

3D MICROANATOMY OF A GASTROPOD ‘WORM’, *RHODOPE ROUSEI*  
N. SP. (HETEROBRANCHIA) FROM SOUTHERN AUSTRALIA

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ABSTRACT

The turbellarian-like, radula-lacking *Rhodope* has been a mystery to taxonomists for over 160 years and was considered a specialized off-shoot of either opisthobranch or pulmonate Euthyneura. Occasionally reported from intertidal waters and sand habitats from all continents, most species of these minute slugs are poorly known and characterized mainly by differences in pigmentation. To understand the evolution of heterobranch microslugs, we established a morphological dataset for *Rhodope* by describing a new species found in the temperate waters of southern Australia. To set a standard for rhodopids, all major organ systems of *R. rousei* n. sp. are reconstructed three-dimensionally from series of semithin sections using the software Amira. Microanatomy confirms the loss of many general gastropod features such as foot, cephalic tentacles, shell, radula, mantle cavity, gill and heart. Excretory and digestive systems are heavily modified, with free rhogocytes in the presumed position of the heart, and a secondary buccal bulb replacing the function of the vestigial pharynx. Structural details of the monaulic but hermaphroditic genital system suggest cutaneous fertilization via spermatophores formed in specialized glands. The highly concentrated central nervous system is compared to those of other species of the genus and targets of all detectable nerves are summarized. These characters are compared with adaptations shown by other interstitial gastropods.

INTRODUCTION

The tiny, worm-like Rhodopomorpha are one of the true enigmas of gastropod systematics and have puzzled taxonomists since the description of the turbellarian-like *Rhodope veranii* (Kölliker, 1847) from intertidal algae in the Mediterranean. Originally considered to be a nudibranch, its molluscan nature was questioned shortly afterwards (Schultze, 1854 described the same species as a flatworm; Bergh, 1882). The anatomy of the millimetre-sized species is characterized by the absence of many typical gastropod features (shell, head tentacles, foot, gill, heart and radula) and the reduction of the excretory system. On the other hand, anatomical features that are present include spicules, a monaulic genital system with separate male and female follicles, and a subepidermal ‘vesicle’ system of unknown function (e.g. Graff, 1883; Böhmig, 1893; Riedl, 1960; Haszprunar & Künz, 1996). In particular, the asymmetry of organ systems and the ‘derived’ architecture of the nervous system led to the conclusive placement of *Rhodope* among euthyneuran gastropods (Böhmig, 1893; Riedl, 1960; see Riedl, 1959 and Salvini-Plawen, 1970 for reviews).

Special emphasis has been placed on the highly condensed central nervous system (CNS) when developing phylogenetic hypotheses. For example, the possession of five ganglia on the visceral cord and a parapedal commissure place the genus

within the Heterobranchia (*sensu* Haszprunar, 1985), and the high concentration of the ganglia was used to include *Rhodope* among ‘higher’ groups such as gymnomorph pulmonates (Salvini-Plawen, 1970) or nudibranch opisthobranchs (with double cerebro-rhinophoral connectives, see Haszprunar & Huber, 1990; Haszprunar & Künz, 1996). The possession of many features typical for meiofaunal opisthobranchs (e.g. worm-like shape, subepidermal spicules, adhesive gland; Swedmark, 1968) led to a grouping with the largely interstitial Acochlidia (Wägele & Klussmann-Kolb, 2005). On the other hand, Salvini-Plawen (1991) erected the taxon Rhodopomorpha—including the even more elongate worm-like and interstitial *Helminthope* Salvini-Plawen, 1991—as a “specialized off-shoot from the lower opisthobranchs” on the basis of the free visceral ganglion and its presumably primitive monaulity.

All these morphology-based assumptions must be reexamined in the light of new molecular results, which have led to reorganization of traditional euthyneuran relationships (Jörger *et al.*, 2010; Schrödl *et al.*, 2011), and specific results indicating that *Rhodope* may not belong to Euthyneura (Wilson, Jörger & Schrödl, 2010), but instead form a clade with the former pyramidellids *Ebala* and *Murchisonella* (referred to herein as Murchisonellidae). The exclusion of Murchisonellidae from true (panpulmonate) pyramidellids to the ‘lower heterobranchs’ was indicated only by molecular analyses (Dinapoli

& Klussmann-Kolb, 2010); internal anatomy is only fragmentarily known and no synapomorphies are yet known to support a relationship with the Rhodopemorpha.

To date, rhodopemorphs are known from occasional records from intertidal to subtidal sand habitats, from temperate and subtropical waters, on all continents (Rieger & Sterrer, 1975; Salvini-Plawen, 1991; Haszprunar & Heß, 2005 for review). Besides the clearly interstitial *Helminthope* and '*Rhodope*' *crucispiculata* Salvini-Plawen, 1991 (with cross-shaped spicules), there are four nominal species of *Rhodope*, including a species from southern Australia showing three conspicuous orange bands (Burn, 1990, 1998, 2006; *Rhodope* sp. 'E' in Haszprunar & Heß, 2005). *Rhodope* species are generally distinguished by characteristic external colour patterns consisting of transverse bands; at least seven colour forms are known, including European *R. veranii* and *R. roskoi* Haszprunar & Heß, 2005, Indian Ocean *R. transtrosa* Salvini-Plawen, 1991 and undescribed species from the Caribbean and Thailand (own unpublished data). However, there also are uniformly white species (Brazilian *R. marculsi* Salvini-Plawen, 1991 and several undescribed ones).

Anatomical knowledge about species of *Rhodope* is very heterogeneous. There are detailed studies of the CNS (Haszprunar & Huber, 1990), and ultrastructure of the epidermis and excretory system (Haszprunar & Künz, 1996; Haszprunar, 1997). Another organ system of taxonomic significance, the hermaphroditic genital system, is known only from schematic representations of *R. transtrosa* and *R. marculsi* (Marcus & Marcus, 1952; Salvini-Plawen, 1991). However, the most detailed anatomical (and the only histological description including the genital system) was carried out by Böhmig (1893) on *R. veranii* from Trieste, Italy; the distal genital system has not been examined in detail since.

The use of microanatomical methods such as computer-based three-dimensional reconstruction from series of semithin sections has proved to be a useful tool for unravelling features of internal anatomy of small to microscopic gastropods (DaCosta *et al.*, 2007; Neusser & Schrödl, 2007, 2009; Brenzinger, Wilson & Schrödl, 2010; Brenzinger *et al.*, 2011; Martynov *et al.*, 2011). Herein, we use these methods to describe the above-mentioned three-banded *Rhodope* in order to establish a modern anatomical dataset as a basis for further studies of the Rhodopemorpha. This species is known from Edithburgh, South Australia and San Remo, Victoria (present study; Burn, 1990, 1998, 2006).

## MATERIAL AND METHODS

### *Specimen sampling*

Specimens of *Rhodope rousei* n. sp. were collected from subtidal sand at Edithburgh Jetty, South Australia (35°5'5.15"S, 137°44'58.73"E; 4–8 m; 2004–2007). Specimens were isolated from bulk samples using elutriation and the concentrated sample was observed under a dissecting microscope. Specimens were photographed under a stereo-microscope. One specimen of an additional undescribed *Rhodope* (principally orange) was found in the same samples.

For histological study, a 2-mm specimen (paratype, ZSM Mol-20110168) was anaesthetized using 7% MgCl<sub>2</sub>·6H<sub>2</sub>O in fresh water (although it remained contracted) and fixed in 4% paraformaldehyde buffered with 2 M sodium cacodylate buffer (pH 7.4) with 0.3 M sucrose. The preserved specimen was later postfixed in 1% osmium tetroxide, dehydrated in a graded ethanol series and embedded in Spurr's resin.

### *Serial sectioning and 3D reconstruction*

Serial semithin sections of 1.5 µm thickness were obtained using a Histo Jumbo diamond knife (Diatome, Biel, Switzerland), a Microm HM 360 rotation microtome (Zeiss, Germany) and contact cement applied to the lower edge of the specimen block, following the method described by Ruthensteiner (2008). Ribbons of serial sections were collected on microscopy slides, stained with methylene blue/azure II dyes (Richardson, Jarett & Finke, 1960) and sealed with cover slips and Araldite resin.

