

Between Vanuatu tides: 3D anatomical reconstruction of a new brackish water acochlidian gastropod from Espiritu Santo

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ABSTRACT

The majority of known acochlidian sea slug species are marine mesopsammic, while some others are limnic. The structural, functional and evolutionary background of the invasion of freshwater systems was hardly explored. During the expedition SANTO 2006 to Espiritu Santo, Vanuatu, we discovered a unique new acochlidian species in a brackish water habitat. *Pseudunela espiritusanta* n. sp. inhabits the underside of intertidal rocks deeply embedded into coarse sand, the interstices of which are filled with a mixture of fresh subsoil and seawater. *Pseudunela espiritusanta* n. sp. is herein described in full external and anatomical detail using computer-based 3-dimensional reconstruction techniques from serial histological sections. This new species possesses a typical acochlidian central nervous and digestive system; it is a simultaneous hermaphrodite with a special androdiaulic reproductive system and complex, stylet-bearing copulatory organs with associated glands. Such penial features may indicate a relationship with marine mesopsammic *Pseudunela* (Pseudunelidae) species, while e.g., the larger body size, the broad foot, and the presence of a special ventricular cell layer may be potential synapomorphies with limnic, benthic Acochliidiidae (*Strubellia* and Acochliidiidae s.s.). *Pseudunela espiritusanta* n. sp. shares its special shape of head tentacles with both *Pseudunela* and *Strubellia*, while other characters are potentially synapomorphic with either one or the other taxon. Regardless of its unresolved exact systematic position, *Pseudunela espiritusanta* n. sp. evidently links marine and limnic taxa by its intermediate ecological and morphological features. Its considerable body size and well-developed heart and kidney can be considered as preadaptations to overcome osmotic challenges when colonising rivers from brackish coastal sands.

KEY WORDS

Mollusca,
Opisthobranchia,
marine,
freshwater,
limnic,
morphology,
histology,
penial stylet,
Vanuatu,
new species.

RÉSUMÉ

Dans la zone intertidale du Vanuatu : reconstruction anatomique 3D d'un nouveau gastéropode acochlidiacé d'eau saumâtre d'Espiritu Santo.

La majorité des acochlidiacés sont des espèces marines mésopsammiques, bien que quelques espèces vivent dans les eaux douces. Le contexte structural, fonctionnel et évolutif de la conquête des eaux douces a rarement été exploré. Au cours de l'expédition SANTO 2006, menée dans l'île d'Espiritu Santo au Vanuatu, nous avons découvert une nouvelle espèce remarquable d'acochlidiacé vivant dans un milieu saumâtre. *Pseudunela espritusanta* n. sp. vit sous les blocs de la zone intertidale profondément enfoncé dans un sable grossier, dont les espaces sont remplis d'un mélange d'eau de mer et d'eau douce résurgente. La morphologie et l'anatomie de *Pseudunela espritusanta* n. sp. sont décrites ici de manière détaillée à partir de sections histologiques sériées et d'un logiciel de reconstruction 3D utilisant des séries de coupes histologiques. Le système nerveux central et le système digestif sont typiques des acochlidiacés ; l'espèce est hermaphrodite simultané, avec un appareil reproducteur androdiaulique particulier et des organes copulateurs comportant un stylet et des glandes associées. Ces caractéristiques du pénis pourraient traduire une parenté avec des espèces mésopsammiques de *Pseudunela* (Pseudunelidae), alors que d'autres caractères comme par exemple la grande taille du corps, un pied large, et la présence d'une couche particulière de cellules ventriculaires pourraient représenter des synapomorphies potentielles avec des Acochliidiidae limniques benthiques (*Strubellia* et Acochliidiidae s.s.). *Pseudunela espritusanta* n. sp. présente la même forme particulière des tentacules de la tête que *Pseudunela* et *Strubellia*, mais d'autres caractères sont communs avec l'un ou l'autre de ces taxons. Indépendamment de sa position taxonomique incertaine, *Pseudunela espritusanta* n. sp. relie de manière évidente taxons marins et taxons d'eaux douces, tant par son écologie que par ses caractères morphologiques intermédiaires. La très grande taille du corps et le développement du cœur et du rein pourraient représenter des préadaptations pour faire face aux changements osmotiques rencontrés lors de la colonisation des rivières depuis les sables littoraux saumâtres.

MOT CLÉS

Mollusca,
Opisthobranchia,
marin,
eau douce,
limnique,
morphologie,
histologie,
stylet pénien,
Vanuatu,
espèce nouvelle.

INTRODUCTION

Among the otherwise marine Opisthobranchia, the Acochlidia comprise the only opisthobranch group that successfully invaded freshwater systems (Neusser & Schrödl 2007; Strong *et al.* 2008). First phylogenetic studies based on morphological characters indicate that colonisation of limnic habitats occurred twice independently within acochliidiids; once by the ancestor of an array of large-sized, benthic species distributed over different Indo-Pacific Islands (*Strubellia* Odhner, 1937, *Acochlidium* Strubell, 1892, *Palliohedyle* Rankin, 1979), and second in the small, interstitial Caribbean *Tantulum elegans*

Rankin, 1979 from St. Vincent Island (Schrödl & Neusser in press). However, up to now, we have no exact information about 1) how the osmotic challenges presented by the colonisation of limnic environments were overcome, and 2) what are the morphological adaptations in limnic acochliidian species. Studying species living in marine habitats that are temporarily or permanently influenced by freshwater input may provide a clue.

Recently, a new acochliidian species was discovered in a brackish water environment, inhabiting the intertidal off Luganville, Espiritu Santo Island, Vanuatu. The goal of this study is to describe this species externally and internally by computer-aided

3D reconstruction of serial histological sections and to compare it with externally similar marine and limnic representatives of the genera *Pseudunela* Salvini-Plawen, 1973 and *Strubellia*.

MATERIAL AND METHODS

The material (six specimens) was collected during the SANTO 2006 Expedition to the island of Santo, Vanuatu, in October 2006. For a narrative of the expedition, see Bouchet *et al.* (2008), and for a review of the geography and natural history of Santo, we refer to Bouchet *et al.* (in press). The specimens were collected by brushing the embedded surfaces of intertidal boulders and relaxed by a solution of isotonic MgCl₂. One specimen was fixed in 80% EtOH. Three specimens were fixed in 95% and 99% EtOH for molecular studies. The pharynx of one of the latter specimens was removed for analysis of the radula by SEM and macerated in 10% KOH. Remaining tissue was separated using an ultrasonic bath (Sonorex TK52, Bandelin, Berlin, Germany) for 20 minutes. The radula was mounted on a SEM stub, sputter-coated with gold for 120 s (SEM-Coating-System, Polaron) and analyzed using a LEO 1430 VP SEM (15 kV).

Two specimens were fixed in 4% glutardialdehyde in 0.2 M sodium cacodylate buffer (0.1 M NaCl, 0.35 M sucrose, pH 7.2), followed by postfixation in 1% OsO₄ buffered in 0.2 M sodium cacodylate (0.3 M NaCl, pH 7.2) for 2 h in the dark. Subsequently the specimens were decalcified in 1% ascorbic acid overnight and dehydrated in an acetone series (30, 50, 70, 90, 100%), embedded in Spurr's low viscosity resin (Spurr 1969) and serially sectioned (thickness: 1.5 µm) using a diamond knife (Histo Jumbo, Diatome, Biel, Switzerland) and contact cement on the lower cutting edge to form ribbons (Ruthensteiner 2008). Sections were stained with methylene-azure II (Richardson *et al.* 1960). Every second section (for cns: every section) of series no. 20080791 was photographed with a CCD microscope camera (Spot Insight, Diagnostic instruments, Sterling Heights, USA) on a Leica DMB-RBE (Leica Microsystems, Wetzlar, Germany) microscope. Computer-aided 3D reconstruction

of all major organ systems was performed with the software AMIRA 4.1 (TGS Europe, Mercury Computer Systems, Mérignac, France). Sections were deposited at the ZSM, Mollusca Section (no. 20070968 and 20080791).

ABBREVIATIONS

cns	central nervous system;
MNHN	Muséum national d'Histoire naturelle, Paris;
SEM	Scanning electron microscopy;
ZSM	Zoologische Staatssammlung, München.

SYSTEMATICS

Family PSEUDUNELIDAE Rankin, 1979
Genus *Pseudunela* Salvini-Plawen, 1973

Pseudunela espritusanta n. sp.

TYPE MATERIAL. — Holotype: ZSM 20080115, 3.5 mm preserved body length, stored in 75% EtOH. Paratypes: ZSM 20070968 and 20080791 (two serially sectioned specimens), ZSM 20080116 (head removed for radula analysis, stored in 96% EtOH), ZSM 20080117 and 20071118 (used for molecular studies).

TYPE LOCALITY. — Vanuatu, Espiritu Santo Island, leaving Luganville to Palikulo Bay. Expedition SANTO 2006, stn VM 53, 15°30'58"S, 167°11'52"E.

ETYMOLOGY. — *Pseudunela espritusanta* n. sp. is named according to the type locality on the island of Espiritu Santo.

DISTRIBUTION. — Known only from the type locality.

