

MICROANATOMY OF *HEDYLOPSIS BALLANTINEI*, A NEW INTERSTITIAL ACOCHLIDIAN GASTROPOD FROM THE RED SEA, AND ITS SIGNIFICANCE FOR PHYLOGENY

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ABSTRACT

This is the first description of an acochlidian gastropod species from the Red Sea. More than 20 specimens of *Hedylopsis ballantinei* n. sp. have been extracted from subtidal coral sand in the Gulf of Aqaba. As a model organism for this poorly known, highly aberrant and enigmatic opisthobranch group, the anatomy of this new species is described in detail. Major organ systems have been reconstructed from histological semithin serial sections. The central nervous system has also been studied by means of immunocytochemical staining and confocal laser scanning techniques; dorsal bodies and rhinophoral ganglia are described for the first time for Acochlidia. Hardparts were examined using SEM, the midpiece of an acochlidian sperm is described with aid of TEM for the first time. Our results show *Hedylopsis ballantinei* n. sp. to be similar to the European *H. spiculifera* (junior synonym *H. suecica*). Differences include tropical occurrence, larger rhinophoral ganglia, different arrangement of ampulla and genital glands, and the probable absence of anterior male genitalia. *Hedylopsis ballantinei* n. sp. plays an important role in understanding acochlidian phylogeny, since it combines an array of morphological features that appear plesiomorphic for Acochlidia, e.g. a (small) mantle cavity, a unidirectional stomach lacking any cuticular elements, a tube-like, holohepatic digestive gland, a prepharyngeal, epiathroid central nervous system with separate cerebral, pleural and pedal ganglia, dorsal bodies associated with the cerebral ganglia, a (short) visceral loop with three ganglia, a monaulic and hermaphroditic reproductive system, and elongate spiral sperm. On the other hand, the absence of anterior male genitalia appears to be a derived condition that is shared with microhedylacean species. The aberrant possession of three glycogen helices in the sperm midpiece appears unique for opisthobranchs. With present, limited knowledge, the Acochlidia is regarded as a monophyletic group that is supported by several autapomorphies, with a hypothetical origin from acteonoidean opisthobranchs, i.e. diaphanid-like ancestors.

INTRODUCTION

Only a few gastropod groups have succeeded in colonizing the marine interstitial by adaptation to this extreme habitat. Among the most diverse and specialized taxa are the Acochlidia, poorly known and enigmatic opisthobranchs. So far, acochlidians have mainly been reported from marine interstitial sands on European coasts. Additional, sparse, records exist from virtually every coast on earth with coarse sand where acochlidians have been searched for (see review by Arnaud, Poizat & Salvini-Plawen, 1986). Uniquely for opisthobranchs, certain acochlidian species inhabit freshwater localities on several tropical West-Pacific islands and on one Caribbean island. At present, about 30 valid species are known. The acochlidians show a total body length from 0.8 mm (*Asperspina loricata* Swedmark, 1968) to 2–4 mm in most marine species, while most brackish and freshwater species are much larger, reaching 35 mm in *Acochlidium amboinense* Strubell, 1892.

All known acochlidians show external bilateral symmetry (i.e. they appear completely detorted), with an anterior partition of the body into head plus foot, and a sac-like visceral hump. All have a narrow radula with a central tooth and 0–2 lateral teeth in each half row, one or two pairs of tentacles, lack any shell, but several species possess calcareous spicules (Rankin, 1979). Many acochlidian species are hermaphrodites as usual for opisthobranchs, but several are gonochoristic. Literature descriptions of acochlidians vary in the amount of details given. Early studies

suffer from inadequate methodology, e.g. the light microscopical description of the tiny radula of *Hedylopsis suecica* by Odhner (1937) and that of the sperm structure of *Hedylopsis spiculifera* by Kowalevsky (1901). Rankin (1979) published a comparative review including most characters of acochlidians known at that time, but her revision is flawed due to not correctly adopting literature information and to uncritical interpretations, e.g. regarding the structure of the pericardial complex (see Fahrner & Haszprunar, 2002), the position of the nephropore and the structure of the central nervous system (Wawra, 1989).