For 3D reconstruction, photographs of sections containing all of the specimen and later only the CNS (taken at higher magnification) were taken using a SPOT CCD camera (Spot Insight, Diagnostic Instruments, Inc., Sterling Heights, MI, USA) mounted on a Leica DMB-RBE microscope (Leica Microsystems, Wetzlar, Germany). Photographs were converted to 8-bit greyscale TIF files prior to importing into AMIRA 4.1 and 5.1 software (TGS Europe, Mercury Computer Systems, Mérégnac, France; Visage Imaging GmbH, Berlin, Germany), resulting in aligned picture stacks of the body (487 photos, downsized to resolution of 1,024 × 768 pixels) and the CNS (66 photos at 1,600 × 1,200 pixels). Organ systems were labelled in the aligned series by hand, using interpolation and surface-smoothing tools to create the rendered 3D models shown. The histological series and AMIRA files are deposited at the Mollusca Department, Bavarian State Collection of Zoology, Munich, Germany.

The series was compared with identically prepared histological series of *Rhodope veranii* from Rat Kamenjak, Istria, Croatia, and an undescribed *Rhodope* from the Caribbean.

## SYSTEMATIC DESCRIPTION

### **Heterobranchia sensu Haszprunar, 1985**

### **Rhodopemorpha Salvini-Plawen, 1970**

### **RHODOPIDAE von Ihering, 1876**

### ***Rhodope* Kölliker, 1847**

### ***Rhodope rousei* new species**

(Figs 1–4)

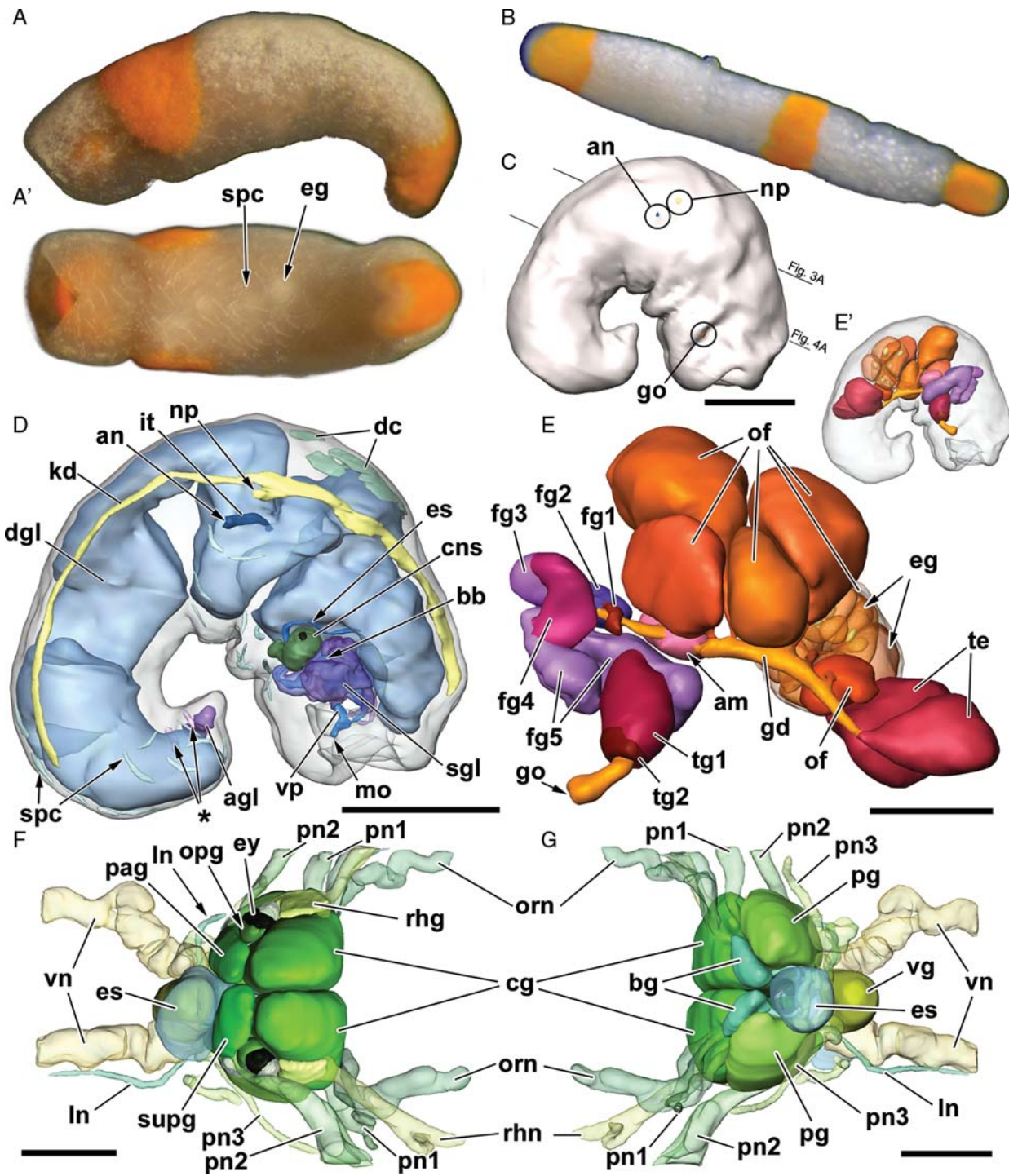
*Rhodope* sp. Burn, 1990: 9–15. Burn, 1998: 960–961. Burn, 2006: 1–42.

*Rhodope* sp. Brenzinger *et al.*, 2010: 269.

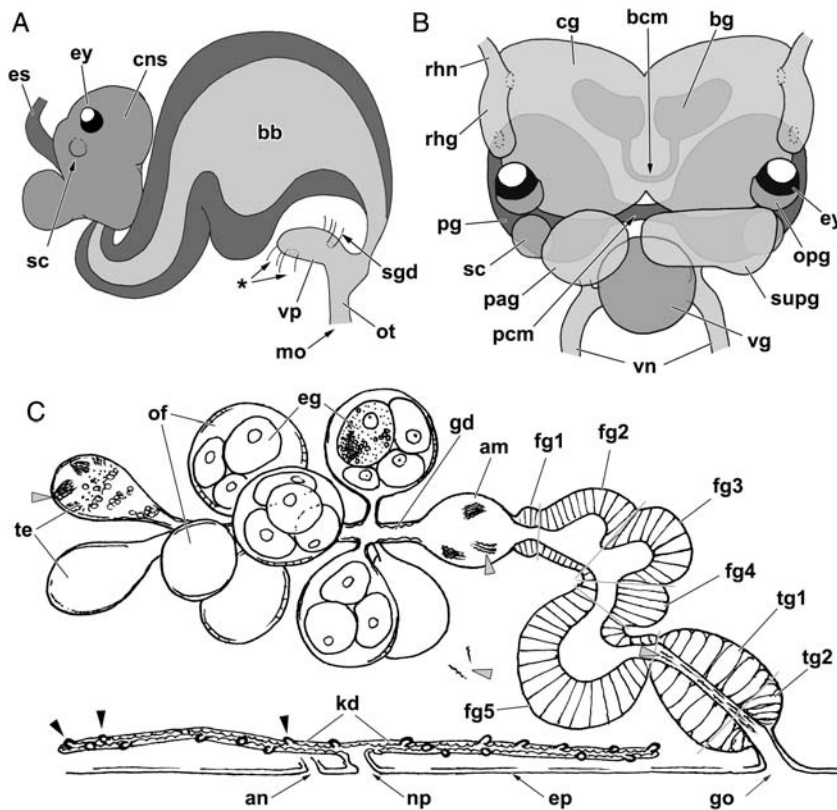
*Type material*: Holotype: complete specimen, anterior retracted, fixed in 10% formalin, stored in 75% ethanol; 2 mm preserved body length, collected under Edithburgh Jetty, South Australia, 27 February 2004, by N.G.W. and G. Rouse, deposited in Australian Museum, AM C.469551.

*Paratypes*: (1) Complete specimen, anterior retracted, fixed in 4% paraformaldehyde, serially sectioned by B.B. and used for 3D reconstruction (five slides). Preserved body length 2 mm, collected under Edithburgh Jetty, 21 March 2007, by N.G.W. and G. Rouse. Deposited in Mollusca Department, Bavarian State Collection of Zoology, Munich, Germany (ZSM Mol-20110168). (2) Complete specimen, anterior retracted, fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide and stored in buffer. Preserved body length 1.5 mm, collected under Edithburgh Jetty, 31 March 2006, by N.G.W. and G. Rouse, deposited in South Australian Museum, SAM D19405.