DESCRIPTION

Habitat

Intertidal zone of type locality characterized by rocks, dead coral pieces and coarse sand (Fig. 1A). During high tide, seawater reaches the bank beside the road. *Pseudunela espritusanta* n. sp. inhabits lower intertidal in brackish environment. It lives underside of rocks deeply embedded into coarse sand (Fig. 1B); interstices filled with mixture of sea and emerging fresh subsoil water. Habitat keeps wet during low tide. Despite further sampling, the new species could not be found in neighbouring sand and gravel patches. Other molluscan species associated with *P. espritusanta* n. sp. in the same

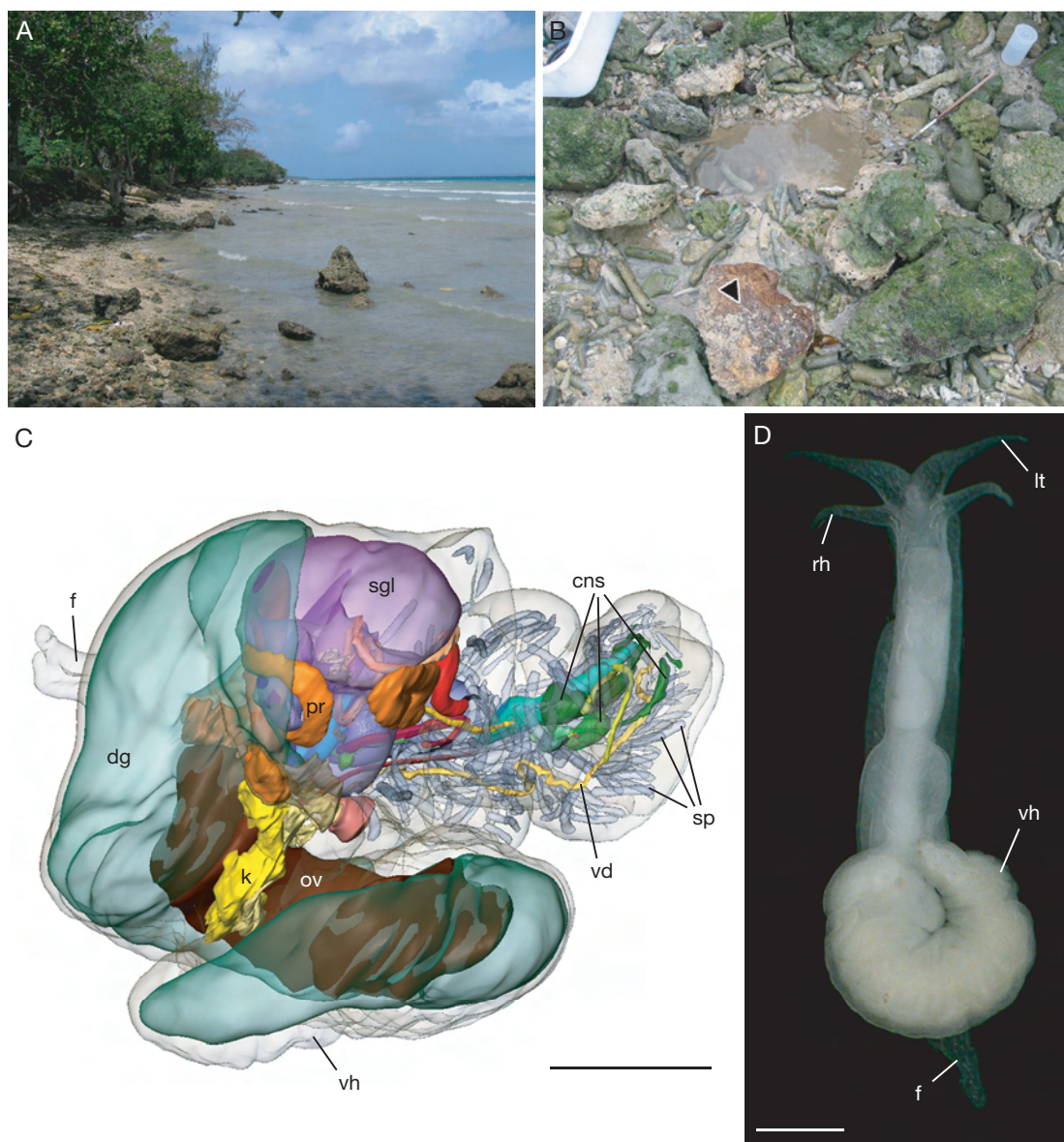


FIG. 1. — Habitat, external morphology and general anatomy of *Pseudunela espritusanta* n. sp.: **A**, type locality when tide is just coming in; **B**, habitat of *P. espritusanta* n. sp.: underside of rocks embedded in coarse sand (arrowhead points to exact place where specimens were found); **C**, 3D reconstruction, position of internal organs: green, central nervous system; blue/lilac, digestive system; yellow, circulatory and excretory systems; red/brownish, reproductive system; **D**, photograph of living specimen. Abbreviations: **cns**, central nervous system; **dg**, digestive gland; **f**, foot; **k**, kidney; **lt**, labial tentacle; **ov**, ovotestis; **pr**, prostate; **rh**, rhinophore; **sgl**, salivary gland; **sp**, spicule; **vd**, vas deferens; **vh**, visceral hump. Scale bars: C, 500 μ m; D, 1 mm.

habitat were at least *Neritilia littoralis* Kano, Kase & Kubo, 2003 and two undescribed *Neritilia* spp. (Kano pers. comm.).

External morphology

Body divided into anterior head-foot complex and posterior elongated visceral hump (vh) (Fig. 1C, D).

Head-foot complex partially retractable into temporary cavity of visceral hump. Length of crawling specimen, 9 mm. Body colour of living specimens translucent-whitish, digestive gland yellowish. Labial tentacles (lt) broad at base (Fig. 1D), tapering to distal end, usually held at 45° to mid line. Rhinophores (rh) slightly shorter and narrower, tapering (Fig. 1D). Pigmented eyes, only clearly visible laterally. Densely ciliated foot (f) broader than anterior head-foot complex (Fig. 1D), tail extending about two-thirds of visceral hump; tip pointed. Visceral hump in living specimens usually curved (Fig. 1D). Heart visible within prominent bulb at anterior right side of visceral hump. Subepidermal spicules (sp) bean-shaped (Figs 1C; 8A), small (70–135 µm) in tentacles, larger (200–300 µm) in foot and around CNS and pharynx (Fig. 3D).

Microanatomy

Central nervous system. CNS eothyneurous. Paired rhinophoral (rhg), cerebral (cg), pedal (pg), pleural (plg), optic (og), buccal (bg) and gastro-oesophageal (gog) ganglia; three distinct separated ganglia on visceral nerve cord, plus presumed osphradial ganglion (osg) (Fig. 2). All ganglia excluding buccal and gastro-oesophageal ganglia pre-pharyngeal (Fig. 3C, D). Large cerebral ganglia (c. 130 µm in diameter) with short commissure (Figs 2; 3A, B). Labiotentacular nerve (lt) emerging anteroventrally from cerebral ganglion, ramifying into (at least) 6 branches within labial tentacles (Figs 2; 3A). Rhinophoral ganglion (c. 40 µm in diameter) anterodorsal to each cerebral ganglion (Figs 2; 3A); short, single cerebro-rhinophoral connective. Rhinophoral nerve (rh) emerging from rhinophoral ganglion (Figs 2; 3A). Thin nerve arising at base of rhinophoral nerve (Fig. 2), extending towards ciliated ridge posterior to rhinophore. Optic ganglion (Figs 2; 3A, B) (c. 35 µm in diameter) situated posterior to rhinophoral ganglion, attached to cerebral ganglion. Thin optic nerve (on) (Figs 2; 3B) innervating pigmented eye (ey) (Figs 2; 3A, B) (c. 45 µm in diameter). Anterior accessory ganglia absent.

Pedal ganglia (c. 100 µm in diameter) connected by thin, long commissure (Figs 2; 3B). Statocyst (st) (Figs 2; 3B) posterior to each pedal ganglion.

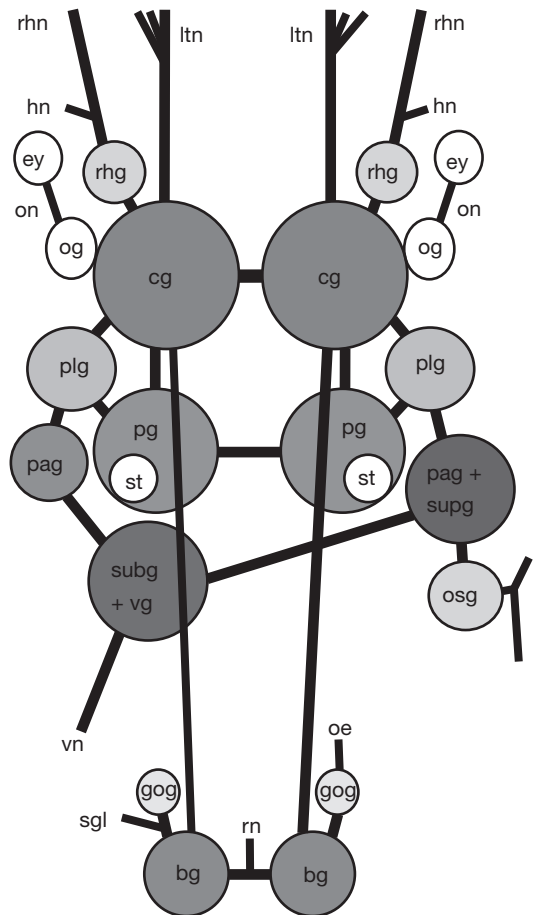


Fig. 2. — Central nervous system of *Pseudunela espiritusanta* n. sp. (schematic, dorsal view). Abbreviations: **bg**, buccal ganglion; **cg**, cerebral ganglion; **ey**, eye; **gog**, gastro-oesophageal ganglion; **hn**, Hancock's nerve; **lt**, labial tentacle nerve; **oe**, nerve innervating oesophagus; **og**, optic ganglion; **on**, optic nerve; **osg**, osphradial ganglion; **pag**, parietal ganglion; **pg**, pedal ganglion; **plg**, pleural ganglion; **rhg**, rhinophoral ganglion; **rh**, rhinophoral nerve; **rn**, radula nerve; **sgl**, nerve innervating salivary gland; **st**, statocyst; **subg**, subintestinal ganglion; **supg**, suprainintestinal ganglion; **vg**, visceral ganglion; **vn**, visceral nerve. Not to scale.

