophoral, pedal, pleural, buccal and gastro-oesophageal ganglia and three distinct ganglia plus presumed osphradial ganglion on visceral loop (Figs 2, 3B). Accessory ganglia absent. Ganglia identified and named according to pentaganglionate hypothesis of Haszprunar (1985) and in agreement with recent studies of acochlidians (see e.g. Neusser & Schrödl, 2007; Neusser, Martynov & Schrödl, 2009b). Size of ganglia given as approximate diameter.

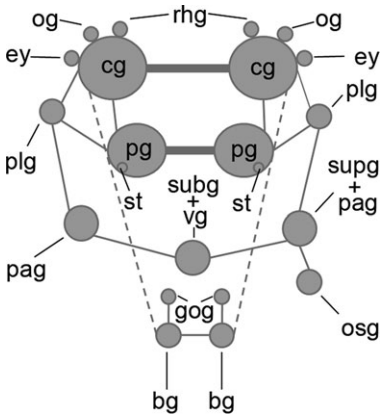

Figure 2. Schematic drawing of the central nervous system of *Helicohedyle dikiki* n. sp. (dorsal view). Abbreviations: bg, buccal ganglion; cg, cerebral ganglion; ey, eye; gog, gastro-oesophageal ganglion; og, optic ganglion; osg, osphradial ganglion; pag, parietal ganglion; pg, pedal ganglion; plg, pleural ganglion; rhg, rhinophoral ganglion; st, statocyst; subg, subintestinal ganglion; supg, supraintestinal ganglion; vg, visceral ganglion. Not to scale.

Prepharyngeal nerve ring formed by cerebral, pleural and pedal ganglia (Figs 2, 3B–D, 4B). Large cerebral ganglia (91 µm) connected through thick cerebral commissure (Figs 2, 3C, D). Small rhinophoral ganglia (Figs 2, 3C) (37 µm) attached to cerebral ganglia anteriorly. Just posteriorly, small optic ganglia nestled on cerebral ganglia (39 µm) (Figs 2, 3B, C). Small, unpigmented eye (Figs 2, 3C, F) (35 µm) posterior to optic ganglia. Rhinophoral and optic ganglia and eye closely attached laterally to cerebral ganglia (Fig. 3C). Rhinophoral, labial tentacular and optic nerves and Hancock's organ not detected due to withdrawn head of examined specimen and resulting compressed state of nervous tissue. Pedal ganglia (91 µm) connected through thick, short commissure; parapedal commissure not detected (Figs 2, 3C). Statocysts attached posteriorly to pedal ganglia (Figs 2, 3D, E). Pleural ganglia (48 µm) connected to visceral loop ganglia (Figs 2, 3D, E, 4B, C), i.e. left parietal ganglion (52 µm), fused subintestinal/visceral ganglion (85 µm), fused supraintestinal/right parietal ganglion (72 µm); presumed unpaired osphradial ganglion (Figs 2, 3D, 4C) (42 µm) connected to last-named. Buccal ganglia (44 µm) (Figs 2, 3B, D, F) linked by short commissure ventral to oesophagus. Cerebrobuccal connectives not detected. Small gastro-oesophageal ganglia (Figs 2, 3B, D) (30 µm) connected to buccal ganglia.

Digestive system: Mouth opening between labial tentacles. Oral tube short (Fig. 3A, B), unciliated, leading to muscular pharynx (Figs 3A, B, E, F). Radula J-shaped and *c.* 100 µm long (Fig. 3A). Radula formula not determined from histological sections. Gizzard and jaws lacking. Large, paired salivary glands (Figs 3A, E, F, 4B, C), secretory cells with dark blue-stained granules. Salivary glands discharging via paired, short salivary gland ducts into posterior pharynx. Proximal salivary gland ducts developed as muscular salivary pumps (Fig. 3A, F). Oesophagus long, ciliated (Fig. 3A, B, E), emerging posterodorsally from pharynx. Stomach (Fig. 3A) developed as anterior lobe of digestive gland, separated only by deep groove; histologically as for digestive gland. Highly coiled and voluminous holohepatic digestive gland without diverticula (Figs 1C, 4D). Digestive gland dorsal to ovotestis (Fig. 1C). Intestine short, densely ciliated (Figs 3A, 4B, C). Anus (Fig. 4B) opens ventrolaterally on right side, slightly anterior to, but separated from, nephropore.

Circulatory and excretory systems: Circulatory and excretory systems positioned on right side of body (Fig. 1D). No heart could be detected. Thin-walled pericardium nearly collapsed (Fig. 4A, C). Renopericardial duct (Fig. 4A, C) highly ciliated connecting pericardium with anterior of kidney. Kidney sac-like (Figs 1D, 4A), epithelial cells appear vacuolized histologically (Fig. 4C, D). Nephroduct short (Fig. 4A, B). Nephropore (Fig. 4B) opens ventrolaterallly on right side, just posterior to, but separated from, anus.

Reproductive system: Reproductive system hermaphroditic and androdiaulic (Fig. 5). Ovotestis sac-like (Figs 5, 6A) with follicles, positioned ventrally to digestive gland. No mature oocytes developed. Preampullary gonoduct (Fig. 6C) leading to tube-like ampulla (Fig. 6C, E) filled with autosperm. No receptaculum seminis developed at least at this ontogenetic stage. Different tube-like female glands (Fig. 6C, E) not distinguished histologically, stained light blue over entire length. Hermaphroditic duct glandular, dividing into vas deferens (Fig. 6C, E) and female distal gonoduct (Figs 4B, C, 5, 6C). Female gonopore (Fig. 5) opening ventrally on right side, considerably anterior to anus. Large bursa copulatrix (Figs 4B, C, 5, 6C) connected to female gonoduct via bursa stalk. Vas deferens leading anteriorly on right side of body to male copulatory organs