Contradictory ideas exist about the possible phylogenetic position of the Acochlidia. Due to the possession of a cladohepatic digestive gland with two or three separate ducts into the stomach, the presence of a median radular tooth, and the absence of a blood gland, Bergh (1895) believed that acochlidians are cladobranch nudibranchs. Odhner (1937) regarded Acochlidia as its own order within the opisthobranchs, separate from nudibranchs because of the pre-pharyngeal position of the central nervous system. Zilch (1959) proposed that Acochlidia is related to Diaphanidae (Cephalaspidea s.l.). The similarity of radular structures lead Gosliner (1994) to suspect the monophyly of acochlidians, sacoglossans and diaphanids. Salvini-Plawen & Steiner (1996) held acochlidians to be the sister group of the equally enigmatic Rhodopemorpha, since members of both groups share the presence of spicules and a monaulic genital system. Up to now, besides being opisthobranchs, the systematic position of Acochlidia is unresolved by morphological data (Dayrat & Tillier, 2002). Molecular analyses by Vonnemann *et al.* (2005) indicate Acochlidia to be a monophyletic offshoot of

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a basal, so far unresolved, opisthobranch grade, rather than being aberrant members of one of the well-supported clades Nudibranchia, Pleurobranchioidea, Sacoglossa, Tylodinoidea, Cephalaspidea (*s.s.*) and Anaspidea. Successful phylogenetic, and especially cladistic, analysis is hindered by: (1) poor morphological information on most known acochlidian species; (2) poor taxon sampling, with many as yet undescribed species expected from the virtually unexplored marine interstitial habitat in the Indo-Pacific (see Schrödl, Eheberg & Burghardt, 2003); (3) the generally high degree of parallel evolution in opisthobranchs (Gosliner, 1994) that is especially true for taxa adapted to extreme environments; (4) a mix of highly derived and apparently very plesiomorphic features within Acochlidia that led Gosliner (1994) to suspect that paedomorphosis was a major force during evolution of the acochlidian family Ganitidae. Due to their unknown origin, there is also poor knowledge on phylogenetic relationships within the Acochlidia. The classification proposed by Rankin (1979) was contradicted by Wawra (1987) and by Arnaud *et al.* (1986) who largely adopted Wawra's system. While Rankin's classification was based on not always reliable data and phenetic analysis, Wawra (1987) discussed potential apomorphies to support the taxa established in his system. Recently, Fahrner & Haszprunar (2002) suggested that the Hedylopsidae should be placed at the base of the Acochlidia due to the presence of a small mantle cavity in some of its members.

In October 1999 we found an acochlidian species from the Red Sea that, according to Arnaud *et al.* (1986), belongs to the genus *Hedylopsis*. Externally, our specimens do not differ significantly from the other known, northeastern Atlantic and Mediterranean congeners *Hedylopsis spiculifera* (Kowalevsky, 1901) and *Hedylopsis suecica* (Odhner, 1937). These two latter species were considered to be conspecific due to similar external morphology (Marcus, 1953), radula (Cobo-Gradin, 1984) and genital system (Wawra, 1989).

The Red Sea specimens were chosen as a model system for acochlidian microanatomy (Sommerfeldt *et al.*, 2000); a detailed description of the excretory system and the mantle cavity has been already presented by Fahrner & Haszprunar (2002). In the present study, reconstructions of further major organ systems are given from semithin serial sections (2 µm). In addition, SEM, TEM and immunocytochemical staining and confocal laser scanning techniques are used to describe cuticular structures, sperm ultrastructure and the nervous system. These results, together with microanatomical re-examination of specimens formerly assigned to *H. spiculifera* and *H. suecica*, also allow further light to be shed on the taxonomy of the genus *Hedylopsis*. The detailed structural information available is used to re-evaluate relationships within Acochlidia and to discuss the position of this group.

MATERIAL AND METHODS

Twenty-six specimens of *Hedylopsis ballantinei* n. sp. were collected from Dahab, Gulf of Aqaba, northern Red Sea, in October 1999. They were extracted from medium-sized (grain size *ca* 1–2 mm) coral sands from 15 m depth. After narcotization with 7% MgCl₂ some specimens were fixed in 70% ethanol for later analysis of spicules and anatomical dissections, 14 others were fixed with 4% glutaraldehyde (in 0.2 M cacodylate buffer with 0.1 M NaCl and 0.35 M sucrose) for semithin sectioning and TEM study. Six specimens were fixed with 4% paraformalin for immunocytochemical study.