Four pedal nerves (pn) per ganglion (Fig. 3A), extending anteriorly, posteriorly and ventrally. Pleural ganglion (Figs 2; 3A, B) (c. 55 µm in diameter) posterior to and equidistant from cerebral and pedal ganglion, connected to both by short connectives forming pre-pharyngeal nerve ring. Visceral nerve cord short with three distinct ganglia: left parietal (pag) (Figs 2; 3B) (c. 55 µm in

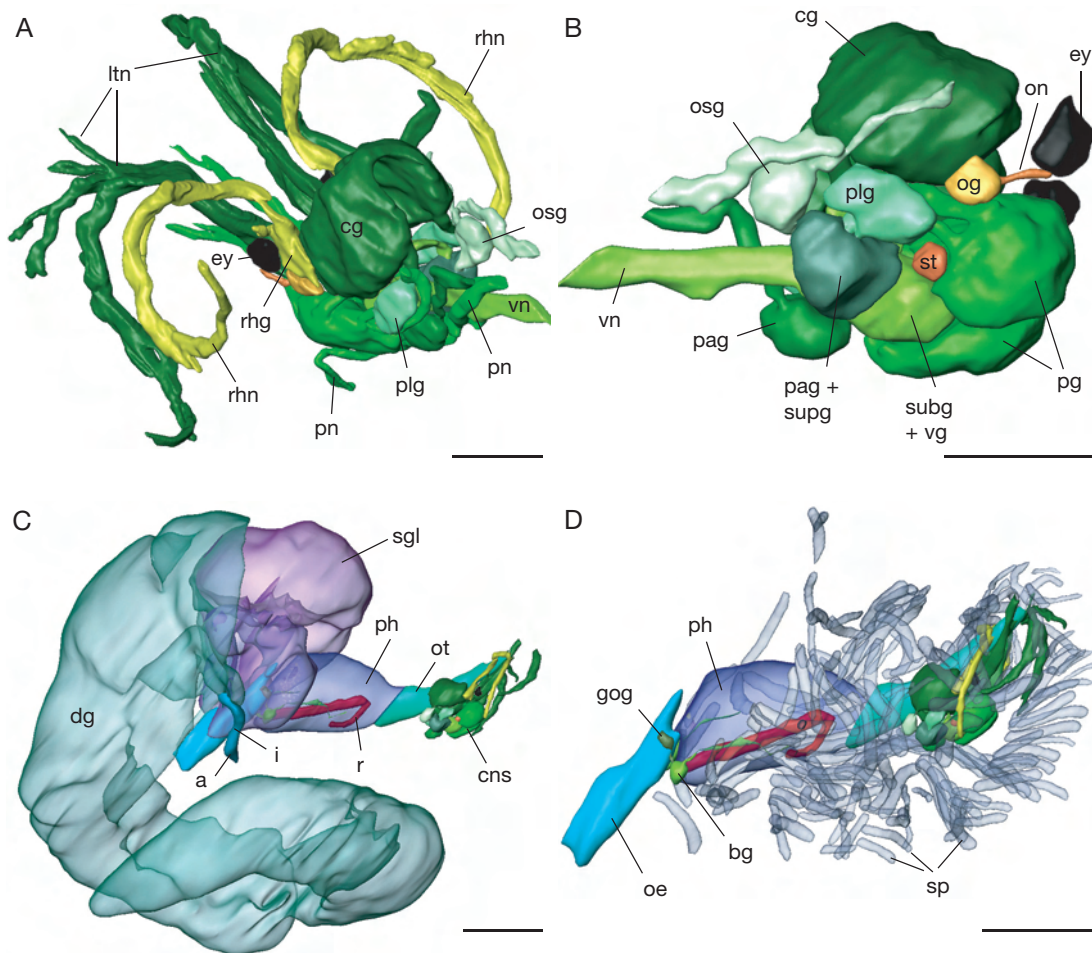


Fig. 3. — 3D reconstruction of the central nervous system and digestive system of *Pseudunela espiritusanta* n. sp.: **A**, CNS, dorsolateral left view; **B**, CNS without cerebral nerves, right view; **C**, CNS and digestive system, right view; **D**, spicules surrounding CNS and buccal mass, right view. Abbreviations: **a**, anus; **bg**, buccal ganglion; **cg**, cerebral ganglion; **CNS**, central nervous system; **dg**, digestive gland; **ey**, eye; **gog**, gastro-oesophageal ganglion; **i**, intestine; **ltn**, labial tentacle nerve; **oe**, oesophagus; **og**, optic ganglion; **on**, optic nerve; **osg**, osphradial ganglion; **ot**, oral tube; **pag**, parietal ganglion; **pg**, pedal ganglion; **ph**, pharynx; **plg**, pleural ganglion; **pn**, pedal nerve; **r**, radula; **rhg**, rhinophoral ganglion; **rhn**, rhinophoral nerve; **sgl**, salivary gland; **sp**, spicule; **st**, statocyst; **subg**, subintestinal ganglion; **supg**, supraintestinal ganglion; **vg**, visceral ganglion; **vn**, visceral nerve. Scale bars: A, B, 100 μ m; C, D, 300 μ m.

diameter), fused subintestinal/visceral (subg+vg) (Figs 2; 3B) (*c.* 75 μ m in diameter) and fused right parietal/supraintestinal (pag+supg) ganglia (Figs 2; 3B) (*c.* 70 μ m in diameter). Left pleuro-parietal, parietal-subintestinal/visceral and right pleuro-parietal/supraintestinal connectives short, subintestinal/visceral-parietal/supraintestinal connective longer. Left parietal ganglion producing one nerve. Robust nerve emerging from subintestinal/visceral

ganglion extending to visceral hump (Fig. 3A, B). Tentative osphradial ganglion (Figs 2; 3A, B) (*c.* 45 μ m in diameter) with one bifurcating nerve linked to parietal/supraintestinal ganglion. Buccal ganglion (*c.* 65 μ m in diameter) posterior to pharynx (ph) (Figs 2; 3D; 7B), thin buccal commissure situated ventral to oesophagus. Radular nerve (rn) thin, branching from buccal commissure (Fig. 2). Thin cerebro-buccal connective emerging anteriorly