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

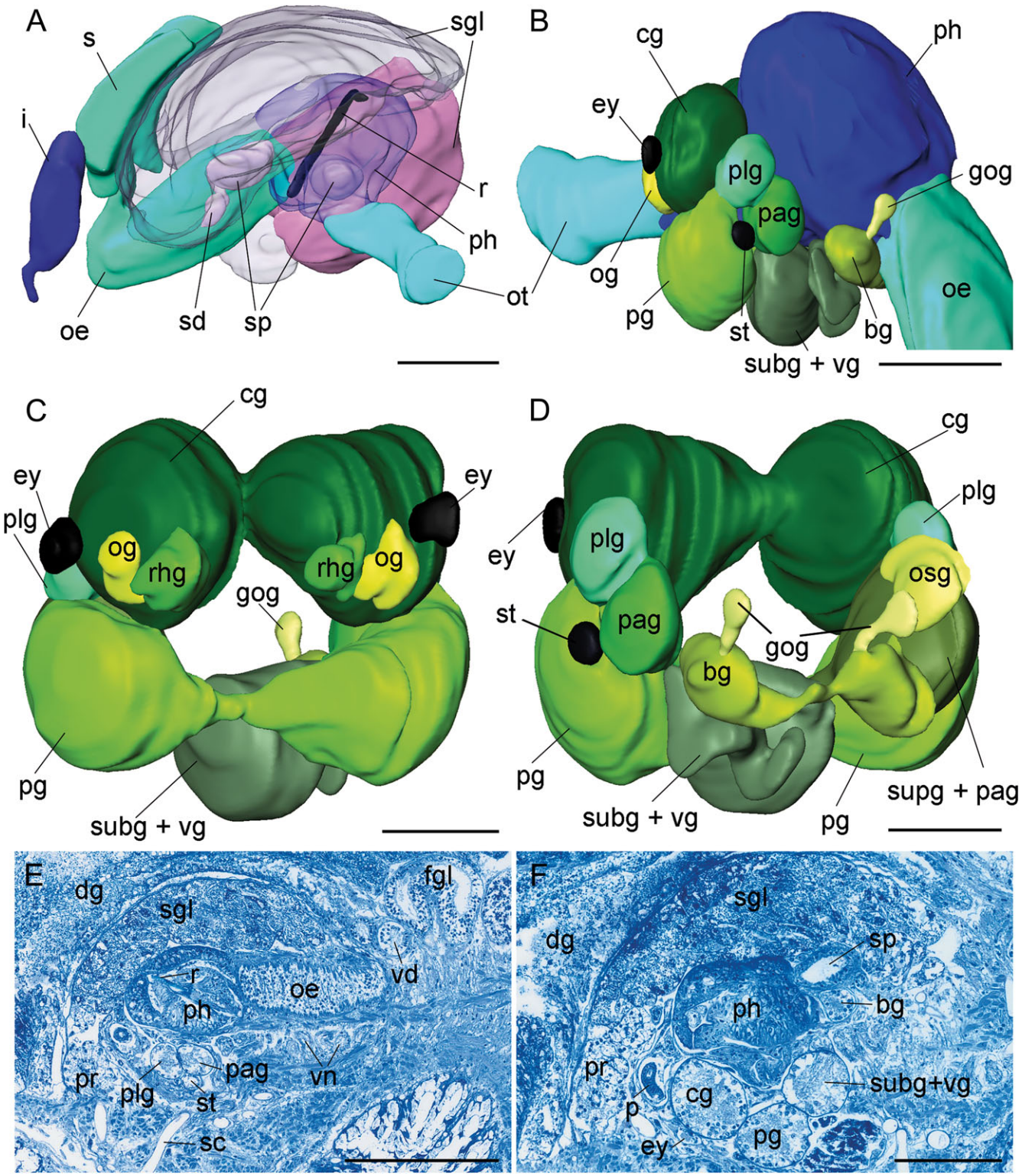

Figure 3. 3D reconstructions **(A–D)** and histological sections **(E, F)** of the digestive system and central nervous system (CNS) of *Helicohedyle dikiki* n. sp. **A.** Anterior digestive system, digestive gland omitted (right view). **B.** Prepharyngeal CNS (left view). **C.** CNS (anterior view). **D.** Visceral loop ganglia (posterior view). **E.** Radula and oesophagus. **F.** Salivary pump and ganglia. Abbreviations: bg, buccal ganglion; cg, cerebral ganglion; dg, digestive gland; ey, eye; fgl, female gland; gog, gastro-oesophageal ganglion; i, intestine; oe, oesophagus; og, optic ganglion; osg, osphradial ganglion; ot, oral tube; p, penis; pag, parietal ganglion; pg, pedal ganglion; ph, pharynx; plg, pleural ganglion; pr, prostate; r, radula; rhg, rhinophoral ganglion; s, stomach; sc, spicule cavity; sd, salivary gland duct; sgl, salivary gland; sp, salivary pump; st, statocyst; subg, subintestinal ganglion; supg, supraintestinal ganglion; vd, vas deferens; vg, visceral ganglion; vn, visceral nerve. Scale bars: **A, F** = 100 μm; **B** = 75 μm; **C, D** = 50 μm; **E** = 40 μm.