For serial sectioning, four specimens were decalcified with 2% EDTA or Bouin's solution, dehydrated in an acetone series and embedded in Araldite (Fluka). Complete, ribboned serial sections (2 µm) were prepared using 'Ralph' glass knives and contact cement ('Pattex-compact') at the lower cutting edge

(Henry, 1977). The sections were stained with methylene-blue-azure II according to Richardson *et al.* (1960) and deposited in the Zoologische Staatssammlung München (ZSM; Mollusca section; inventory numbers 20004766/1, 20004767, 20004768, 20004769); colours of tissues mentioned within the description refer to staining reactions. The nervous, genital, digestive and excretory systems were reconstructed graphically by hand.

Ethanol-fixed specimens were used for SEM examinations of whole specimens, spicules and radulae. Whole specimens were dehydrated in an ethanol series and critical-point dried with a BAL-TEX CPD 030 (Lange & Blödorn, 1981). Radula and spicules were separated as far as possible from surrounding tissue, and remaining tissue was macerated in 10% KOH. Objects were sputtered with gold for 135 s and examined using a Philips XL 20 SEM.

Specimens for TEM study were decalcified with 2% EDTA, dehydrated in an ethanol series and embedded in low viscosity resin (Spurr, 1969). Ribboned serial sections (1–2 µm) were prepared for light microscopy. Ultrathin sections (80 nm) were made with diamond knives, stained with uranyl acetate and lead citrate and examined using a Philips CM 10 TEM.

Specimens for immunocytochemical study were transferred into phosphate buffer (pH 7.3) (PBS) plus 0.1 % NaN₃, decalcified with 2% EDTA and rinsed with PTA buffer (0.1 M PBS, 0.1% NaN₃, 0.1–0.2% Triton X-100). Unspecific fluorescence was blocked with 6% goat serum (GSA; Jackson ImmunoResearch Laboratories, West Grove, USA). Anti-FMRF-Amid (DiaSorin, Stillwater, USA) in a concentration of 1:400 in PTA plus 6% GSA was used as primary antibody (24 h at 4 °C), followed by multiple rinsing with PTA for 10 h. Goat-antirabbit-immunoglobulin G coupled with TRITC as fluorescence marker in a concentration of 1:100 in PTA was applied as secondary antibody for 20 h, followed by multiple rinsing with PBS for 20 h under red light. The specimens were embedded with Vectashield (Vector Laboratories, Burlingame, USA) and studied with a confocal laser microscope (Leica DM IRBE) using 518 nm wavelength. Serial optical sections (1 µm) and red-green stereoimages were generated using Leica TCS NT 4D software. Rotated projections of stacks were created with Scion Image (Scion Corp., Frederick, MD, USA) software.

In addition, serial histological sections of *H. spiculifera* (det. Wawra) were obtained from the Museum of Natural History Vienna (NHMW). A specimen of *H. suecica* (det. Odhner) from the Swedish Museum of Natural History (SMNH 27211; fixed and preserved in 70% ethanol) was decalcified, embedded in Araldite, and serially sectioned.

The taxon names used herein follow Wawra (1987).

SPECIES DESCRIPTION

Family Hedylopsidae Odhner, 1952

Genus *Hedylopsis* Thiele, 1931

Hedylopsis ballantinei new species (Figures 1–6)

Hedylopsis sp. Sommerfeldt *et al.*, 2000: 11.

Hedylopsis sp. Fahrner & Haszprunar, 2002: 87–94.

Etymology: *Hedylopsis ballantinei* n. sp. is named in honour of our dear Scottish friend Mr Ballantine.

Type material: Holotype: ZSM 20040549; 2 mm preserved body length; collected from 'INMO-reef' Dahab, Gulf of Aqaba, Red Sea, at 15 m depth, in coral sand, 17 October 1999.