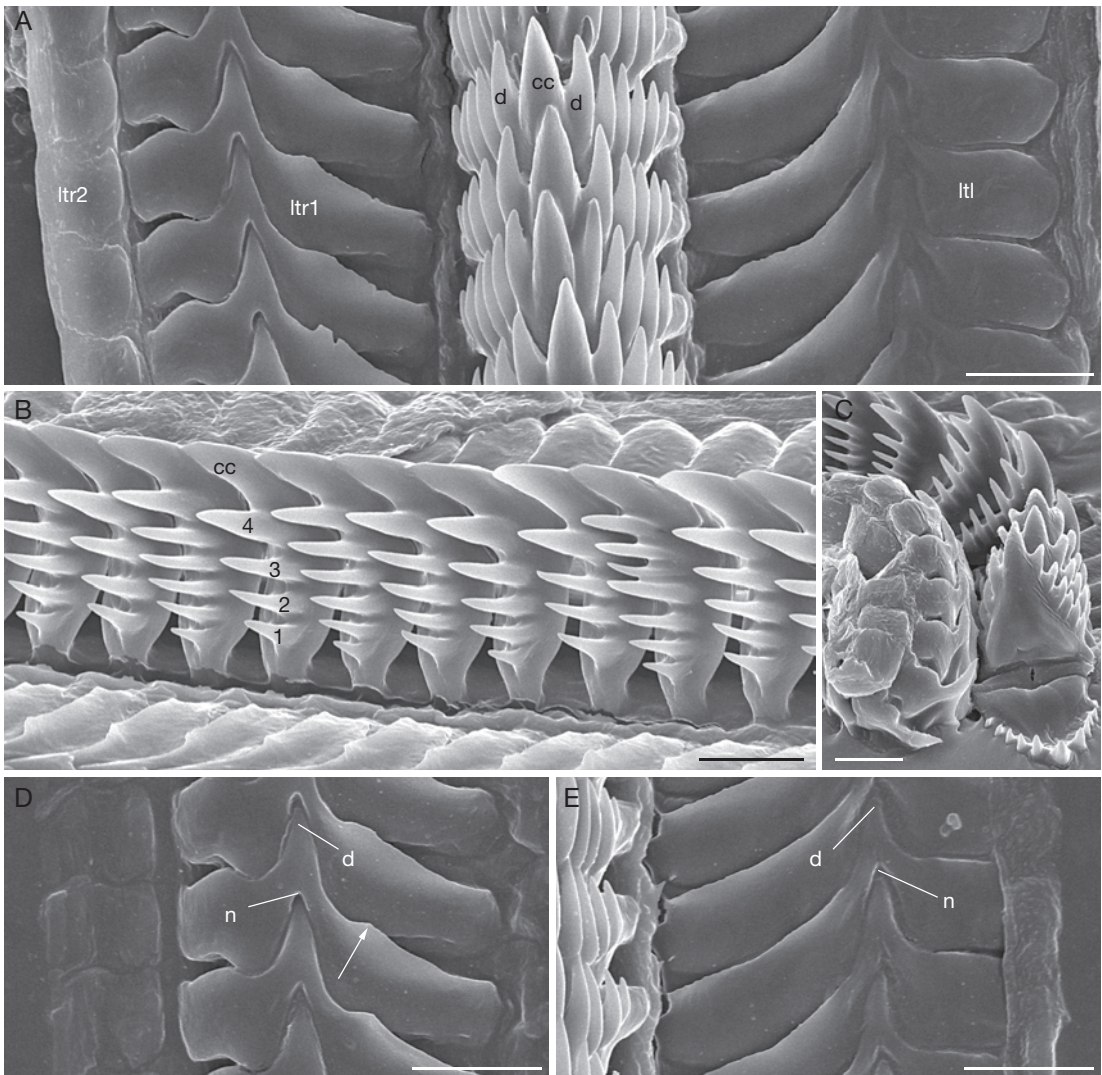


FIG. 4. — Radula of *Pseudunela espritusanta* n. sp., SEM-micrographs: **A**, row of radular teeth; **B**, rhachidian teeth, right view; **C**, rhachidian tooth, anterior view; **D**, right lateral teeth, arrow points to blunt protrusion; **E**, left lateral teeth. Abbreviations: **cc**, central cusp; **d**, denticle; **ltl**, left lateral tooth; **ltr1**, first right lateral tooth; **ltr2**, second right lateral tooth; **n**, notch; **1-4**, lateral denticle on rhachidian tooth. Scale bars: 10 μ m.

from each buccal ganglion, not traceable along entire length. Small gastro-oesophageal ganglion (Figs 2; 3D; 7B) (*c.* 30 μ m in diameter) slightly dorsally to each buccal ganglion, innervating oesophagus (oe). Thin nerve innervating the salivary gland (sgl), branching from buccal-gastro-oesophageal connective (Fig. 2).

Digestive system. Mouth opening ventrally between labial tentacles. Anterior pedal gland (apg) (Fig. 8A) opening ventral to mouth. Oral tube (ot) long, unciliated (Fig. 3C). Paired oral glands (otg) flanking oral tube (Fig. 8A). Pharynx (ph) bulbous and muscular (Figs 3C, D; 7A). Jaws absent. Radula (r) *c.* 575 μ m long, hook-shaped

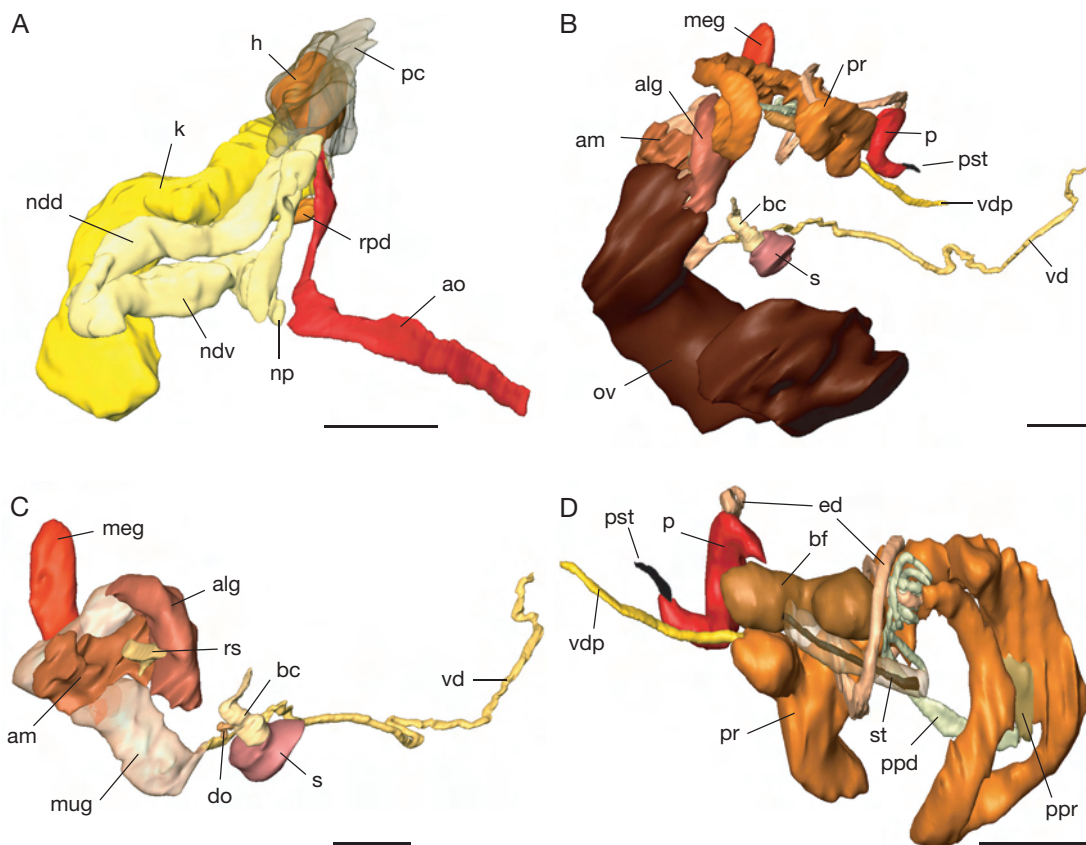


FIG. 5. — 3D reconstruction of the circulatory, excretory and reproductive systems of *Pseudunela espritusanta* n. sp.: **A**, circulatory and excretory systems, right view; **B**, complete reproductive system, dorsolateral view from right; **C**, nidamental glands, sperm storing receptacles and sphincter, right view; **D**, anterior male copulatory organs, left view. Abbreviations: **alg**, albumen gland; **am**, ampulla; **ao**, aorta; **bc**, bursa copulatrix; **bf**, basal finger; **do**, oviduct; **ed**, ejaculatory duct; **h**, heart; **k**, kidney; **meg**, membrane gland; **mug**, mucus gland; **nnd**, nephroduct dorsal branch; **ndv**, nephroduct ventral branch; **np**, nephropore; **ov**, ovotestis; **p**, penis; **pc**, pericardium; **ppd**, paraprostatic duct; **ppr**, paraprostate; **pr**, prostate; **pst**, penial stylet; **rpd**, renopericardioduct; **rs**, receptaculum seminis; **s**, sphincter; **st**, stylet of basal finger; **vd**, vas deferens; **vdp**, posterior-leading vas deferens. Scale bars: 200 μ m.

(Fig. 3C, D) and asymmetric with formula $67 \times 1.1.2$; upper ramus with 48 rows (*c.* 410 μ m long), lower ramus with 19 rows (*c.* 165 μ m long). Rha-chidian tooth triangular (21 μ m high, 18 μ m wide) with prominent central cusp (cc) and 4-7 thinner denticles (d) per side (Fig. 4A-C). Lateral teeth plate-like (Fig. 4A). Left lateral tooth (ltl) (5-7 μ m high, 29 μ m wide) with prominent, cuspid denticle on anterior margin 6 μ m in length; deep triangular notch (n) on posterior margin of tooth (Fig. 4E) receiving denticle of subsequent tooth. First/inner right lateral tooth (ltr1) similar to left

lateral but slightly smaller (5-6 μ m high, 25 μ m wide), with small blunt protrusion at inner side of denticle (Fig. 4D). Outer margin of left lateral tooth rounded; outer margin of first right lateral tooth straight (Fig. 4A). Second/outer right lateral tooth (ltr2) small and quadratic plate (8 \times 8 μ m) (Fig. 4D).

Ciliated oesophagus (oe) long, emerging posterodorsally from pharynx (Figs 3C, D; 7B), flanked by longitudinal muscles. Paired, large salivary glands (sgl) (Figs 3C; 7B) discharging into posterior pharynx via salivary gland ducts (sgd) (Fig. 7B).

Digestive gland (dg) sac-like (Fig. 3C), inner surface heavily folded, lumen empty, situated dorsally of ovotestis (ov), extending to end of visceral hump (Fig. 1C). Stomach continuous with broad, anterior, lobed part of digestive gland. Intestine (i) short (Fig. 3C), densely ciliated (Fig. 8C, D). Anus (a) (Fig. 3C) ventrolaterally on right side of visceral hump, slightly anterior to nephropore.