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

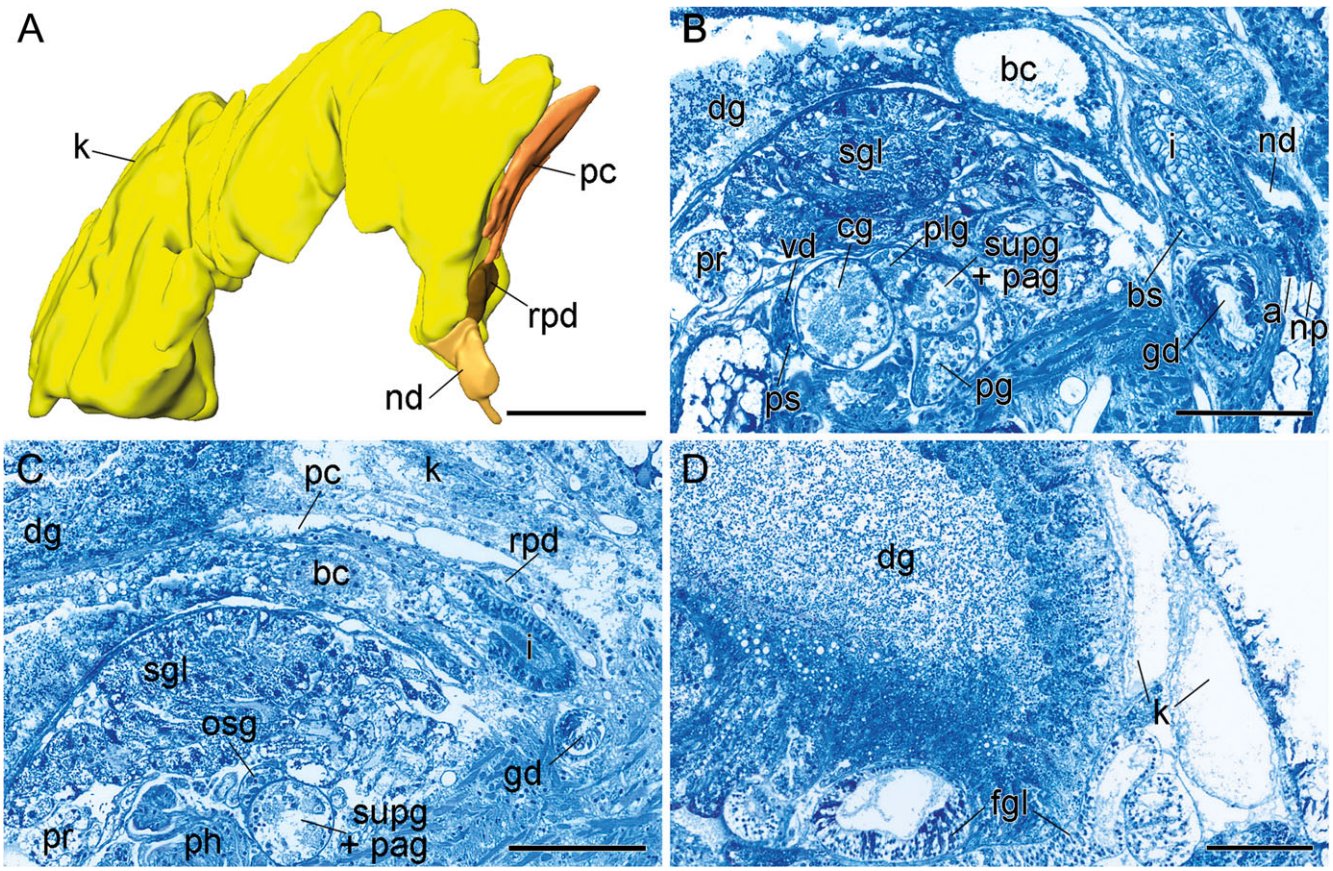

Figure 4. 3D reconstruction **(A)** and histological sections **(B–D)** of the circulatory and excretory systems of *Helicohedyle dikiki* n. sp. **A.** Right view. **B.** Nephroduct and nephropore. **C.** Pericardium and renopericardioduct. **D.** Kidney. Abbreviations: a, anus; bc, bursa copulatrix; bs, bursa stalk; cg, cerebral ganglion; dg, digestive gland; fgl, female gland; gd, gonoduct; i, intestine; k, kidney; nd, nephroduct; np, nephropore; osg, osphradial ganglion; pag, parietal ganglion; pc, pericardium; pg, pedal ganglion; pgl, pleural ganglion; ph, pharynx; pr, prostate; ps, penial sheath; rpd, renopericardioduct; sgl, salivary gland; supg, supraintestinal ganglion; vd, vas deferens. Scale bars: **A** = 150 μm; **B** = 50 μm; **C, D** = 100 μm.

(Figs 5, 6B). Vas deferens not traced over entire length. Posterior-leading vas deferens closely attached to penial sheath (Figs 4B, 5, 6B). Prostate tube-like (Figs 5, 6B), highly glandular (Figs 3E, F, 6D). Ejaculatory duct (Figs 5, 6B, D) connecting to muscular penis (Figs 3F, 5, 6B, D) surrounded by thin-walled penial sheath (Figs 5, 6B, D). Penis simple, without cuticular stylets or thorns. Muscular basal swelling at base of penis and second paraprostatic glandular system absent. Male gonopore (Fig. 5) opening posterior to right rhinophore.

Distribution and habitat: Known from type locality and Anae Island (Table 1). Sandy patches of reef flat.

DISCUSSION

Phylogenetic placement of Helicohedyle

Intuitively, this novel sea slug was assigned to the heterobranch order Acochlidiida based on external characters, despite its unique, coiled visceral hump (Carlson & Hoff, 1998, 2003). The subdivision of the body into head-foot complex and separated visceral hump and the ability to retract the head-foot complex at least partially into the latter has been discussed as a potential apomorphy of Acochlidiida (Schrödl & Neusser, 2010), while modifications in limnic and terrestrial lineages have been interpreted as secondary adaptations to these novel environments (e.g., Brenzinger *et al.*, 2011a, b; Neusser *et al.*, 2011a). *Helicohedyle* shows this typical body subdivision and direct observations indicate that animals can at least partially retract into the visceral hump. Furthermore, our microanatomical study reveals additional characters which, though not unique to, are nevertheless typical for marine Acochlidiida, such as the presence of calcareous subepidermal spicules, the reduction of the mantle cavity and the presence of solid rhinophores innervated by the rhinophoral nerve only (Sommerfeldt & Schrödl, 2005; Schrödl & Neusser, 2010).

Initially, the enigmatic slug was classified as '*Microhedyle* sp.' (Carlson & Hoff, 1998) and thus considered part of the Microhedylacea. Our data however, suggest that it is related to the other major acochlidian lineage, the Hedylopsacea *sensu* Wawra (1987), because of the presence of male anterior copulatory organs (Schrödl & Neusser, 2010), which are absent in all Microhedylacea (Neusser *et al.*, 2006, 2009b; Jörger *et al.*, 2008, 2009, 2014a). Additionally, *Helicohedyle* lacks accessory ganglia, which are absent in Hedylopsacea (except *Tantulum elegans*) and typically present in Microhedylacea. Hedylopsacea comprise marine mesopsammic Hedylopsidae (Wawra, 1989; Sommerfeldt & Schrödl, 2005) and Pseudunelidae (Challis, 1970; Neusser, Heß & Schrödl, 2009a; Neusser, Jörger & Schrödl, 2011b), deep-sea benthic Bathyhedylidae (Neusser *et al.*, 2016), limnic interstitial Tantulidae (Rankin, 1979; Neusser & Schrödl, 2007), limnic benthic Acochlidiidae (Wawra, 1979, 1980; Haynes & Kenchington, 1991; Brenzinger *et al.*, 2011a, b) and (semi)terrestrial Aitengidae (Swennen & Buatip, 2009; Neusser *et al.*, 2011a; Kano *et al.*, 2015). Externally, *Helicohedyle dikiki* resembles most closely members of the marine Pseudunelidae by its relatively small body size