*Other material*: (1) Complete specimen, anterior retracted, fixed in RNAlater. Preserved body length 1 mm, collected under



**Figure 1.** Live specimens of *Rhodope rousei* n. sp. (A, B) and 3D reconstructions of internal anatomy (C–G). **A.** Holotype (AM C.469551), left view, *c.* 2 mm long. Head at left, retracted. **A'.** Same as **A**, ventral view. Note subepidermal spicules and whitish eggs visible through the body wall. **B.** Dorsolateral view of crawling specimen (AM C.469553), fully extended, *c.* 6 mm long. Head at right; note whitish epidermal glands in anterior portion of body. **C.** Three-dimensional reconstruction of paratype (ZSM Mol-20110168), showing external aspect and localization of body openings, right view. Bars show section planes of Figures 3A and 4A. **D.** Internal organ systems, genital system omitted. Right view. **E.** Genital system, left view. **E'.** Dimensions of genital system in the body, right view. **F.** CNS, dorsal view, anterior side to the right. Nerves are displayed slightly transparent. **G.** CNS, ventral view, anterior side to the left. Note several nerves projecting from the intersection between two ganglia. Abbreviations **A–E:** agl, caudal adhesive gland (asterisk: ciliated openings of adhesive gland); am, ampulla; an, anus; bb, buccal bulb; cns, central nervous system; dc, patches of subepidermal, spherical ‘dorsal cells’; dgl, digestive gland; eg, egg; es, oesophagus; fg1–fg5, nidamental glands (proximal to distal); gd, gonoduct; go, ciliated genital opening; it, intestine; kd, two-branched kidney; mo, mouth opening; np, nephropore; of, ovarian follicles; sgl, salivary gland; spc, subepidermal spicules; te, testes; tg1, barrel-shaped terminal gland; tg2, ring-shaped terminal gland; vp, putative vestigial pharynx. **F, G:** bg, buccal ganglion; cg, cerebropleural ganglion; ey, eye; es, oesophagus; ln, lateral nerves originating between pedal and ‘visceral’ ganglion; orn, oral nerve; opg, optic ganglion; pg, (left) parietal ganglion; pn1–pn3, pedal nerves; rhg, rhinophoral ganglion; rhn, rhinophoral nerve; supg, putative combined suprainstestinal and (right) parietal ganglion; vg, ‘visceral ganglion’ = putative combined subintestinal and visceral ganglion; vn, ‘visceral’ nerves. Scale bars: **C, D** = 250  $\mu$ m; **E** = 150  $\mu$ m; **F, G** = 50  $\mu$ m.



**Figure 2.** Schematic illustrations of anterior digestive, central nervous and genital systems of *Rhodope rousei* n. sp. paratype (ZSM Mol-20110168). **A.** Anterior digestive system and CNS, right view. Salivary glands omitted, openings of salivary ducts indicated by thin lines. **B.** CNS, showing organization of ganglia. Note that dorsal ganglia are separated only superficially. Dorsal view (see Fig. 1F). **C.** Genital system. Dorsal view, body wall below. Abbreviations: am, ampulla; an, anus; bb, buccal bulb; bcm, buccal commissure; bg, buccal ganglion; CNS, central nervous system; cg, cerebropleural ganglion; fg1–fg5, nidamental glands (proximal to distal); gd, gonoduct; eg, egg; es, oesophagus; ep, epidermis; ey, eye; kd, kidney; mo, mouth opening; np, nephropore; of, ovarian follicles; opg, optic ganglion; ot, oral tube; pag, (left) parietal ganglion; pcm, pedal commissure; pg, pedal ganglion; rhg, rhinophoral ganglion, note double cerebro-rhinophoral connectives; rhn, rhinophoral nerve; sc, statocyst; sgd, insertion point of salivary duct; supg, putative combined supaintestinal and (right) parietal ganglion; vg, ‘visceral ganglion’ = putative combined subintestinal and visceral ganglion; vn, visceral nerves; vp, putative vestigial pharynx; te, testes; asterisk in **A**: possible second pair of salivary ducts; arrowheads in **C**: bulbs of pseudo-protonephridia; grey arrowheads: (screw-shaped heads of) spermatozoa.

Edithburgh Jetty, 27 February 2004, by N.G.W. and G. Rouse, deposited in Australian Museum, AM C.469550. (2) Two complete specimens, both anterior retracted, fixed in 3% glutaraldehyde. Preserved body lengths 1.5–2.5 mm, collected under Edithburgh Jetty, 29 February 2004, by N.G.W. and G. Rouse, deposited in Australian Museum, AM C.469552. (3) Complete specimen, anterior retracted, fixed in 96% ethanol. Preserved body length 1 mm, collected under Edithburgh Jetty, 22 March 2007, by N.G.W. and G. Rouse, deposited in Australian Museum, AM C.469554. (4) DNA from one specimen, live crawling length 6 mm, collected under Edithburgh Jetty, 22 March 2007, by N.G.W. and G. Rouse, deposited in Australian Museum, AM C.469553.

*Other records:* Four individuals collected 11–15 February 2005, under Edithburgh Jetty. Photo record only, specimens lost.

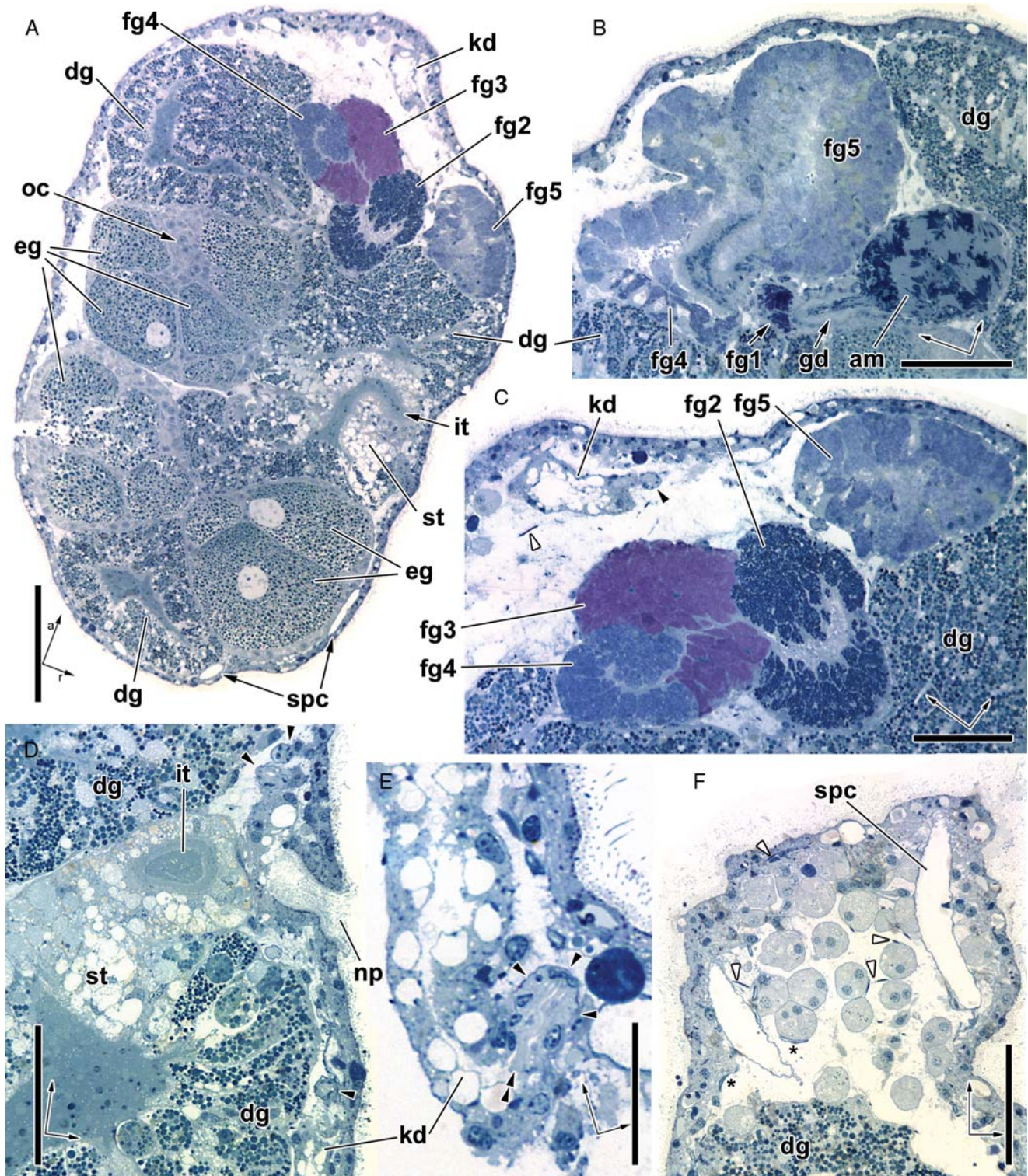
*Etymology:* The species is named for Greg Rouse, who introduced N.G.W. to the interstitial world, and who helped collect many specimens of interstitial heterobranchs.