Circulatory and excretory systems. Circulatory and excretory systems situated at right of body (Fig. 1C), at anterior end of visceral hump. Heart (h) (c. 160 μm) with one well-developed muscular ventricle (Figs 5A; 8D), thin valve at posterior end (Fig. 8D). Atrium not apparent. Aggregations of discrete cells in lumen of ventricle (v) (Fig. 8D). Aorta (ao) (Figs 5A; 8C) emerging anteriorly from ventricle, extending to anterior head region. Heart surrounded by thin-walled pericardium (pc) (Fig. 5A); thick layer of blue stained epicardial cells of unknown function embracing ventricle (Fig. 8D). Kidney (k) elongated (Fig. 5A), internally divided into narrow lumen (kn) defined by tissue with small vacuoles, and wide lumen (kw) bordered by highly vacuolated tissue (Fig. 8C, D). Both lumina joining in posterior part of kidney. Renopericardial duct (rpd) well developed and densely ciliated (Figs 5A; 8C). Connection between kidney and nephroduct narrow and ciliated (Fig. 8B). Nephroduct long; dorsal branch (nnd) extending posteriorly, continuing anteriorly via ventral branch (ndv) (Fig. 5A); distally looped. Nephropore (np) ventrolateral at right side of visceral hump, slightly posterior to anus.

Reproductive system. Simultaneously hermaphroditic, androdiaulic. Ovotestis (ov) sac-like (Figs 1C; 5B; 6), extending over almost entire visceral hump, ventral to digestive gland; not separated into follicles, but spermatocytes situated more anteriorly and ventrally, oocytes dorsally and posteriorly. Details of individual oocytes obscured by large amount of yolk. Anterior to ovotestis sac-like ampulla (am) filled with unorientated autosperm in disorder (Figs 5B, C; 6; 7D). Small receptaculum seminis (rs) with orientated allosperm (Figs 5C; 6; 7D). Sperm heads short. Three female nidamental glands: tubular albumen (alg), sac-like membrane (meg)

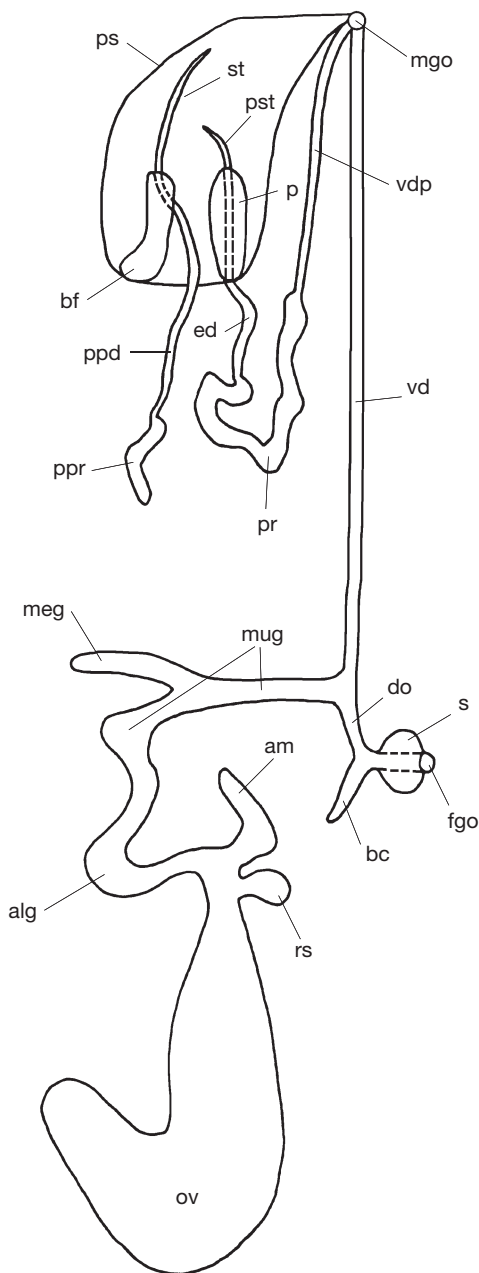


FIG. 6. — Reproductive system of *Pseudunela espritusanta* n. sp. (schematic drawing). Abbreviations: **alg**, albumen gland; **am**, ampulla; **bc**, bursa copulatrix; **bf**, basal finger; **do**, oviduct; **ed**, ejaculatory duct; **fgo**, female gonopore; **meg**, membrane gland; **mgo**, male gonopore; **mug**, mucus gland; **ov**, ovotestis; **p**, penis; **ppd**, paraprostatic duct; **ppr**, paraprostate; **pr**, prostate; **ps**, penial sheath; **pst**, penial stylet; **rs**, receptaculum seminis; **s**, sphincter; **st**, stylet of basal finger; **vd**, vas deferens; **vdp**, posterior-leading vas deferens. Not to scale.

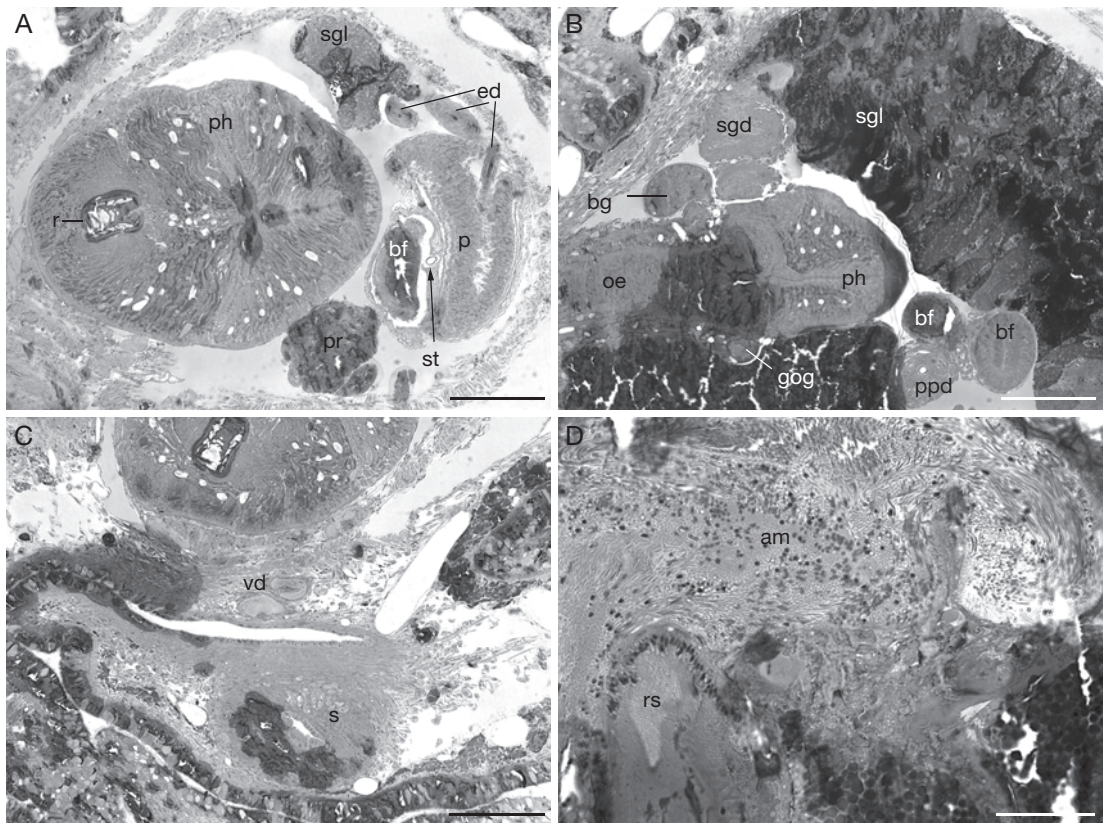


FIG. 7. — Transverse histological sections of *Pseudunela espritusanta* n. sp.: **A**, pharynx with radula, penis, basal finger; **B**, salivary gland, buccal ganglion; **C**, sphincter; **D**, sperm storing receptacles. Abbreviations: **am**, ampulla; **bf**, basal finger; **bg**, buccal ganglion; **ed**, ejaculatory duct; **gog**, gastro-oesophageal ganglion; **oe**, oesophagus; **p**, penis; **ph**, pharynx; **ppd**, paraprosthetic duct; **pr**, prostate; **r**, radula; **rs**, receptaculum seminis; **s**, sphincter; **sgd**, salivary gland duct; **sgl**, salivary gland; **st**, stilet of basal finger; **vd**, vas deferens. Scale bars: A-C, 100 µm; D, 25 µm.

and tubular mucus gland (mug) (Figs 5B, C; 6). Membrane gland branching off in middle of mucus gland. Albumen gland characterized by cells containing dark blue-stained granules; cells of mucus and membrane glands with violet-staining contents. All nidamental glands densely ciliated. Distal part of mucus gland extending to right side of body wall where hermaphroditic duct separates into vas deferens (vd) and oviduct (do) (Figs 5C; 6). Oviduct short, narrow (Fig. 5C; 6), dividing into long slender, blind duct (bc) (bursa copulatrix or bursa stalk) (Fig. 5B, C; 6) and distal gonoduct extending to female gonopore (fgo). Large muscular sphincter (s) surrounding female gonopore (Figs 5B, C; 6;

7C). Female gonopore ventrolateral at right side of visceral hump, anterior to anus and nephropore.