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

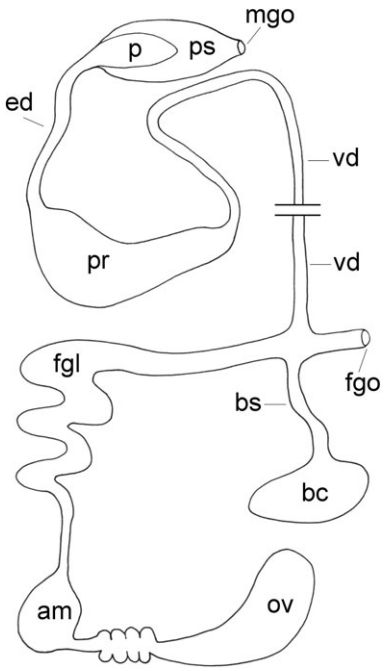

Figure 5. Schematic drawing of the reproductive system of *Helicohedyle dikiki* n. sp. Abbreviations: am, ampulla; bc, bursa copulatrix; bs, bursa stalk; ed, ejaculatory duct; fgl, female gland; fgo, female gonopore; mgo, male gonopore; ov, ovotestis; p, penis; pr, prostate; ps, penial sheath; vd, vas deferens. Not to scale.

and a clearly visible pericardial swelling (Table 2). The more or less digitiform head tentacles have the same shape in Pseudunelidae and Tantulidae, but differ in length, whereas the cephalic tentacles are of the same length in *H. dikiki* and the rhinophores are shorter than the labial tentacles in Pseudunelidae (Neusser & Schrödl, 2009; Neusser *et al.*, 2009a, 2011b) and Tantulidae (Rankin, 1979). In contrast, the labial tentacles of members of marine *Hedylopsis* are flattened, rounded lobes (Sommerfeldt & Schrödl, 2005).

The internal microanatomy of *H. dikiki* does not entirely resemble that of Pseudunelidae; instead there is a mosaic of features shared with marine and brackish Pseudunelidae and with freshwater Tantulidae (Table 2). *Helicohedyle* shares the marine habitat, the absence of accessory ganglia, the presence of three visceral loop ganglia and the short nephroduct with *Pseudunela*. On the other hand, the unpigmented eyes, the presence of salivary pumps, the stomach separated by a groove and the unarmed reproductive system suggest an affinity of *Helicohedyle* with *Tantulum*. The most eye-catching and unique external characteristic of *H. dikiki* is the very long and highly coiled visceral sac (Table 2) with thin ventral muscle fibres. This is usually considerably shorter and simply elongated in all aquatic, mesopsammic as well as benthic, acochlidians. In the brackish *Pseudunela espiritusanta* the elongated visceral sac is bent forward in living specimens, but never coiled or forming whorls (Neusser & Schrödl, 2009).

A cladistic analysis based on morphological characters (data on *H. dikiki* added to character set of Schrödl & Neusser, 2010) did not reveal the family affiliation of the present species. With the present state of anatomical knowledge, *Helicohedyle* is a new hedylopsacean taxon with a completely new shape of the visceral sac, which does not fit within any known family- or genus-level acochlidian lineage; this justifies the erection of a new species and new genus. Future molecular analyses and additional morphological investigation of the mature female reproductive system and ultrastructure of the radula will reveal if the new species should be assigned to one of the existing acochlidian families or represents a novel evolutionary lineage. Until new material is available, *H. dikiki* is provisionally considered *incertae sedis*, but with affinity to Pseudunelidae and Tantulidae.

Interpretation of the evolution of a helicoid acochlidian

In the absence of a phylogenetic tree, we can only hypothesize the relationships of the new genus *Helicohedyle* based on the available microanatomical data and speculative evolutionary scenarios that need to be tested once molecular and additional morphological data are available. Based on current knowledge, there are two evolutionary scenarios that could account for a helicoid acochlidian. *Helicohedyle dikiki* was collected from coarse sand samples and, in the absence of direct behavioural observations, we conclude from the external morphology and microanatomical characters that this species is sand-dwelling and not truly mesopsammic, i.e. it does not inhabit the interstitial spaces between sand grains. *Helicohedyle dikiki* exceeds the typical size of interstitial organisms and shows none of the adaptations considered as characteristic for mesopsammic slugs, i.e. worm-shaped body, adhesive glands or modifications in the reproductive system towards rapid modes of sperm transfer via hypodermic injection and spermatophores (Swedmark, 1968; Jörger *et al.*, 2014b). Observations of living specimens showed no tendency towards unwinding the large and clumsy coiled visceral sac and we found no indication of a distinct musculature in the coiled visceral hump that could allow for complex movements. Thus, we consider it unlikely that *H. dikiki* extends into a worm-like shape in the interstitial spaces and only coils up occasionally.

In the first evolutionary scenario, *Helicohedyle* originated from a common mesopsammic acochlidian ancestor with Pseudunelidae and Tantulidae, and secondarily inhabited benthic sandy habitats. The helicoid visceral sac of *Helicohedyle* is then interpreted as a derived character—whether achieved by 'reactivation' of a nonexpressed ancestral condition, or as a novel secondary coiling, possibly achieved late in development. Switching to a benthic lifestyle, the coiled visceral sac might be advantageous for stability and movement, or as strategy against benthic predators, minimizing visible length and surface, in contrast to an elongated worm-shape. The new species does rather resemble a faecal coil, which may be biologically relevant as a kind of mimesis. The coiled visceral sac of *Helicohedyle* encloses a highly voluminous digestive system and provides space for comparably enlarged gonads. It might thus be related to the acquisition of a different food source requiring an enlarged digestive gland and/ or indicate a high reproductive output. So far, histological sections of the digestive gland do not allow for inferences on the food source and collection of specimens in the mature female phase is needed to measure egg sizes and count the number of eggs to evaluate reproductive output and larval type.