*Distribution:* Species known from two localities in southeast Australia. Known from subtidal sand at Edithburgh Jetty, South Australia (present study); previous record and

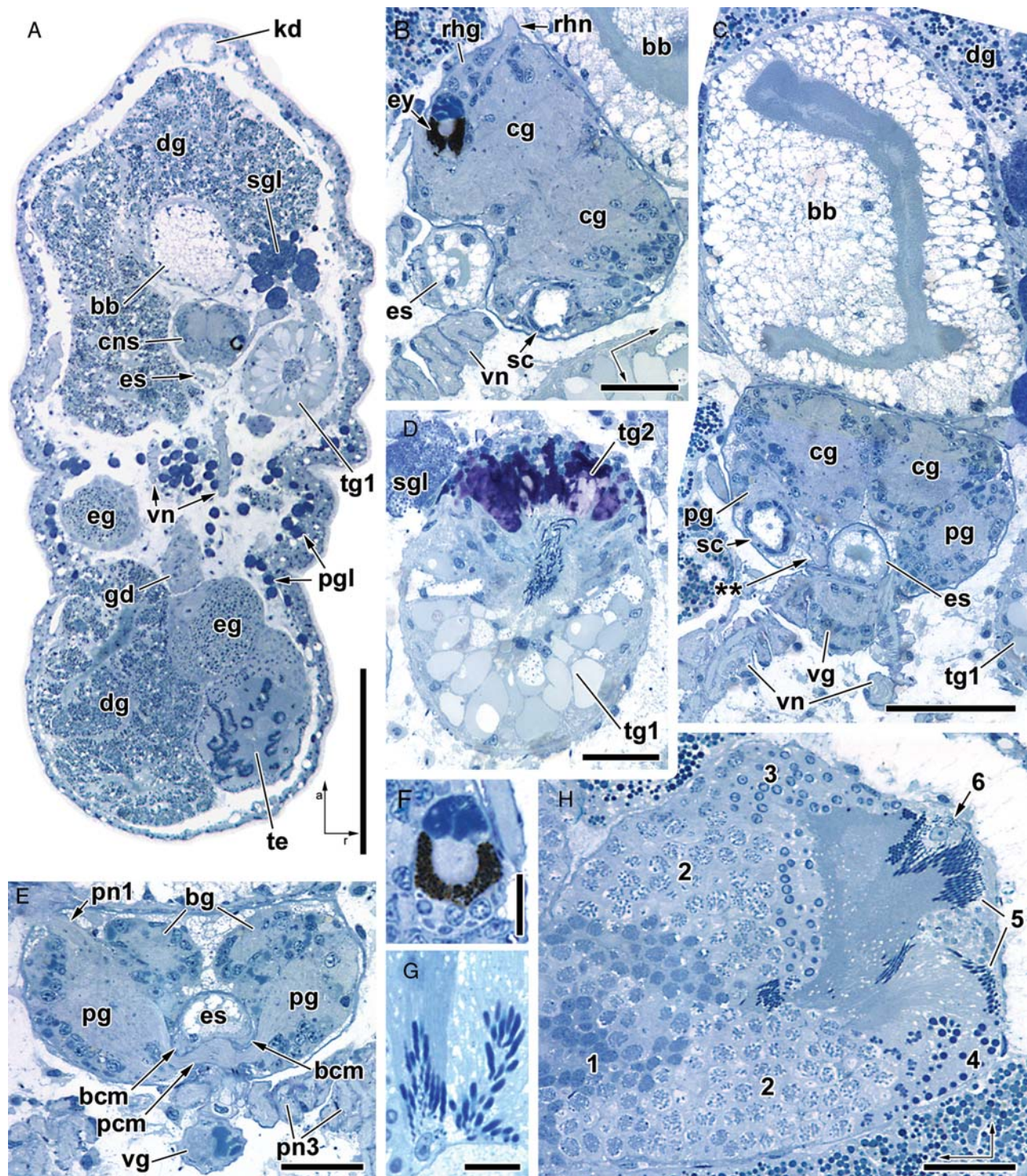
illustration of a single three-banded *Rhodope* “crawling on intertidal *Zostera* on a reef flat” at San Remo, Westernport, Victoria (Burn, 1990, 1998) is believed to refer to the same species.

*External morphology* (Fig. 1A–C): Body elongate and cylindrical in cross-section, with no marked cephalic appendages, mantle cavity, visceral hump or foot. Snout rounded with terminal mouth opening, retractable together with anterior quarter of body. Tail end sometimes broader and slightly flattened in crawling specimens, with slightly concave underside (position of the adhesive gland).

Distinguishable from other *Rhodope* species by characteristic orange pigmentation of snout, tail end, and transversal dorsal band at anterior third of body (a constriction of body visible just anterior to this band in contracted specimens; see Fig. 1A'). Rest of dorsal side opaque with white pigment; ventral side of body colorless, translucent. Genital opening at right side, close to anterior border of median transversal band; anus dextral and close to posterior border of band (middle of animal), nephropore slightly anterodorsal to anus. Subepidermal spicules and eggs visible through ventral epidermis, whitish spherical glands through dorsal side.



**Figure 3.** Semithin histological sections from *Rhodope rousei* n. sp. paratype (ZSM Mol-20110168). Anterior/right pictograms next to scale bars indicate orientation of section relative to animal. **A.** Longitudinal section through midsection of curved body (level of section indicated in Fig. 1C). **B.** More anteroventral section showing both female gland 1 and 5, and ampulla. **C.** Female glands 2 to 4, enlarged from **A.** **D.** Right body side showing stomach (light wall), intestine and nephropore. **E.** Longitudinal section through kidney and pseudo-protonephridium, showing ciliary flame. Epidermis at right. **F.** Putative rhogocytes (spherical 'dorsal cells'; note double nuclei in some) and parts of the 'vesicle system' below the dorsal epidermis. Abbreviations: am, ampulla filled with batches of autosperm; dg, digestive gland; eg, egg; fg1–fg5, midamental glands (proximal to distal); gd, gonoduct; it, intestine; kd, kidney; np, nephropore; oc, oocytes; spc, spicule; st, stomach (wall); arrowheads in **C–E**: cross-section of pseudo-protonephridium, characterized by strong basal lamina; double arrowhead in **E**: ciliary flame of pseudo-protonephridium; white arrowheads in **F**: spermatozoon in body cavity; asterisks in **F**: putative tubes of 'vesicle system'. Scale bars: **A** = 100  $\mu$ m; **B–D, F** = 50  $\mu$ m; **E** = 20  $\mu$ m. This figure appears in colour in the online version of *Journal of Molluscan Studies*.