Subepidermal, ciliated vas deferens (Figs 5C; 6; 7C) extending along right body side to opening at base of right rhinophore. Anterior male copulatory organs cephalic. Posterior-leading vas deferens (vdp) connecting to large tubular prostate (pr) (Figs 5D; 6; 7A). Ejaculatory duct (ed) connecting to elongated, muscular penial papilla (p) (Figs 5D; 6; 7A). Curved hollow penial stilet (pst) (*c.* 80 µm long) partially retractable into muscular penial bulb (Figs 5D; 6). Blind glandular paraprosthetic (ppr) (Figs 5D; 6) considerably smaller than prostate, connected by paraprosthetic duct (ppd) to muscular

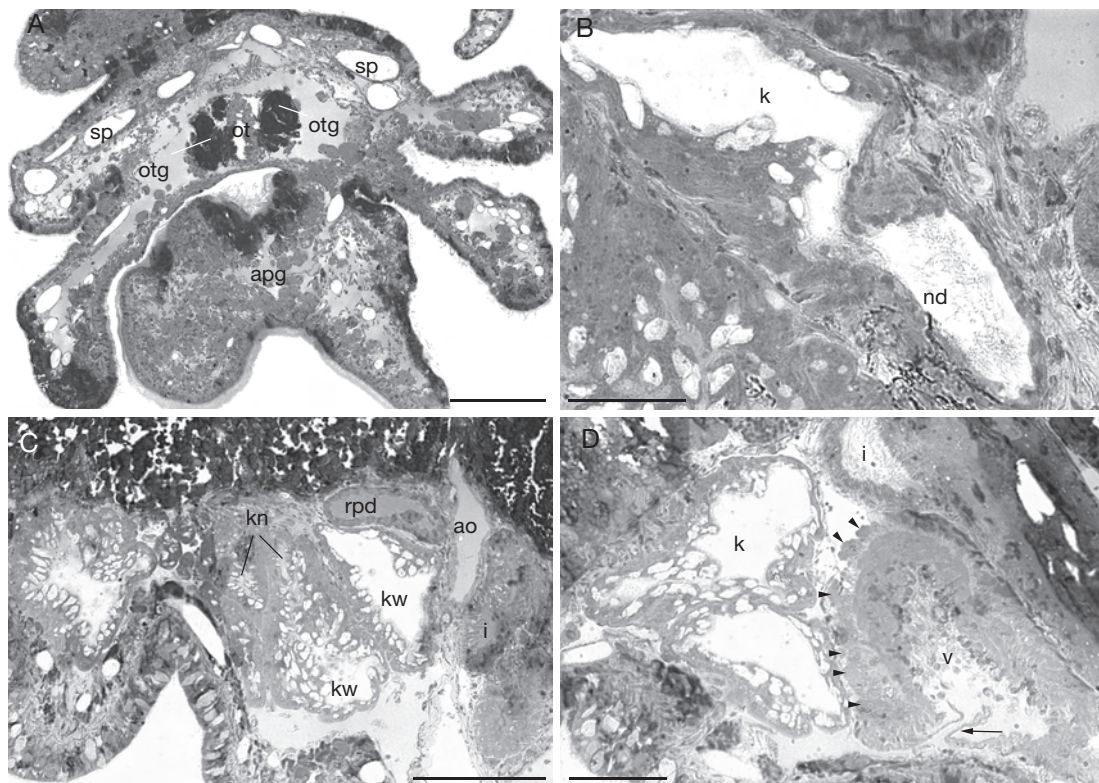


FIG. 8. — Transverse histological sections of *Pseudunela spiritusanta* n. sp.: **A**, oral tube gland, anterior pedal gland; **B**, transition kidney-nephroduct; **C**, renopericardial duct; **D**, heart, arrow points to valve; arrowheads point to epicardial cells of unknown function. Abbreviations: **ao**, aorta; **apg**, anterior pedal gland; **i**, intestine; **k**, kidney; **kn**, narrow lumen of kidney; **kw**, wide lumen of kidney; **nd**, nephroduct; **ot**, oral tube; **otg**, oral tube gland; **rpd**, renopericardioduct; **sp**, spicule cavity; **v**, ventricle. Scale bars: A, C, 100 μ m; B, 25 μ m; D, 50 μ m.

basal finger (bf) (Figs 5D; 6; 7A, B). Paraprostatic duct entering basal finger subapically and opening apically via a long hollow, slightly curved stylet (st) (c. 340 μ m long). Penis and basal finger not connected, surrounded by common penial sheath (ps) (Fig. 6).

DISCUSSION

HABITAT

Most of the Acochlidia are marine mesopsammic including *Pseudunela cornuta* (Challis, 1970) and *P. eirene* Wawra, 1988; only six of 27 valid species are limnic. *Palliohedyle weberi* (Bergh, 1895) is reported to live in a “brackish” habitat “in a river

mouth on [...] Flores” (Bergh 1895). However, despite a detailed sampling at the type locality, we could not find any acochlidian species near the sea (own unpubl. data). While all marine species are mesopsammic, the limnic species live benthically in coastal rivers of Indo-Pacific Islands or, i.e. the only Caribbean limnic species *Tantulum elegans*, in the interstices of a muddy mountain spring swamp on St. Vincent Island. *Pseudunela spiritusanta* n. sp. is thus unique regarding its habitat, not only by dwelling in a true brackish-water environment but also by living on the underside of large intertidal rocks. Kano *et al.* (2003) already described a neritiliid gastropod from this special, rarely explored habitat. Feeding habits of *P. spiritusanta* n. sp. are as yet unknown. Potential prey organisms living on the underside

TABLE 1. — Comparison of the external morphology within the genera *Pseudunela* Salvini-Plawen, 1973 and *Strubellia* Odhner, 1937. Abbreviations: **cns**, central nervous system; **vh**, visceral hump; **?**, no data available.

	<i>Pseudunela cornuta</i>	<i>Pseudunela eirene</i>	<i>Pseudunela espiritusanta</i> n. sp.	<i>Strubellia paradoxa</i>	<i>Strubellia paradoxa</i>
Data source	Challis 1970; Neusser <i>et al.</i> 2009b	Wawra 1988a	present study	Küthe 1935	Wawra 1988b
Body size (mm)	3; 3	4 (fixed)	9	20 (fixed)	20-30
Colour of body	translucent-whitish; translucent-whitish	?	translucent-whitish	light brown	
Colour of digestive gland	?; orange-brownish	?	yellowish	red-brownish	
Eyes visible externally	no; no	?	small, visible lateral	large, visible lateral	
Foot width	as broad as head; as broad as body	as broad as body	broader than body	broader than body	broader than body
Foot length	slightly longer than head; 1/2 of vh	?	2/3 of vh	2/3 of vh	2/3 of vh
Visceral hump	elongated; elongated	?	recurved	elongated	elongated
Heart bulb visible	?; yes	?	yes	yes	yes
Subepidermal calcareous spicules	absent; few in vh	?	bean-shaped; in cephalic tentacles, foot, vh, around cns	present	absent (after fixation)

of stones may be limited. *Pseudunela espiritusanta* n. sp. thus may prey upon mesopsammic organisms, although it was not yet found in the adjacent sandy interstices. Reporting a limpet-like brachiopod species attached to rubble deeply embedded in coarse sand in the mid intertidal, Kato (1996) emphasized potential advantages of this habitat, which provides protection from ultraviolet rays, desiccation, physical turbulence caused by wave action and benthic predators. Subsoil freshwater influence additionally may deter marine predators, but also requires mechanisms to cope with osmotic stress for all species including potential prey.

EXTERNAL MORPHOLOGY

The external appearance of *Pseudunela espiritusanta* n. sp. resembles that of the marine *Pseudunela cornuta* and *P. eirene* and the limnic *Strubellia paradoxa* (Strubell, 1892). While the shape of body and tentacles coincide in all four species (Küthe 1935; Challis 1970; Wawra 1974, 1988a), there are some differences regarding body sizes and colour, foot

width and length, shape of the visceral hump and the absence or presence of subepidermal calcareous spicules (Table 1).

MICROANATOMY

The anatomy of the cns of *P. espiritusanta* n. sp. broadly agrees with the typical acochlidian nervous system and resembles in different features the cns of *P. cornuta*, *S. paradoxa* (Challis 1970; Wawra 1988b) and *Tantulum elegans* (Neusser & Schrödl 2007). While anterior accessory ganglia were described as present in *T. elegans* and possibly *P. eirene*, they are absent in *P. cornuta*, *S. paradoxa* and our new species (Table 2) (Wawra 1988a, b; Neusser & Schrödl 2007; Neusser *et al.* 2009b). The optic ganglion innervating the eye is reported only from *T. elegans* and *P. espiritusanta* n. sp. (Neusser & Schrödl 2007), while the optic nerve emerges from the rhinophoral ganglion in *P. cornuta*, although an optic ganglion is present in this species as well (Neusser *et al.* 2009b). The thin nerve emerging at the base of the rhinophoral nerve and leading

TABLE 2. — Comparison of different anatomical characteristics within the genera *Pseudunela* Salvini-Plawen, 1973 and *Strubellia* Odhner, 1937. ?, no data available.