In the second evolutionary scenario, the coiled visceral sac of *Helicohedyle* is a plesiomorphic character retained from a shelled and coiled panpulmonate ancestor. Most established families among Acochlidiida are well supported monophyla in current molecular phylogenetic hypotheses, but the basal interrelationships between hedylopsacean clades are still poorly resolved and the discovery and inclusion of novel evolutionary lineages might change previous phylogenetic hypotheses (Jörger *et al.*, 2014a). The origin of Acochlidiida among Panpulmonata was ambiguous (Jörger *et al.*, 2010, 2014a; Schrödl, 2014), but recent phylogenomic analyses by Teasdale (2017) recovered Acochlidiida as sister to a combined clade of limnic Hygrophila and largely terrestrial (but also marine or limnic) Eupulmonata. While details await future analyses, it appears that Acochlidiida evolved from aquatic, benthic snails which bore a helicoid shell with an accordingly coiled visceral hump. Thus, the evolution of Acochlidiida involved reduction of the shell and the transformation of a coiled visceral hump towards a short and straight one, which has usually been

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

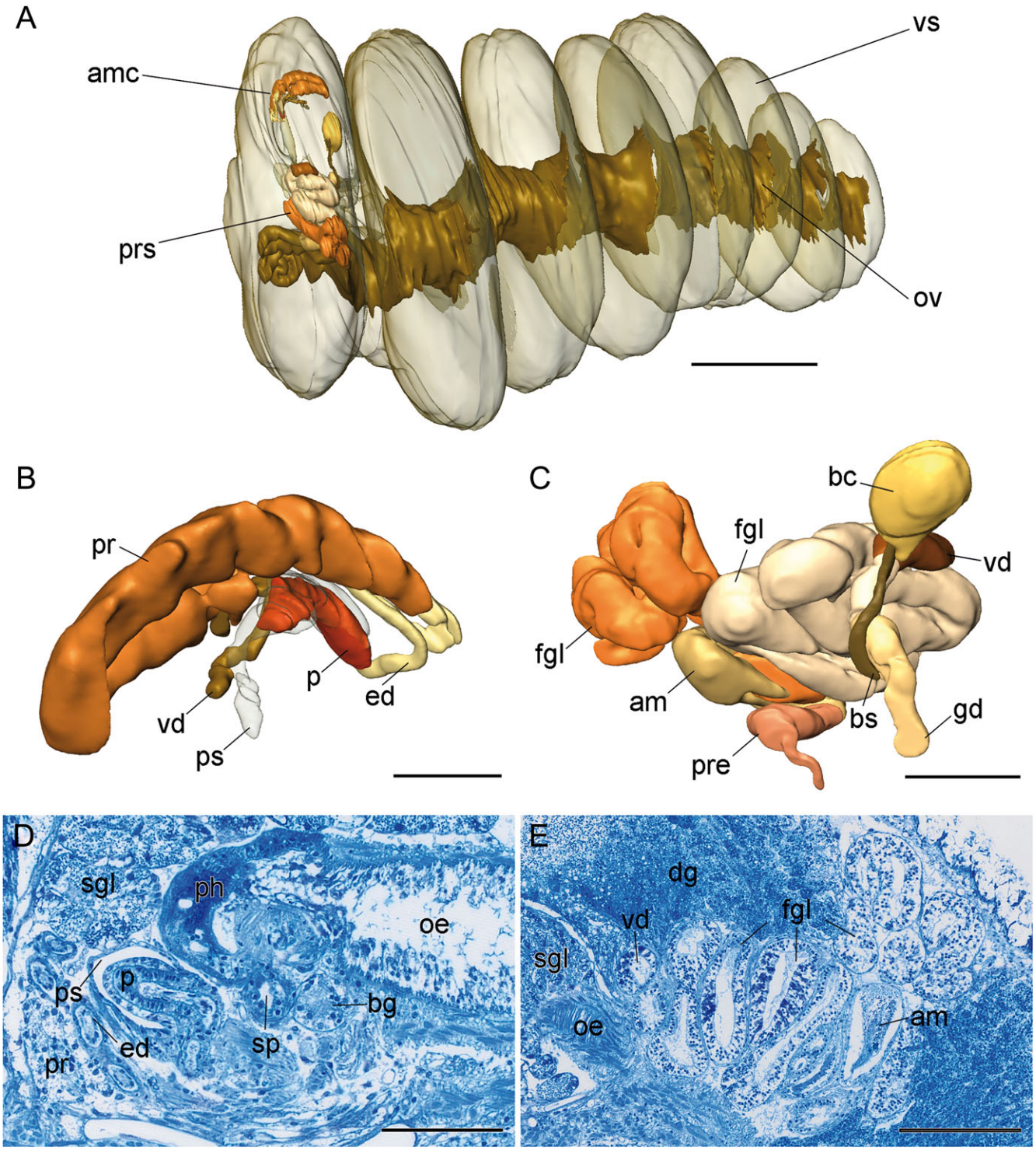

Figure 6. 3D reconstructions **(A–C)** and histological sections **(D, E)** of the reproductive system of *Helicohedyle dikiki* n. sp. **A.** Position of the reproductive system (lateral view). **B.** Anterior male copulatory organs (ventral view). **C.** Posterior reproductive system without ovotestis (ventral view). **D.** Penis and prostate. **E.** Ampulla and female gland. Abbreviations: am, ampulla; amc, anterior male copulatory organs; bc, bursa copulatrix; bg, buccal ganglion; bs, bursa stalk; dg, digestive gland; ed, ejaculatory duct; fgl, female gland; gd, gonoduct; oe, oesophagus; ov, ovotestis; p, penis; ph, pharynx; pr, prostate; pre, preampullary duct; prs, posterior reproductive system; ps, penial sheath; sgl, salivary gland; sp, salivary gland pump; vd, vas deferens; vs, visceral sac. Scale bars: **A** = 500 µm; **B, D** = 75 µm; **C, E** = 150 µm.

interpreted as a result of progenesis correlated with the invasion of the supposedly ancestral interstitial habitat (Jörger *et al.*, 2010, 2014a). However, *Helicohedyle* exhibits a series of likely plesiomorphic characters, i.e. a simple sac-like kidney with short nephroduct and an unarmed copulatory organ; with its coiled visceral sac and arguably benthic lifestyle it might thus represent a basal acochlidian offshoot prior to the invasion of the interstitial environment. According to this scenario, an early juvenile could have lost its coiled shell and

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

Table 2. Comparison of external and internal anatomical characteristics of *Helicohedyle dikiki* n. sp. with species of *Pseudunela* and *Tantulum*.