**Figure 4.** Further semithin histological sections from *Rhodope rousei* n. sp. paratype (ZSM Mol-20110168). See anterior/right pictogram next to scale bar in **A** for orientation (omitted in others if orientation the same). **A.** Longitudinal section through curved body (see Fig. 1C for level of section; other sections are more ventral). **B.** Longitudinal section through CNS, rather dorsal level. **C.** Buccal bulb and CNS, middle level. **D.** Terminal glands of genital system at intersection between first and second part. Note spermatozoa inside lumen. **E.** CNS, rather ventral level. **F.** Right eye. Note corneal lens lacking a cornea, optic ganglion below pigment cup. **G.** Enlarged area of testis wall showing almost ripe (right) next to ripe spermatozoa with nutritive cell. **H.** Section through testis showing densely packed areas of premeiotic spermatogonia/spermatids (1,2), postmeiotic spermatocytes (3,4), and ripe spermatozoa (5) crowding around nutritive cell (6). Abbreviations: bb, buccal bulb; bcm, buccal commissure; bg, buccal ganglion; cg, cerebropleural ganglion; ens, central nervous system; dg, digestive gland; eg, egg; es, (distal part of) oesophagus; ey, eye; gd, gonoduct; kd, kidney; pcm, pedal commissure; pg, pedal ganglion; pgl, pedal ganglion; pn1, anterior pedal nerve; pn3, posterior pedal nerve; rhg, rhinophoral ganglion; rhn, rhinophoral nerve; sc, statocyst; sgl, salivary gland; te, testis; tg1, barrel-shaped terminal gland; tg2, ring-shaped terminal gland; vg, 'visceral ganglion' = putative combined subintestinal and visceral ganglion; vn, visceral nerves; double asterisk in **C**: connective between left parietal and 'visceral ganglion'; numbers in **H**: see above. Scale bars: **A** = 200 μm; **B, D, E, H** = 25 μm; **C** = 50 μm; **F, G** = 10 μm. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

**Body wall** (Figs 3, 4): Epidermis *c.* 8  $\mu\text{m}$  thick, strongly ciliated all around. Cells with large vacuoles interspersed. Body wall musculature indistinct. Extent of orange pigmentation not detectable in histological sections. Subepidermal spicules scattered below epidermis, oriented roughly at 45° angle to body axis. Spicules *c.* 100–120  $\mu\text{m}$  long, curved, narrowing towards tips (Fig. 3F). Spicule body dissolved in histological sections, surrounding layer hints at slightly rough surface. Dark-staining nucleus of spicule cell located in middle of concave side (Fig. 3F). Thin tubes of ‘vesicle system’ visible in sections below the dorsum, close to patches of spherical ‘dorsal cells’ (see Excretory system; Fig. 3F). Numerous monocellular pedal glands (diam. to 20  $\mu\text{m}$ ) below ventral epidermis (Fig. 4A), staining dark blue, each oval cell opening through individual apical duct. Subepidermal adhesive gland in ventral side of tail end appearing as aggregation of smaller glandular cells with grainy blue-staining interior. Adhesive gland opening through paired ciliated grooves situated lateroventrally (Fig. 1D). No aggregated muscle fibres spanning or delimiting body cavities.

**Digestive system** (Figs 1D, 2A): Mouth opening a transversal slit terminal on snout, followed by very short oral tube. Blind sac of about 30  $\mu\text{m}$  length projecting from ventral side of oral tube; pair (possibly two) of salivary ducts opening into supposedly vestigial pharynx. Large salivary glands aggregations of oval, droplet-filled cells (staining dark blue, some with violet tinge; Fig. 4A), located left and right of oesophagus. Oesophagus lined with thick layer of irregularly sorted, vacuolated cells and mostly basal nuclei; anterior portion thin and curved. Middle part of oesophagus greatly enlarged (buccal bulb), forming an elongate oval bulb with very thick cushion-like wall and flat, ciliated lumen (Fig. 4C). Posterior part of oesophagus rather long and very thin, curving upward through cerebral nerve ring and leading into digestive gland. Tubular digestive gland with irregular inner surface of columnar, droplet-filled epithelial cells (e.g. Fig. 3B, D); short branch of gland extending

anteriorly from where oesophagus enters, long and undulated posterior branch extending to tail end of animal (Fig. 1D). Vacuolate, not droplet-filled, area of digestive gland wall at right body side (stomach); short and ciliated intestine exits stomach and opens at right body side (Fig. 3A, D).

**CNS and sensory organs** (Figs 1D, F, G, 2B, 4, Table 1): CNS a dense mass of ganglia posterior to buccal bulb, encapsulated within thin connective sheath, gaps filled with loose tissue (Fig. 4E). In large ganglia, nuclei located along periphery; central medulla a homogeneous mass, slightly fibrous, similar to nerves in histology (Fig. 4B, C). Nerves and their targets are summarized in Table 1.

Very large paired anterodorsal ganglia (cerebropleural ganglia) touching medially and separated only by slight superficial groove; cerebral commissure detectable as broad connection of medulla (Fig. 4B). Pigment-cup eyes (diam. 20  $\mu\text{m}$ ) located at posterior sides of cerebropleural ganglia; eyes face dorsally, lacking cellular cornea but with lens consisting of discrete cells (Fig. 4B, F). Eyes cradled by cup-shaped optic ganglia containing less than 10 nuclei; optic nerves not detectable. Elongate rhinophoral ganglia located anterior to eyes, with double cerebro-rhinophoral connectives: one connective close to the base of the ganglion, the second at tip leading into rhinophoral nerve (Figs 2B, 4B). Paired oral nerves very thick (Fig. 1G), numerous nuclei surrounding nerve fibres at nerve’s base similar to rhinophoral ganglia; oral nerves extend anteroventrally from superficial gap in cerebropleural ganglia.

Paired medium-sized ganglia connecting broadly to posterior side of cerebropleural ganglia, divisible externally by shallow dorsal constrictions; left ganglion less wide (left parietal ganglion) than right (combined suprintestinal and right parietal ganglion) (Figs 1F, 2B). Medium-sized, spherical posterior ‘visceral’ ganglion (combined subintestinal and visceral ganglion) joined to latter ganglia posteroventrally by connectives of medium length extending around oesophagus (see Fig. 4C). Two thick, double-rooted ‘visceral’ nerves extend from intersection of latter ganglia and pedal ganglia (Fig. 4C); thick root inside ‘visceral’ ganglion, thin root in region of cerebropleural and parietal ganglia. ‘Visceral’ nerves very thick, undulated especially at base, containing single nuclei interspersed along their length; nerves extend parallel along ventral side of body to tail.

Large paired pedal ganglia below cerebropleural ganglia; cerebropedal connectives short and wide, pleuropedal connectives not detected, pedal commissure longer, parapedal commissure not detected. Paired spherical statocysts, slightly larger than eyes, embedded in dorsal part of each pedal ganglion (Fig. 4B); hollow capsule of few cells surrounds cavity containing remnants of single statolith. Static nerve not detectable. Three pairs of pedal nerves detectable (Fig. 1: ‘pn1’ to ‘pn3’): First pair rather thick and extending from anterior side of each pedal ganglion (Fig. 4E), second pair very thick and extending from just anterior to statocysts, with thick second root in region of pleural ganglia, third pair extending laterally from close to base of pedal commissure. Fourth pair of thin nerves extending laterally from gap between pedal and visceral ganglia (‘lateral’ nerves in Fig. 1F, G) appears rooted in visceral and possibly parietal ganglia.

Paired buccal ganglia medium-sized, located in anteroventral depression between cerebropleural and pedal ganglia (Fig. 4E). Cerebrobuccal connectives short; buccal commissure rather long and thin, looping around oesophagus close to pedal commissure. Paired buccal nerves medium-sized and with very few nuclei, extending anteriorly along sides of buccal bulb (not shown).

**Table 1.** Summary of nerves in *Rhodope rousei* n. sp. paratype (ZSM Mol-20110168).

Nerve	Abbreviation in Figs	Rooted in	Targets
Rhinophoral nerve	rhn	cg/rhg	Sides of snout, branch into salivary glands, and along dorsal sides of cephalic caecum
Oral nerve	om	cg	Sides of mouth opening, running between salivary glands and buccal bulb
Pedal nerve, anterior	pn1	pg	To anterior ventral side, flanks
Pedal nerve, lateral	pn2	pg + cg	To flanks and running posterior
Pedal nerve, posteroventral	pn3	pg	To anterior ventral side, median side
Lateral nerve	ln	pag + vg	Right side: parallel to right vn Left side: curves anteriorly
Visceral nerve	vn	vg + pag/ supg	Parallel up to tail end/ adhesive gland
Buccal nerve	—	bg	Sides of buccal bulb

**Excretory system** (Figs 1D, 2C, 3C–F): Kidney consisting of two tubular branches (collapsed diameter 50 µm) extending anterior and posterior from ciliated nephropore along right dorsolateral side. Epithelium of kidney containing rounded vacuoles; knob-shaped pseudo-protonephridia (diam. ca. 10 µm) protruding from vacuolate epithelium in irregular intervals. Each knob formed by capsule of few flat cells, outer border discernible in histological sections by conspicuously strong basal lamina (arrowheads in Fig. 3C–E); ciliary flame inside lumen of each knob, directed towards kidney lumen (Fig. 3E). Spherical light-blue-staining cells (diam. ca. 15 µm), some with two nuclei, located in loose aggregations below dorsal epidermis anterior to nephropore ('dorsal cells' = putative rhogocytes; Fig. 3F).