	<i>Pseudunela cornuta</i>	<i>Pseudunela eirene</i>	<i>Pseudunela espiritusanta</i> n. sp.	<i>Strubellia paradoxa</i>	<i>Strubellia paradoxa</i>
Data source	Challis 1970; Neusser <i>et al.</i> 2009b	Wawra 1988a	present study	Kütke 1935	Wawra 1988b
Accessory ganglia	present; absent	?	absent	?	absent
Optic ganglion	absent; present	?	present	absent	absent
Osphradial ganglion	absent; present	present	present	?	present
Gastro-oesophageal ganglia	absent; present	absent	present	absent	present
Radula formula	50 × 1.1.1; ?	52 × 1.1.2	67 × 1.1.2	48-56 × 2.1.2	48-51 × 2.1.2
Rhachidian cusp	projecting; ?	?	projecting	very elongate	very elongate
Rhachidian tooth denticles	3 or 4; ?	3 or 4	4-7	numerous, tiny	numerous, tiny
Anal-genital cloaca	present; absent	?	absent	present	?
Kidney	large, unfolded sac; long, internally divided	?	long, internally divided	long, internally divided	?
Long nephroduct with 2 branches	?: present	?	present	present, branches interconnected	?
Hollow curved penial stylet (µm)	100; 600	200	80	500	1000
Solid basal thorn (µm)	absent; absent	30	absent	present	present
Hollow curved stylet on basal finger (µm)	?: 110	?	340	?	?
Glands associated with copulatory organs	prostate, penial gland; prostate, paraprostate	?	prostate, paraprostate	2 glands	?
Receptaculum seminis	?: present	?	present	present	present
Bursa copulatrix	present; present	?	present	?	present

to a ciliated ridge just posterior to the rhinophore might be interpreted as the nerve leading to the Hancock's organ, just as reported for *T. elegans* (Neusser & Schrödl 2007). The ganglion connected to the suprainstestinal ganglion in *P. cornuta*, *P. eirene*, *P. espiritusanta* n. sp. and *S. paradoxa* is interpreted to be the osphradial ganglion according to Huber (1993), even though an osphradium has never been identified in any acochlidian species so far. Gastro-oesophageal ganglia were reported by Wawra (1988b, 1989) for *Strubellia paradoxa* and *Hedylopsis spiculifera* (Kowalevsky, 1901), as well as for *P. cornuta*, *Tantulum elegans* and *Asperspina murmanica* (Kudinskaya & Minichev, 1978) (Neusser & Schrödl 2007; Neusser *et al.* 2009a, b).

The digestive system of *P. espiritusanta* n. sp. conforms with that of other acochlidian species

studied in detail (Sommerfeldt & Schrödl 2005; Neusser *et al.* 2006, 2009a; Jörger *et al.* 2008). The radula resembles that of *Pseudunela cornuta* (Challis 1970) and *Asperspina murmanica* (Neusser *et al.* 2009a), but is considerably larger reflecting the overall larger body size. Both latter species show a rhachidian tooth with large, well-defined denticles and a large denticle on the first lateral tooth which has a pronounced notch at least in *A. murmanica*. While *P. cornuta* was described to have a symmetric radula with one lateral tooth (Challis 1970), *P. eirene* shows an asymmetric radula (Wawra 1988a) like the new species. The radula of *P. cornuta* should be re-examined in detail, as well as the radula of *Strubellia paradoxa* which was reported as symmetric with two lateral teeth on each side (Kütke 1935).

Only sparse data on the circulatory and excretory systems of Acochlidia are available. *Pseudunela cornuta*, *Microhedyle remanei* (Marcus, 1953) and *Pontohedyle milaschewitchii* (Kowalevsky, 1901) were found to possess well-developed, two-chambered hearts (Neusser *et al.* 2006, 2009b; Jörger *et al.* 2008). The heart of *P. spiritusanta* n. sp. is large, and the ventricle is strongly muscular. The valve detected at the posterior, thin-walled margin of the ventricle might be the connection to an equally thin-walled atrium, which possibly is collapsed or compressed and, thus, could not be detected. Highly conspicuous is the layer of irregular bulbous (epicardial?) cells covering the ventricle. Kütke (1935) has previously reported a layer of bulbous cells covering the heart of *S. paradoxa*. Their weak similarity to so-called pericardial glands of doridoidean nudibranchs (see e.g., Schrödl & Wägele 2001), which were shown to be podocytes by Fahrner & Haszprunar (2002a), might indicate that they are involved in ultrafiltration. The unique ventricular podocyte-like cells are thus shared by *Pseudunela spiritusanta* n. sp. and at least one of the limnic Acochliidiidae. If they are indeed podocytes, they likely enhance production of primary urine in species exposed to brackish or limnic conditions. Furthermore, *P. spiritusanta* n. sp. shows a complex excretory system as was reported from limnic acochlidian species, e.g., *Acochlidium amboinense* (Strubell, 1892), *S. paradoxa* and *T. elegans* (Bücking 1933; Kütke 1935; Rankin 1979; Neusser & Schrödl 2007). While marine acochlidians usually show a simple sac-like kidney with a short nephroduct (Neusser *et al.* 2006, 2009a; Jörger *et al.* 2008), all the freshwater species have a well-developed, complex kidney. The kidney of *P. spiritusanta* n. sp. is divided into two inter-connected compartments, and also has a long looped nephroduct which increases the surface area for resorption. *Strubellia paradoxa* was reported to have an internally divided kidney just as our new species (Kütke 1935); whether or not the branches of the nephroduct really show anastomose (Table 2) requires re-examination. Challis (1970) described the kidney of *P. cornuta* as a “large unfolded sac”; unfortunately without mentioning the (shape and length of the) nephroduct. Re-examination of *P. cornuta* from the type locality shows this species

has in fact a large, internally divided kidney and a long looped nephroduct (Neusser *et al.* 2009b). The marine *Hedylopsis ballantinei* was reported to possess a long, sac-like kidney extending almost over the entire visceral sac (Fahrner & Haszprunar 2002b; Sommerfeldt & Schrödl 2005); however, our re-examination revealed a complex kidney with a narrow duct extending posteriorly and a wide one leading anteriorly (own unpubl. data), just as in *P. cornuta*. Functionally, the well-developed excretory system of *P. spiritusanta* n. sp. clearly enhances excretion and resorption and can be regarded as an adaptation to their brackish-water habitat. Species adapted to subsoil freshwater influence such as *P. spiritusanta* n. sp. or intertidal mesopsammic species like *P. cornuta* which at least temporarily tolerate freshwater input (e.g., during heavy rainfalls) may have evolved preadaptations necessary for colonising limnic systems. Consequently, these habitats may have served as stepping stones for the colonisation of freshwater. Neusser *et al.* (2009b) proposed that the complex kidney has evolved in the mesopsammic ancestor of the marine Hedylopsacea and is assumed to be a preadaptation and key feature to both, independent invasions of a limnic habitat known from opisthobranchs.

The posterior reproductive system of *P. spiritusanta* n. sp. is interesting in possessing a proximal receptaculum seminis and a bursa-like duct in the typical distal position. In acochlidians, a full set of allosperm receptacles is only known from *Strubellia paradoxa* from Solomon Islands (Wawra 1988b), but is also present in *Pseudunela cornuta* (Neusser *et al.* 2009b). A sac-like ampulla is only reported from *Asperspina murmanica*, *Tantulum elegans* and *P. cornuta* (Neusser & Schrödl 2007; Neusser *et al.* 2009a, b), while the ampulla is a tubular swelling in *Pontohedyle milaschewitchii* and *Microhedyle remanei* (Neusser *et al.* 2006; Jörger *et al.* 2008). The nidamental glands were identified based on their position in the reproductive system from proximal to distal, i.e. the albumen, membrane and mucus gland, respectively. The mucus gland may be tubular as in *A. murmanica* and *P. milaschewitchii* but is a blind sac in *M. remanei* and *P. cornuta* (Neusser *et al.* 2006, 2009a, b; Jörger *et al.* 2008). In contrast, the membrane gland is tubular in all acochlidian species

examined except *P. spiritusanta* n. sp. A sphincter at the female gonoduct has never been observed in acochlidians, the function is unknown. A similar structure is present in the nudibranch *Goniodoris castanea* Alder & Hancock, 1845 (Wägele & Cervera 2001). The complex anterior male genitalia of *P. spiritusanta* n. sp. presents some similarities to marine species of the genus *Pseudunela* (Table 2). While *P. eirene* and the limnic *Strubellia paradoxa* seem to possess one larger, hollow penial stylet and a second smaller solid thorn (Küthe 1935; Wawra 1998a, b), the re-examination of *P. cornuta* revealed a hollow penial stylet connected to the prostate and an additionally stylet on the basal finger connected to the glandular paraprostata (Neusser *et al.* 2009b). Küthe (1935) observed two glands associated with the penial muscle in *S. paradoxa*, but did not identify them. There are no data concerning male glands in *P. eirene* available. A detailed (re-)examination of the basal marine genera *Hedylopsis* and *Pseudunela* and of the limnic *Strubellia paradoxa* is essential to homologise structures of the complex copulatory organs and associated glands.