	Helicohedyle dikiki	*Pseudunela cornuta* Challis, 1970	*Pseudunela viatoris* Neusser *et al.*, 2011b	*Pseudunela marteli* Neusser *et al.*, 2011b	*Pseudunela espiritusanta* Neusser & Schrödl, 2009	*Tantulum elegans* Rankin, 1979
Type locality	Bile Bay, Guam	Guadalcanal, Solomon Is	Viti Levu, Fiji	Guadalcanal, Solomon Is	Espiritu Santo, Vanuatu	St Vincent I.
Data source	this study	Neusser *et al.* (2009a)	Neusser *et al.* (2011b)	Neusser *et al.* (2011b)	Neusser & Schrödl (2009)	Rankin (1979), Neusser & Schrödl (2007)
Habitat	Marine	Marine	Marine	Marine	Brackish	Freshwater
Body size (mm)	3.5–6	3	3	3	9	2.5
Cephalic tentacles	**Of same length**	rh shorter than lt	rh shorter than lt	rh shorter than lt	rh shorter than lt	rh shorter than lt
Eyes visible externally	No	No	No, weakly	Well, weakly	Well	No
Foot width	**Slightly broader than body**	As broad as body	As broad as body	As broad as body	Broader than body	As broad as body
Visceral hump	**Very long, highly coiled**	Elongated	Elongated	Elongated	Bent, recurved	Elongated
Pericardial swelling/heart bulb visible	Yes	Yes	Yes	Yes	Yes	No
Subepidermal calcareous spicules	In head-foot, anterior vh	Few in vh	In cephalic tentacles, foot and vh	In cephalic tentacles, foot, vh	Bean-shaped; in cephalic tentacles, foot, vh, around CNS	Around CNS
Accessory ganglia	–	–	–	–	–	+
Origin of optic nerve	?	Rhinophoral nerve	Optic ganglion	Optic ganglion	Optic ganglion	Optic ganglion
Eye pigment	–	–	–, +	+	+	–
Eye diameter (μm)	20	20	30–35	30–35	45	20
Hancock's organ	?	?	+	+	+	+
Common opening of digestive and excretory system	–	–	+	+	–	–
Salivary pumps	+	–	–	–	–	+
Stomach anterior lobe of dg	+	–	–	–	+	+
Heart	?	Atrium and ventricle	Ventricle	Ventricle	Ventricle	Atrium and ventricle
Kidney	Sac-like	Long, internally divided	Long, internally divided	Long, internally divided	Long, internally divided	Long, internally divided
Nephroduct	Short	Long, looped	Short	Short	Long, looped	Long, looped
Hollow curved penial stylet (μm)	–	+	+	+	+	–
Penis associated structure	–	–	–	–	–	+
Basal finger with stylet	–	+	+	+	+	–
Paraprostate	–	+	+	+	+	–

Abbreviations: CNS, central nervous system; dg, digestive gland; lt, labial tentacle; rh, rhinophore; vh, visceral hump; +, present; –, absent; ?, no data available. Characters in bold are unique for *H. dikiki*.

then developed a coiled and hypertrophied visceral hump, which was later reduced in mesopsammic acochlidians.

Conclusion and outlook

Additional specimens allowing for molecular analyses and placement within a phylogenetic framework are needed to confirm or reject our hypotheses on the evolution of this uniquely helicoid sea slug. During the evolution of heterobranch gastropods the shell has been reduced various times independently, usually accompanied by different degrees of decoiling (Gosliner, 1994; Wägele *et al.*, 2008). As a putative secondary development, the coiled visceral sac of *Helicohedyle* represents yet another example of the flexibility of a slug's body plan or—if plesiomorphic—a 'missing link' on the evolutionary pathway from coiled panpulmonate snails to their uncoiled slug descendants.

Our study shows how even shallow and easily accessible habitats in the Indo-Pacific still harbour novel genus- or family-level

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

lineages which might play a key role in our understanding of the evolution of certain taxa and their morphologies. This highlights the urgent need to describe formally the already discovered diversity to make the taxa accessible to further scientific study. Extraordinarily exciting species which require deep species descriptions are of course just the tip of the iceberg of all undescribed species, especially in the Indo-Pacific hotspot of marine biodiversity; funding of systematics, streamlining of description, and recognizing the merit of modern, integrative α-taxonomy is a crucial challenge—not only for malacology.

ACKNOWLEDGEMENTS

We thank the organizing team of the World Congress of Malacology 2016 in Malaysia and are grateful to Kathe Jensen (Denmark) for organizing the symposium 'Integrating molecules, morphology, biology and biogeography in "opisthobranch" research', at which this paper was first presented. The authors are grateful to two anonymous referees and to Guest Editor Kathe Jensen for improving the manuscript. This project was funded by a 'Lehre@LMU-award for student research projects' to KD and by the 'Award for equal opportunities for women in research/Ludwig-Maximilians-University' to TPN.

REFERENCES

AVILA, C., BALLESTEROS, M., SLATTERY, M., STARMER, J. & PAUL, V.J. 1998. *Phyllodesmium guamensis* (Nudibranchia: Aeolidoidea), a new species from Guam (Micronesia). *Journal of Molluscan Studies*, **64**: 147–160.

BOUCHET, P., ROCROI, J.-P., HAUSDORF, B., KAIM, A., KANO, Y., NÜTZEL, A., PARKHAEV, P., SCHRÖDL, M. & STRONG, E. E. in press. *Revised classification, nomenclator and typification of gastropod and monoplacophoran families*. *Malacologia*.

BRENZINGER, B., NEUSSER, T.P., GLAUBRECHT, M., HASZPRUNAR, G. & SCHRÖDL, M. 2011a. Redescription and three-dimensional reconstruction of the limnic acochlidian gastropod *Strubellia paradoxa* (Strubell, 1892) (Gastropoda: Euthyneura) from Ambon, Indonesia. *Journal of Natural History*, **45**: 183–209.

BRENZINGER, B., NEUSSER, T.P., JÖRGER, K.M. & SCHRÖDL, M. 2011b. Integrating 3D microanatomy and molecules: natural history of the Pacific freshwater slug *Strubellia* Odhner, 1937 (Heterobranchia: Acochlidia), with description of a new species. *Journal of Molluscan Studies*, **77**: 351–374.