**Genital system** (Figs 1E, 2C, 3A–C, 4A, D, G, H): Monaulic genital system hermaphroditic, with spermatozoa and oocytes in separate acini. Posterior two acini (testes) drop shaped, containing spermatozoa and their precursors in distinct stages of development, sorted in batches (Fig. 4H). Following gonoduct a muscular (circular fibres) and ciliated tube, six roughly spherical ovarian acini of different sizes extending on thin stalks (Fig. 1E). Ovarian acini containing dense batches of oocytes close to the epithelial wall and 2–10 yolk-rich developing eggs (diam. to 120 µm) inside, each egg with clear nucleus and darker nucleolus (Fig. 3A). Last ovarian acinus followed by roughly spherical ampulla filled with irregularly sorted bundles of spermatozoa (Fig. 3B). Epithelium of postampullary gonoduct developed into five distinct (nidamental) glands, separable in sections by constitution of tissue and its staining properties: First nidamental gland a very small ring of small cells staining dark blue, second gland a larger sac-like extension with higher epithelium stained by dark blue granules, third gland an equally sized sac staining homogeneously pink, fourth gland a medium blue-staining tube with regular epithelium and fifth gland largest, a sac-like extension with rather loose epithelium staining light blue (Fig. 3A–C). Ciliated lumen of nidamental glands followed by compound tube of two 'terminal' glands surrounding gonoduct: first terminal gland barrel-shaped and circular in cross-section, formed by regular epithelium of apparently holocrinous glandular cells containing light blue staining vacuoles and basal nuclei; second terminal gland a short ring of columnar, irregularly dark violet staining cells (Fig. 4A, D). Gonoduct inside terminal glands filled densely with autospermatozoa (sperm heads pointing distally). Gonoduct following terminal glands short and thick, forming the strongly ciliated genital opening. Allospermatozoa with screw-shaped heads found freely distributed in entire haemocoel (highlighted in Fig. 3C, F), sometimes lodged in lining of organs, such as CNS.

## DISCUSSION

### *Taxonomic remarks*

The latest review of rhodopid species by Haszprunar & Heß (2005: table 1) recognized four described *Rhodope* (the type *R. veranii*, *R. marcusii*, *R. transtrosa* and *R. roskoi*) besides at least five undescribed species. These included *Rhodope* sp. 'E' which differs from all other known species by its possession of three orange bands. We regard our *Rhodope rousei* n. sp. from Edithburgh, Victoria to be conspecific with the aforementioned one from Westernport, Victoria (Burn, 1990, 1998, 2006), because they share the unique three-banded pattern and are both distributed in temperate southeastern Australia. Comparing it with the few *Rhodope* species known in anatomical detail, *R. rousei* n. sp. most resembles the (presumed)

Indo-Pacific *R. transtrosa* (with a single orange band) in general morphology of the CNS (superficial gaps between the cerebropleural and parietal ganglia), in the length of the pedal and buccal commissures (comparatively long) and in the size of the eyes and statocysts (relatively large in comparison to the CNS) (Haszprunar & Huber, 1990). The set of nerves identified herein corresponds well to what is known for *R. veranii* and *R. transtrosa* (Haszprunar & Huber, 1990; Huber, 1993); differences are the lack of a "clearly detectable" parapetal commissure as in *R. transtrosa* and the presence of three pairs of pedal nerves instead of only one (including the double-rooted nerve termed 'pn2' herein). Two nerves leaving the 'visceral' ganglion present another shared character with *R. transtrosa*, but in *R. rousei* n. sp. the two 'visceral' nerves appear more symmetrical in their size and origin. The thin lateral nerves herein were not shown for the other species but, judging from its position, could as well refer to the right 'pallial' nerve. The genital system differs from that of *R. transtrosa* (described by Salvini-Plawen, 1991) in its possession of distinct terminal glands in the gonoduct and of more than three nidamental glands.

The nervous system presents a difficult object of study due to its strong fusion, but there appear to be morphologically 'derived' species with strongly fused ganglia (i.e. *R. veranii* in Haszprunar & Huber, 1990; *R. roskoi*: own observation) and those with superficially separated ones (*R. transtrosa*; *R. rousei* n. sp.). The genital system appears not to show much interspecific variation except in the number of ovarian follicles. The needle-like spicules were previously regarded as species-specific, e.g. by Haszprunar & Huber (1990), but have not been used to delimit species and appear not to show much interspecific variation.

We conclude that the pigmentation of *Rhodope* is still the best means to separate species but, with more data available, micro-anatomical information may be of taxonomic use in future. In this study we intend to set a new standard for anatomical comparison of rhodopemorph species.

### *Digestive system*

The digestive system of *Rhodope* is highly modified due to the lack of a radula and the tubular digestive gland with a branch leading into the head (Böhmig, 1893). Especially the parts between mouth opening and digestive gland appear to be specialized for sucking soft and liquid food using the conspicuous buccal bulb; this structure appears to represent a shared feature of the Rhodopemorpha (also present in *Helminthope*; Salvini-Plawen, 1991). Judging from histology and anatomy of *Rhodope rousei* n. sp. we conclude that (1) this buccal bulb is a specialized part of the oesophagus—i.e. not homologous with the otherwise muscular pharynx of other heterobranchs as was previously assumed, and (2) that a vestige of the original pharynx is present as the blind sac close to the mouth opening into which the salivary glands open. The first is supported by essentially identical histological properties of the buccal bulb and the adjoining thinner parts of the oesophagus (the bulb is not muscular or otherwise differentiated except for its size) and that it lacks the insertion of salivary glands typical for the pharynx (see below). Regarding the second, the vestigial pharynx (mentioned by Böhmig, 1893 and Salvini-Plawen, 1991 as an "outlet of the oral glands") can be identified as such from the salivary ducts entering there, and from the observation during ontogeny of *R. veranii* that buccal ganglia develop from ectoderm just next to the mouth opening, just next to a pharyngeal anlage with a rudimentary radula (Riedl, 1960).

Pumping of the buccal bulb by dilation might be facilitated by the densely vacuolated epithelium forming an elastic wall,



although ingestion of food appears to be strongly dependent on ciliary motion (Riedl, 1959). Riedl observed *R. veranii* to be specialized for feeding on the planula-like placozoan *Trichoplax* (which is not a sponge larva, as assumed by Burn, 1998); however, bacterial assemblages, large protists or soft-shelled eggs might also fit within the food spectrum.

So far the described variation of the digestive system of *Rhodope* relates to the presence of oral glands opening next to the mouth (not obvious herein, but histologically separable from salivary glands: Böhmig, 1893; Marcus & Marcus, 1952; own observation on *Helminthope*), the form of the salivary glands (sac-like: Marcus & Marcus, 1952; or consisting of numerous acini: Graff, 1883; present study), and where the salivary ducts open (directly into the buccal bulb: Marcus & Marcus, 1952; or close to the mouth: Böhmig, 1893; present study). Judging from semithin sections, the connection to the buccal bulb is likely a mass of salivary glands that opens into the short blind sac protruding from the oral tube just behind the mouth opening. Since there appears to be more than one pair of ducts leading there, *R. rousei* n. sp. might have oral glands that are histologically similar to the salivary glands and embedded within those.

#### Central nervous system

The highly condensed, euthyneuran CNS of *Rhodope* has repeatedly been used to place the taxon among ‘derived’ heterobranchs, i.e. Euthyneura such as nudibranchs or gymnomorph pulmonates (Salvini-Plawen, 1970; Haszprunar & Huber, 1990). Judging from molecular results by Wilson *et al.* (2010), many previously assumed synapomorphies (strong fusion of ganglia, double cerebro-rhinophoral connective) are thus either analogies or simply plesiomorphic for Heterobranchia, and not synapomorphies for opisthobranchs and pulmonates, as suggested by Jörger *et al.* (2010).