TAXONOMIC REMARKS

External features such as the special tentacle shape leave no doubt that *P. spiritusanta* n. sp. is associated with the acochlidian genera *Pseudunela* and *Strubellia*, according to Schrödl & Neusser (in press) belonging to the hedylopsacean families Pseudunelidae Rankin, 1979 and Acochliidiidae *sensu* Wawra (1987), respectively. Comparing external and anatomical features clearly shows that *P. spiritusanta* n. sp. differs in many significant ways from other known *Pseudunela* and *Strubellia* species (Tables 1; 2).

A sketch of an acochlidian specimen found in marine sands of Lizard Island was provided by Burn (1998: fig. 16.42B as *Microhedyle cornuta*). Its tentacle shape is characteristic for both marine *Pseudunela* and limnic *Strubellia* species. While its broad foot contradicts identification as *P. cornuta* or *P. eirene*, the marine environment and still smaller body size (10 mm) argues against a placement into *Strubellia*. Burn's specimen resembles our new species by its size and body shape, and especially by also having a bent visceral hump. However, the

foot appears considerably shorter, and no spicules were recognized by Burn. In contrast to our species, Burn's species was found in marine sand samples (R. Burn pers. comm.) and most likely represents an undescribed *Pseudunela* species.

Although the specimens from Espiritu Santo clearly represent a new species, the generic placement of this species is problematic. From a taxonomic point of view, *P. spiritusanta* n. sp. does not correspond to any of the current generic diagnoses by Wawra (1987). According to the acochlidian phylogeny based on morphological data (Schrödl & Neusser in press), *P. spiritusanta* n. sp. possesses a mixture of features that are apomorphic for acochlidians (e.g., the asymmetric radula with relatively small rhachidian teeth), hedylopsaceans (short sperm head), and a clade composed of *Pseudunela* (marine interstitial), *Strubellia*, and Acochliidiidae (both limnic, benthic). Unique synapomorphies of *P. spiritusanta* n. sp., *Pseudunela* and Acochliidiidae *sensu* Arnaud *et al.* (1986), i.e. *Strubellia* plus Acochliidiidae *sensu* Wawra (1987), are the well-developed and externally visible heart bulb, and the fusion of the anterior portion of the visceral hump with the headfoot without showing a discernable mantle border (Schrödl & Neusser in press). With present, limited knowledge the only potential synapomorphies for *Pseudunela* and *P. spiritusanta* n. sp. refer to the very similar and special structure and arrangement of copulatory organs and associated glands in *P. spiritusanta* n. sp. and *P. cornuta* (Neusser *et al.* 2009b). On the other hand, the presence of bean-shaped spicules, a broad foot, and the layer of bulbous cells around the ventricle may be synapomorphic for *P. spiritusanta* n. sp. and *Strubellia*. For several features, *P. spiritusanta* n. sp. displays intermediate conditions, e.g., it is still marine but under freshwater influence, it is presumably neither mesopsammic nor lives benthically on stones, it is larger than marine interstitial species but still smaller than limnic ones. We conclude that the generic placement of our new species is uncertain; it could be either a basal offshoot of *Pseudunela* or of the *Strubellia*-Acochliidiidae clade (see Schrödl & Neusser in press). Its exact position will depend on a detailed re-examination of the inadequately known *Pseudunela* (Pseudunelidae) and *Strubellia* (Acochliidiidae *s.l.*)

species and a subsequent phylogenetic analysis. At the moment, we do not wish to introduce new generic or familiar names; we thus place our new and very special species into *Pseudunela*, with the caveats expressed above.

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REFERENCES

- ARNAUD P. M., POIZAT C. & SALVINI-PLAWEN L. VON 1986. — Marine-interstitial Gastropoda (including one freshwater interstitial species), in BOTOSANEANU L. (ed.), *Stygofauna Mundi*. Brill/Backhuys, Leiden: 153-161.
- BERGH R. 1895. — Die Hedyliiden, eine Familie der kladohepatischen Nudibranchien. *Verhandlungen der kaiserlich königlichen zoologisch-botanischen Gesellschaft Wien* 45: 4-12, 2 pls.
- BOUCHET P., LE GUYADER H. & PASCAL O. 2008. — Des voyages de Cook à l'expédition Santo 2006: un renouveau des explorations naturalistes des îles du Pacifique. *Journal de la Société des Océanistes* 126-127: 167-185.
- BOUCHET P., LE GUYADER H. & PASCAL O. (eds) in press. — The Natural History of Santo. *Patrimoines Naturels*.
- BÜCKING G. 1933. — *Hedyle amboinensis* (Strubell). *Zoologische Jahrbücher Abteilung für Systematik* 64: 549-582.
- BURN R. 1998. — Order Acochlidea, in BEESLEY P. L., ROSS G. J. B. & WELLS A. (eds), *Mollusca: The Southern Synthesis. Fauna of Australia*. Vol. 5, Part B. CSIRO Publishing, Melbourne: 959-960.
- CHALLIS D. A. 1970. — *Hedylopsis cornuta* and *Microhedyle verrucosa*, two new Acochliidae (Mollusca: Opisthobranchia) from the Solomon Islands Protectorate. *Transactions of the Royal Society of New Zealand, Biological Sciences* 12: 29-40.
- FAHRNER A. & HASZPRUNAR G. 2002a. — Ultrastructure of the renopericardial complex in *Hypselodoris tricolor* (Gastropoda, Nudibranchia). *Zoomorphology* 121: 183-194.
- FAHRNER A. & HASZPRUNAR G. 2002b. — Microanatomy, ultrastructure, and systematic significance of the excretory system and mantle cavity of an acochlidian gastropod (Opisthobranchia). *Journal of Molluscan Studies* 68: 87-94.
- HUBER G. 1993. — On the cerebral nervous system of marine Heterobranchia (Gastropoda). *Journal of Molluscan Studies* 59: 381-420.
- 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 and Evolution* 8: 194-214.
- KANO Y., KASE T. & KUBO H. 2003. — The unique interstitial habitat of a new neritilid gastropod, *Neritilia littoralis*. *Journal of the Marine Biological Association of the United Kingdom* 83: 835-840.
- KATO M. 1996. — The unique intertidal subterranean habitat and filtering system of a limpet-like brachiopod, *Discinisca sparselineata*. *Canadian Journal of Zoology* 74: 1983-1988.
- KÜTHE P. 1935. — Organisation und systematische Stellung des *Acochlidium paradoxum* Strubell. *Zoologische Jahrbücher, Abteilung für Systematik* 66: 513-540.
- 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., HASZPRUNAR G., HEß M. & SCHRÖDL M. 2006. — Computerbased 3-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., MARTYNOV A. V. & SCHRÖDL M. 2009a. — Heartless and primitive? 3D reconstruction of the polar acochlidian gastropod *Asperspina murmanica*. *Acta Zoologica* 90: 228-245.
- NEUSSER T. P., HEß M. & SCHRÖDL M. 2009b. — Tiny but complex – interactive 3D visualization

- of the interstitial acochlidian gastropod *Pseudunela cornuta* (Challis, 1970). *Frontiers in Zoology* 6 (doi: 10.1186/1742-9994-6-20).
- 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: 31-323.
- RUTHENSTEINER B. 2008. — Soft Part 3D visualization by serial sectioning and computer reconstruction. *Zoosymposia* 1: 63-100.
- SCHRÖDL M. & NEUSSER T. P. in press. — Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society*.
- SCHRÖDL M. & WÄGELE H. 2001. — Anatomy and histology of *Corambe luca* Marcus, 1959 (Gastropoda, Nudibranchia, Doridoidea), with a discussion of the systematic position of Corambidae. *Organisms, Diversity and Evolution* 1: 3-16.
- 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.
- STRONG E. E., GARGOMINY O., PONDER W. F. & BOUCHET P. 2008. — Global diversity of gastropods (Gastropoda: Mollusca) in freshwater. *Hydrobiologia* 595: 149-166.
- SPURR A. R. 1969. — A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* 26: 31-43.
- WÄGELE H. & CERVERA J. L. 2001. — Histological study of *Goniodoris castanea* Alder and Hancock, 1845 (Nudibranchia, Doridoidea, Goniadorididae). *Journal of Morphology* 250: 61-69.
- WAWRA E. 1974. — The Rediscovery of *Strubellia paradoxa* (Strubell) (Gastropoda: Euthyneura: Acochliidae) on the Salomon Islands. *The Veliger* 17: 8-11.
- WAWRA E. 1987. — *Zur Anatomie einiger Acochlidia (Gastropoda, Opisthobranchia) mit einer vorläufigen Revision des Systems und einem Anhang über Platybedylidae (Opisthobranchia, Ascoglossa)*. Dissertation Nr. 17335, University Vienna, Austria, 167 p.
- WAWRA E. 1988a. — Sand-Opisthobranchia aus dem Golf von Bengalen. *Annalen des Naturhistorischen Museums Wien* 90: 427-431.
- WAWRA E. 1988b. — *Strubellia paradoxa* (Strubell 1892) (Gastropoda: Opisthobranchia) von den Solomon-Inseln. *Zoologischer Anzeiger* 220: 163-172.
- WAWRA E. 1989. — Zur Kenntnis der interstitiellen Opisthobranchierart *Hedylopsis spiculifera* (Kowalevsky) (Mollusca, Gastropoda). *Zoologica Scripta* 18: 397-403.

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