CARLSON, C.H. & HOFF, P.J. 1974. The Gastropteridae of Guam, with descriptions of four new species (Opisthobranchia: Cephalaspidea). *Publications of the Seto Marine Biological Laboratory*, **21**: 345–363.

CARLSON, C.H. & HOFF, P.J. 1993. 3 new *Halgerda* species (Doridoidea, Nudibranchia, Opisthobranchia) from Guam. *Veliger*, **36**: 16–26.

CARLSON, C.H. & HOFF, P.J. 1998. *Microhedyle* from Guam. *Sea Slug Forum*. Available from http://www.seaslugforum.net/find/251. Accessed 20 September 2017.

CARLSON, C.H. & HOFF, P.J. 2003. The opisthobranchs of the Mariana Islands. *Micronesica*, **35–36**: 271–293.

CARPENTER, K.E., BARBER, P.H., CRANDALL, E.D., ABLAN-LAGMAN, M.C.A., AMBARIYANTO, MAHARDIKA, G.N., MANJAJI-MATSUMOTO, B.M., JUINIO-MENEZ, M.A., SANTOS, M.D., STARGER, C.J. & TOHA, A.H.A. 2011. Comparative phylogeography of the Coral Triangle and implications for marine management. *Journal of Marine Biology*, **2011**: 14.

CHALLIS, D.A. 1970. *Hedylopsis cornuta* and *Microhedyle verrucosa*, two new Acochlidiacea (Mollusca: Opisthobranchia) from the Solomon Islands Protectorate. *Transactions of the Royal Society of New Zealand (Biological Sciences)*, **12**: 29–40.

DRAINAS, K., CARLSON, C.H., SCHRÖDL, M. & NEUSSER, T.P. 2016. First record and 3D-reconstruction of a marine mesopsammic acochlidian gastropod from Guam. In: Abstracts. *World Congress of Malacology, Penang*, 2016, p. 235.

GOSLINER, T.M. 1994. Gastropoda: Opisthobranchia. In: *Microscopic anatomy of invertebrates, Mollusca I*, Vol. 5 (F.W. Harrison & A.J. Kohn, eds), pp. 253–355. Wiley-Liss, New York, NY.

GOSLINER, T.M. & DRAHEIM, R. 1996. Indo-Pacific opisthobranch gastropod biogeography: how do we know what we don't know? *American Malacological Bulletin*, **12**: 43.

GOSLINER, T.M., VALDÉS, Á. & BEHRENS, D.W. 2015. *Nudibranchs and sea slugs. Identification. Indo-Pacific*. New World Publications, Jacksonville, FL.

HASZPRUNAR, G. 1985. The Heterobranchia—a new concept of the phylogeny of the higher Gastropoda. *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, **23**: 15–37.

HAYNES, A. & KENCHINGTON, W. 1991. *Acochlidium fijiensis* sp. nov. (Gastropoda: Opisthobranchia: Acochlidiacea) from Fiji. *Veliger*, **34**: 166–171.

HOEKSEMA, B.W. 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. In: *Biogeography, time, and place: distributions, barriers, and islands* (W. Renema, ed.), pp. 117–178. Springer, Netherlands, Dordrecht.

HOFF, P.J. & CARLSON, C.H. 1990. A new Runcinacea from the Mariana Islands (Gastropoda, Opisthobranchia). *Venus*, **494**: 263–269.

JÖRGER, K.M., BRENZINGER, B., NEUSSER, T.P., MARTYNOV, A.V., WILSON, N.G. & SCHRÖDL, M. 2014a. Panpulmonate habitat transitions: tracing the evolution of Acochlidia (Heterobranchia, Gastropoda). *bioRxiv*. doi:https://doi.org/10.1101/010322.

JÖRGER, K.M., HEß, M., NEUSSER, T.P. & SCHRÖDL, M. 2009. Sex in the beach: spermatophores, dermal insemination and 3D sperm ultrastructure of the aphallic mesopsammic *Pontohedyle milaschewitchii* (Acochlidia, Opisthobranchia, Gastropoda). *Marine Biology*, **156**: 1159–1170.

JÖRGER, K.M., NEUSSER, T.P., BRENZINGER, B. & SCHRÖDL, M. 2014b. Exploring the diversity of mesopsammic gastropods: how to collect, identify, and delimitate small and elusive sea slugs? *American Malacological Bulletin*, **32**: 290–307.

JÖRGER, K.M., NEUSSER, T.P., HASZPRUNAR, G. & SCHRÖDL, M. 2008. Undersized and underestimated: 3D-visualization of the Mediterranean interstitial acochlidian gastropod *Pontohedyle milaschewitchii* (Kowalevsky, 1901). *Organisms, Diversity & Evolution*, **8**: 194–214.

JÖRGER, K.M., NORENBURG, J.L., WILSON, N.G. & SCHRÖDL, M. 2012. Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. *BMC Evolutionary Biology*, **12**: 245.

JÖRGER, K.M. & SCHRÖDL, M. 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology*, **10**: 59.

JÖRGER, K.M., STÖGER, I., KANO, Y., FUKUDA, H., KNEBELSBERGER, T. & SCHRÖDL, M. 2010. On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. *BMC Evolutionary Biology*, **10**: 323.

KANO, Y., NEUSSER, T.P., FUKUMORI, H., JÖRGER, K.M. & SCHRÖDL, M. 2015. Sea-slug invasion of the land. *Biological Journal of the Linnean Society*, **116**: 253–259.

KAY, E.A. 1995. Pacific island marine molluscs: systematics. In: *Marine and coastal biodiversity in the tropical island Pacific region*, Vol. 1: *Species systematics and information management properties* (J.E. Maragos, M.N.A. Peterson, L.G. Eldredge, J.E. Bardach & H.F. Takeuchi, eds), pp. 135–159. East-West Center, Honolulu.

KLUSSMANN-KOLB, A., DINAPOLI, A., KUHN, K., STREIT, B. & ALBRECHT, C. 2008. From sea to land and beyond—new insights into the evolution of euthyneuran Gastropoda (Mollusca). *BMC Evolutionary Biology*, **8**: 57.

MARCUS, E. 1965. Some Opisthobranchia from Micronesia. *Malacologia*, **3**: 263–286.