Presence of giant nerve cells, a character of Euthyneura (see Haszprunar, 1985), is not evident from any of the examined material, but might be connected to the miniaturization.

The fusion of cerebral, pleural and visceral-loop ganglia in *Rhodope rousei* n. sp. is striking. While not as extreme as in *R. veranii*, it resembles closely the condition shown in *R. transtrosa* (Haszprunar & Huber, 1990). The cerebral ganglia touch broadly and the cerebral commissure is almost as thick as the contacting zone. The fusion of the pleural ganglia with the posterior part of each cerebral ganglion was observed in adult and larval *R. veranii* by Riedl (1960) and was deduced from the presence of two almost parallel connectives running from the cerebropleural ganglia into the pedal ganglia (Haszprunar & Huber, 1990); we follow this interpretation of fused cerebropleural ganglia, although a distinct pleuro-pedal connective was not detected.

Due to their fusion and close contact with the cerebropleural ganglia, the ganglia of the visceral loop can only be identified with knowledge of the ontogeny. Five separate ganglia have been observed in developmental stages of *R. veranii* (Riedl, 1960) and later fuse in a pattern which can be inferred to be present also in *R. rousei* n. sp.: three of the visceral loop ganglia are joined closely to the posterior end of the cerebropleural ganglia from which they are separated by superficial incisions. The right part is relatively larger than the left one, which can be explained—following the nomenclature used by Haszprunar (1985)—from the (also observed) fusion of both the right parietal and the supraintestinal ganglion to the cerebropleural ganglion, while on the left side only the (left) parietal ganglion is merged with the posterior side of the cerebropleural ganglion. The free ganglion below the oesophagus is ontogenetically derived from the subintestinal and visceral ganglion, which fits with the presence of two nerves leaving this

ganglion, at least one of them likely to be homologous with the ‘true’ visceral nerve. The two nerves appear more or less symmetrical herein, but Haszprunar & Huber (1990) described two functions: a thick ‘pallial’ and a thinner, left, ‘genitovisceral’ nerve.

It should be noted that *Rhodope* is one of few heterobranchs where fusion of ganglia on the visceral loop has not been deduced solely from relative size and emerging nerves, a practice criticized by Dayrat & Tillier (2000). Together with *Helminthope*—which has been described with five free ganglia on the visceral loop (Salvini-Plawen, 1991)—the rhodopids appear to be Pentaganglionata (=Euthyneura) in the literal sense, although they formally fall outside of this taxonomic grouping judging from molecular phylogenetic data.

The pedal ganglia show three nerves, one of which shares a second root with the posterior part of the cerebropleural ganglia; this configuration is similar to that described for *R. veranii*, but not *R. transtrosa* which has been depicted with only a single pedal nerve (Haszprunar & Huber, 1990). The buccal ganglia (long connective in *R. rousei* n. sp. and *R. transtrosa*) are not reduced as suggested by Riedl (1960) and Oberzeller (1969), but are clearly developed and show conspicuously thick nerves which, judging from their position, innervate the buccal bulb and oesophagus.

Some of the very thick nerves of *R. rousei* n. sp. reflect the strong fusion of the ganglia by being rooted within two ganglia (Table 1) or branching close to or from a connective (e.g. the ‘lateral’ nerves herein; also the optic nerve reported by Haszprunar & Huber, 1990). Distinct neurons can be found within e.g. the oral and ‘visceral’ nerves, giving the nerves the appearance of medullary cords. These neurons are however never organized into ‘true’ ganglia (with distinct, external cortex) and also are not aggregated in thicker areas of the nerves (both being the case in *Helminthope*; own observation; Salvini-Plawen, 1991). The presence of neurons within the nerves presents an analogous character to that of other meiofaunal gastropods such as some philinoglossids (Marcus & Marcus, 1954), microhedylacean Acochlidia (Neusser *et al.*, 2006; Jörger *et al.*, 2008) or the sacoglossan *Platyhedyle* Salvini-Plawen (Rückert, Altnöder & Schrödl, 2008). The presence of accessory ganglia in these miniaturized species has been interpreted as adding extra neurons to a CNS that would otherwise be too small (Haszprunar & Huber, 1990).

Again, the CNS of *Rhodope* can be stated to show a mosaic of features that are likely to be ancestral for heterobranchs (double rhinophoral connective, see Neusser, Jörger & Schrödl, 2007; Jörger *et al.*, 2010) and those that appear highly derived (extreme fusion of ganglia) or induced by the aberrant worm-like morphology and miniature size (‘outsourced’ ganglia). Whether giant nerve cells (as a character of Euthyneura *sensu* Haszprunar, 1985) are present in *Rhodope* or not cannot be clarified from the present material.

#### Sensory organs

The eyes and statocysts are the most prominent sensory organs and are visible in live specimens, especially by transmitted light. Both organs are relatively large (compared to the rest of the CNS), differing from *R. veranii* but resembling the condition in *R. transtrosa*, as shown by Haszprunar & Huber (1990).

The peculiar pigment-cup eyes (no cellular cornea, ‘corpuscular’ lens with distinct cell borders) were first shown by Böhmig (1893) and their development—with ingression of primary corneal cells into the lens/vitreous body—was described by Riedl (1960). This peculiar feature appears to be derived in *Rhodope*, since the eyes of *Helminthope* do not show the corpuscular lens (own observations). Whether this

modification of the eyes affects visual performance significantly remains unclear, but it appears that the visual apparatus of *Rhodope* is not subjected to strong selection, as developmental malformations involving the eyes appear to be rather common: examples are the formation of double lenses in one eye with the other one lacking (Graff, 1883) or the formation of four eyes (Riedl, 1959, 1960).

Further sensory structures such as an osphradium or Hancock's organs are not detectable in the present material and are not reported for other species. Haszprunar & Künz (1996) mentioned sensory cells interspersed within the epidermis. According to Riedl (1960), a pit-shaped osphradium and osphradial ganglion are briefly present during early ontogeny and are innervated from the suprainestinal ganglion by what appears to be the right lateral nerve herein. Parts of the rhinophoral and oral nerve have been described to innervate the epidermis of the anterior body sides "corresponding to the anterior and posterior portions of the Hancock's organ" (Haszprunar & Huber, 1990). This follows our observation that the rhinophoral nerve ends at the sides of the snout (Table 1).

### Genital system

The peculiar division into distal male and proximal female acini in the gonad (testes and ovarian follicles herein) has been reported in all previous descriptions of the rhodopid genital system. This is a rare feature in hermaphroditic heterobranchs. Exceptions include the architectonicoid *Omalogyra* and *Heliacus* (see Haszprunar, 1985) and the acochlidian *Asperspina riseri* (Morse, 1976), however ovaries and testes are described as more or less parallel in these cases. The described number of gonad acini, especially those containing sperm, varies in previous reports. While the older accounts mention up to 10 male lobes (Marcus & Marcus, 1952—only two are depicted), it appears that there really are only two in ripe specimens of any species examined more recently (Salvini-Plawen, 1991; this study). The number of developed ovarian acini, on the other hand, seems to be variable among individuals, although most described specimens contain several follicles (up to 10 in Marcus & Marcus, 1952).

The nidamental glands have been described to contain either three (Salvini-Plawen, 1991) or four lobes (Böhmig, 1893; Marcus & Marcus, 1952), the latter likely identical to the condition found in *Rhodope rousei* n. sp. In their histology the glands resemble those of nudibranchs (e.g. Klusmann-Kolb, 2001a, b), but are otherwise not very differentiated—there are no elaborate folds or similar structures. The tiny proximal gland (fg1 herein) has not been described before and appears not to be present in *R. veranii* and Caribbean specimens (own observations); its identity as a nidamental gland is not clear.

Oviposition of egg strings by a circular crawling motion was observed by Riedl (1959) in *R. veranii*; egg masses were described to contain between 6 and 30 eggs, each egg surrounded by a secondary layer and later covered by the adult with algal filaments and detritus. It is not clear if the egg masses show other heterobranch features (Haszprunar, 1985) such as inclusion of the eggs within a characteristic gelatinous capsule or if the eggs are united into strings by so-called chalazae.