NEUSSER, T.P., BOURKE, A.J., METCALFE, K. & WILLAN, R.C. 2015. First record of Aitengidae (Mollusca: Panpulmonata: Acochlidia) for Australia. *Northern Territory Naturalist*, **26**: 27–31.

NEUSSER, T.P., FUKUDA, H., JÖRGER, K.M., KANO, Y. & SCHRÖDL, M. 2011a. Sacoglossa or Acochlidia? 3D reconstruction, molecular phylogeny and evolution of Aitengidae (Gastropoda: Heterobranchia). *Journal of Molluscan Studies*, **77**: 332–350.

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024

NEUSSER, T.P., HEß, M., HASZPRUNAR, G. & SCHRÖDL, M. 2006. Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acochlidian gastropod from Bermuda. *Journal of Morphology*, **267**: 231–247.

NEUSSER, T.P., HEß, M. & SCHRÖDL, M. 2009a. Tiny but complex—interactive 3D visualization of the interstitial acochlidian gastropod *Pseudunela cornuta* (Challis, 1970). *Frontiers in Zoology*, **6**: 20.

NEUSSER, T.P., JÖRGER, K.M., LODDE-BENSCH, E., STRONG, E.E. & SCHRÖDL, M. 2016. The unique deep sea–land connection: interactive 3D visualization and molecular phylogeny of *Bathyhedyle boucheti* n. sp. (Bathyhedylidae n. fam.)—the first panpulmonate slug from bathyal zones. *PeerJ*, **4**: e2738.

NEUSSER, T.P., JÖRGER, K.M. & SCHRÖDL, M. 2011b. Cryptic species in tropic sands—interactive 3D anatomy, molecular phylogeny and evolution of meiofaunal Pseudunelidae (Gastropoda, Acochlidia). *PLoS One*, **6**: e23313.

NEUSSER, T.P., MARTYNOV, A.V. & SCHRÖDL, M. 2009b. Heartless and primitive? 3D reconstruction of the polar acochlidian gastropod *Asperspina murmanica*. *Acta Zoologica*, **90**: 228–245.

NEUSSER, T.P. & SCHRÖDL, M. 2007. *Tantulum elegans* reloaded: a computer-based 3D-visualization of the anatomy of a Caribbean freshwater acochlidian gastropod. *Invertebrate Biology*, **126**: 18–39.

NEUSSER, T.P. & SCHRÖDL, M. 2009. Between Vanuatu tides: 3D anatomical reconstruction of a new brackish water acochlidian gastropod from Espiritu Santo. *Zoosystema*, **31**: 453–469.

PAULAY, G. 2003. Marine biodiversity of Guam and the Marianas: overview. *Micronesica*, **35–36**: 3–25.

RANKIN, J.J. 1979. A freshwater shell-less mollusc from the Caribbean: structure, biotics and contribution to a new understanding of the Acochlidioidea. *Royal Ontario Museum Life Sciences Contributions*, **116**: 1–123.

RICHARDSON, K.C., JARETT, L. & FINKE, E.H. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technology*, **35**: 313–323.

RUTHENSTEINER, B. 2008. Soft part 3D visualization by serial sectioning and computer reconstruction. *Zoosymposia*, **1**: 63–100.

SCHRÖDL, M. 2014. Time to say "Bye-bye Pulmonata"? *Spixiana*, **37**: 161–164.

SCHRÖDL, M. 2016. Slugs, snails and their allies: news and lessons from heterobranch systematics. *Malacologist*, **67**: 21–22.

SCHRÖDL, M. & NEUSSER, T.P. 2010. Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society*, **158**: 124–154.

SOMMERFELDT, N. & SCHRÖDL, M. 2005. Microanatomy of *Hedylopsis ballantinei*, a new interstitial acochlidian gastropod from the Red Sea, and its significance for phylogeny. *Journal of Molluscan Studies*, **71**: 153–165.

SPURR, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research*, **26**: 31–43.

STRUBELL, A.D. 1892. [no title]. *Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande, Westphalens, 49. Jg, Sitzung der niederrheinischen Gesellschaft*, p. 62.

SWEDMARK, B. 1968. The biology of interstitial Mollusca. *Symposium of the Zoological Society of London*, **22**: 135–149.

SWENNEN, C.K. & BUATIP, S. 2009. *Aiteng ater*, new genus, new species, an amphibious and insectivorous sea slug that is difficult to classify (Mollusca: Gastropoda: Opisthobranchia: Sacoglossa(?): Aitengidae, new family). *Raffles Bulletin of Zoology*, **57**: 495–500.

TEASDALE, L.C. 2017. *Phylogenomics of the pulmonate land snails*. PhD thesis, University of Melbourne.

WÄGELE, H., KLUSSMANN-KOLB, A., VONNEMANN, V. & MEDINA, M. 2008. Heterobranchia I: the Opisthobranchia. In: *Phylogeny and evolution of the Mollusca* (W.F. Ponder & D. Lindberg, eds), pp. 385–408. University of California Press, Berkley, CA.

WAWRA, E. 1974. The rediscovery of *Strubellia paradoxa* (Strubell) (Gastropoda: Euthyneura: Acochlidiacea) on the Solomon Islands. *Veliger*, **17**: 8–10.

WAWRA, E. 1979. *Acochlidium sutteri* nov. spec. (Gastropoda, Opisthobranchia, Acochlidiacea) von Sumba, Indonesien. *Annalen des Naturhistorischen Museums in Wien. Serie B. Botanik und Zoologie*, **82**: 595–604.

WAWRA, E. 1980. *Acochlidium bayerfehlmanni* spec. nov. (Gastropoda: Opisthobranchia: Acochlidiacea) from Palau Islands. *Veliger*, **22**: 215–218.

WAWRA, E. 1987. *Zur Anatomie einiger Acochlidia (Gastropoda, Opisthobranchia) mit einer vorläufigen Revision des Systems und einem Anhang über Platyhedylidae (Opisthobranchia, Ascoglossa)*. PhD thesis no. 17335, University of Vienna.

WAWRA, E. 1989. Zur Kenntnis der interstitiellen Opisthobranchierart *Hedylopsis spiculifera* (Kowalevsky) (Mollusca, Gastropoda). *Zoologica Scripta*, **18**: 397–403.

Downloaded from https://academic.oup.com/mollus/article/84/1/1/4480316 by guest on 24 April 2024