The genital system of *R. veranii* following the nidamental glands was originally described as containing an eversible, spiral penis (Kölliker, 1847; Bronn & Keferstein, 1862–1866; Graff, 1883), which Böhmig (1893) interpreted to be a cone-shaped, ciliated fold inside the voluminous distal part of the genital system visible in histological sections. Marcus & Marcus (1952) describe a similar "conical, ciliated, unarmed penis" inside the "penis sheath" which is a wide and muscular

bulb (the latter likely corresponding to the terminal glands herein); in *R. transtrosa*, it was explicitly mentioned to be lacking (Salvini-Plawen, 1991). While all of the specimens examined herein contained the bulbous structure consisting of the two terminal glands, there is never a cone-shaped structure inside (the wall of the terminal glands being clearly glandular and not muscular) and there obviously is no other large spiral or eversible copulatory organ. This leads to the question how sperm are transferred in *Rhodope*. Riedl (1959) assumed copulation to be taking place in specimens he observed with the anterior right side of the body touching ("typical for euthyneuran gastropods", Haszprunar & Künz, 1996) and—due to the monaulic genital system—concluded sperm transfer to be unidirectional (transfer itself was not observed), while Salvini-Plawen (1991) assumed "functional dialy". The presence of free spermatozoa in the haemocoel and the lack of allosperm receptacles, however, imply a hypodermic mode of insemination. "Fertilization by hypodermic injection" was suggested by Haszprunar & Künz (1996), but is linked with the presence of a copulatory, or at least perforating, organ. Judging from the aphillic nature of *Rhodope rousei* n. sp. and the presence of numerous autospermatozoa within the lumen of the terminal glands, we suggest instead that *Rhodope* uses dermal insemination and dermal fertilization via spermatophores, as recently described from the acochlidian *Pontoledyle milaschewitchii* (Jörger et al., 2009). Spermatophores in *R. rousei* n. sp. are likely formed by the terminal glands and applied to the partner's epidermis. Sperm would have to be transferred subepidermally and into the body cavity from this spermatophore, possibly by short-term lysis of a small stretch of epidermis (as in mesopsammic acochlidians, see Swedmark, 1968; Jörger et al., 2009), prior to fertilization of oocytes inside the gonad (or gonoduct). The typical heterobranch spermatozoa (cork-screw-shaped head; Healy, 1996) must hence be able to penetrate the dense basal lamina of the epidermis and the gonad epithelium, as was discussed for microhedylacean acochlidians by Jörger et al. (2009); it remains unclear if this is a purely mechanical process or guided by biochemical activity.

### Kidney and excretory cells

*Rhodope rousei* n. sp. lacks a heart and shows the typical excretory system with 'protonephridium-like' knobs containing ciliary flames interspersed along the paired kidney tubes, as originally described by Graff (1883) and Böhmig (1893). As was shown from previous TEM studies, an ultrafiltration weir appears not to be present in the "pseudo-protonephridia", but only in the free haemocoelic rhogocytes (Haszprunar & Künz, 1996; Haszprunar, 1997). These were described as large, spherical cells "scattered within the body cavity" by Haszprunar & Künz (1996) for *R. transtrosa* (but not *R. veranii*). Assuming that the 'dorsal' cells in *Rhodope rousei* n. sp. are rhogocytes (see Haszprunar, 1996), then they are unusually aggregated in the place where one might expect the heart to have been (namely slightly anterodorsal to the kidney opening).

### *Rhodopemorpha* as infaunal taxa

The rhodopids have repeatedly been treated as part of the interstitial molluscan fauna (e.g. Rieger & Sterrer, 1975; Arnaud, Poizat & Salvini-Plawen, 1986) due to their minute size, vermiform external morphology and their possession of anatomical features that are assumed to be 'typical' adaptations of interstitial molluscs. These include prominent epidermal ciliation, spicules, an adhesive gland and accessory ganglia, but also production of spermatophores (discussed above) and lack of pigmentation (Swedmark, 1968). Some

species—including pigmented ones—have indeed been found in coarse sand (Karling, 1966; Rieger & Sterrer, 1975; Haszprunar & Heß, 2005; own unpublished data), but only *Helminthope* and '*Rhodope*' *crucispiculata*—being even more vermiform and unpigmented—resemble 'full-time' infaunal animals. The anatomically described species of *Rhodope* are so far known only from algal communities on rocks (Marcus & Marcus, 1952; Riedl, 1959). The new species *R. rousei* is the first that has been sampled from both sand and algae.

### Spicules

The subepidermal calcareous spicules of *R. rousei* n. sp. are typical for rhodopids in their curved form and slightly rough surface. Some other species have been shown to have a notch in the middle of the convex side of each spicule (*R. veranii* in Riedl, 1960; own unpublished data on a Caribbean species); this notch (opposite position of spicule cell's nucleus?), which is well visible in microscopic views of complete specimens, has not been mentioned for other species, but might simply have been overlooked. However, this notch is not evident in living specimens or histological sections of *R. rousei* n. sp. If variation exists among *Rhodope* species, the presence of a notch might represent a useful feature for taxonomy besides the thickness of spicules as suggested by Haszprunar & Heß (2005); the notch is clearly lacking at least in *Helminthope* (Salvini-Plawen, 1991; own observations).

Spicules are arranged at an angle of *c.* 45° to the longitudinal axis of the body, similar to what has been described for interstitial solenogasters (or gastrotrichs; Rieger & Sterrer, 1975); their uniform distribution speaks for a skeletal function in supporting the otherwise thin body wall and preventing injury by squeezing, as has been suggested for other meiofaunal gastropods that show this typical adaptation to the interstitial habitat (Swedmark, 1968; Jörger *et al.*, 2008).

### Adhesive gland

The caudal adhesive gland has also been described for *R. veranii* (e.g. Graff, 1883) and *R. marcusii* (Marcus & Marcus, 1952) and appears to be a general feature of Rhodopemorpha (judging from behaviour of live *Helminthope*; own observations), although it might not be easily detectable in fixed material (own observations). It is developed just after metamorphosis in *R. veranii* (Riedl, 1960). In its function as anchoring the animal to the substratum, the gland represents a character convergent with numerous infaunal worms and other organisms that quickly attach to and detach from sand grains if disturbed by quick water movement (Swedmark, 1964, 1968). In *Rhodope*, one can postulate a homology to either monocellular pedal glands, or to a posterior pedal gland as a discrete organ.

### How well do we know *Rhodope*?

Our study on three-banded *R. rousei* n. sp. presents the second rhodopemorph species examined in full anatomical and histological detail after *R. veranii*, confirming several previous records and adding useful detail, e.g. to the knowledge of the genital system. Also, it represents the only temperate water species described so far from the southern hemisphere. However, collecting trips revealed it to be part of a southern Australian rhodopemorph fauna containing further undescribed morphospecies based on colour (N.G.W., unpubl.).

In general, there appears to be much diversity to be discovered among these minute and apparently quite rare slugs. The fact that at least *R. veranii* from Rovinj, Croatia, shows direct development and crawl-away larvae (Riedl, 1960) indicates low dispersal capabilities, strong tendency to localized

speciation and perhaps high numbers of cryptic species, as was recently shown for meiofaunal acochlidians (Neusser, Jörger & Schrödl, 2011). On the other hand, an affinity with algae shown by some species, including *R. rousei* n. sp., might allow for rare long-range dispersal events on floating algae, as is hypothesized for the corambid nudibranchs (Martynov & Schrödl, 2011). This could help explain the presence of several undescribed *Rhodope* recorded on oceanic islands such as Madeira, Guam and the Galapagos (see Graff, 1883; Haszprunar & Heß, 2005).

The likely low dispersive capability of rhodopids, and the fact that coloration still appears to be the most practical means of separating species, hints at a possible taxonomic problem: the type species of *Rhodope*, *R. veranii*, was originally described from Messina, Sicily by Kölliker (1847), who mentioned a red transverse band only (see also Bronn & Keferstein, 1862–1866). All later studies of *R. 'veranii'* were however done with specimens from the northern Adriatic (Trieste, Italy or Rovinj, Croatia), all showing the 'typical' crimson red transverse bar but elongated posteriorly by a longitudinal stripe (Graff, 1883; Böhmig, 1893; Riedl, 1959, 1960). The identity of these specimens as *R. veranii* has not been questioned by previous authors, but it might well be that this best-known *Rhodope* species is not conspecific with the type *R. veranii*.

This demonstrates that the rhodopemorphs still pose many questions and that further anatomical and molecular research is greatly needed.

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