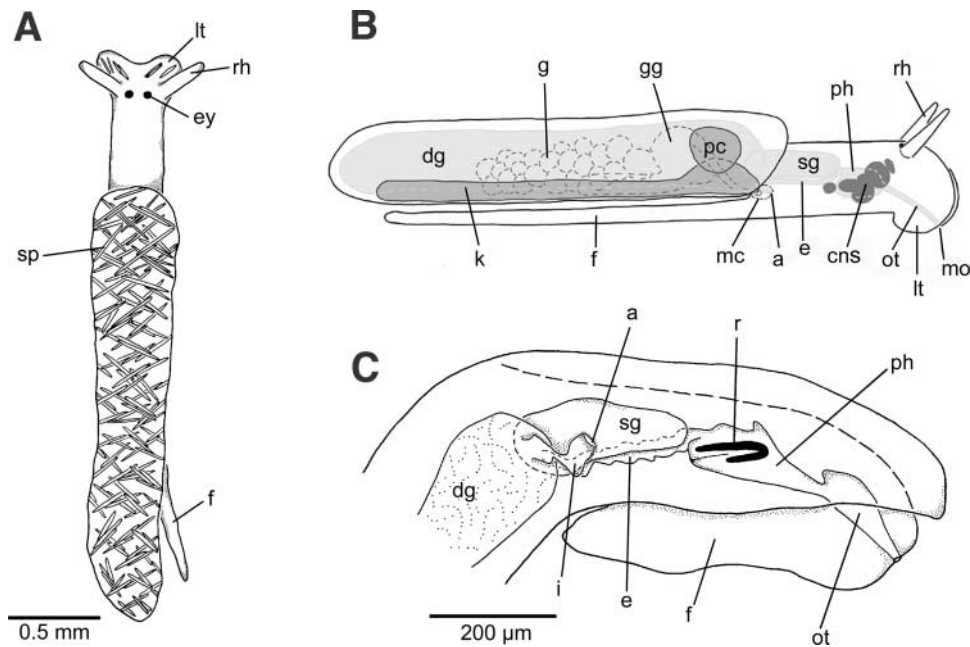


Figure 1. *Hedylopsis ballantinei* n. sp. **A.** Semi-schematic drawing of a living specimen (dorsal view). **B.** Schematic overview of the arrangement of internal organs. **C.** Graphical reconstruction of the digestive system (lateral view; posterior part of digestive gland omitted). Abbreviations: a, anus; cns, central nervous system; dg, digestive gland; e, oesophagus; ey, eye; f, foot; g, gonads; gg, genital glands; i, intestine; k, kidney; lt, oral tentacles; mc, mantle cavity; mo, mouth; ot, oral tube; pc, pericardium; ph, pharynx; r, radula; rh, rhinophore; sg, salivary gland; sp, spicule.

Paratypes: Two entire specimens (ZSM 20040550, 20040552) fixed with 4% glutardialdehyde; four serially sectioned specimens mounted on microscope slides (ZSM 20004766/1, 20004767, 20004768, 20004769); all paratypes were collected together with holotype.

External morphology (Figs 1A, B, 2A): *Hedylopsis ballantinei* n. sp. conforms with the usual body shape of Acochlidia. The body (Fig. 1A) is divided into a cylindrical anterior body (head and foot; ca one-third of whole body length) and a sac-like, elongated visceral hump (ca two-thirds of whole body length) that can be considerably flattened dorsoventrally. When a crawling animal is disturbed, the head-foot complex is partially or totally withdrawn into a cavity formed by the anterior portion of the visceral hump.

Crawling specimens reach up to 5.5 mm body length. The body coloration is uniformly whitish. The head bears two pairs of tentacles, cylindrical rhinophores and large, flattened

oral tentacles. A pair of black eyes is located at the inner side of the rhinophores and is visible through the epidermis. Some needle-like spicules can be detected through the body wall of the head and of the labial tentacles as well. The spicules of the anterior body are smaller than those of the visceral hump. SEM examination of a specimen with 3 mm living body length shows a protruding area of the body wall posterior to the right rhinophore that is richly supplied with cilia, i.e. a ciliary field (Fig. 2A).

The foot is as broad as the anterior body and laterally fused with the latter. The foot is narrower than the visceral hump to which it is not connected. The free posterior portion of the foot extends to about the same length as the visceral mass in crawling animals.

The wall of the visceral hump is rugose due to strong, needle-like spicules embedded within the tissue. Spicules are up to 170 µm long and reach 10 µm in diameter (Fig. 2B). Most spicules form bundles (2–5) with spicules more or less arranged in

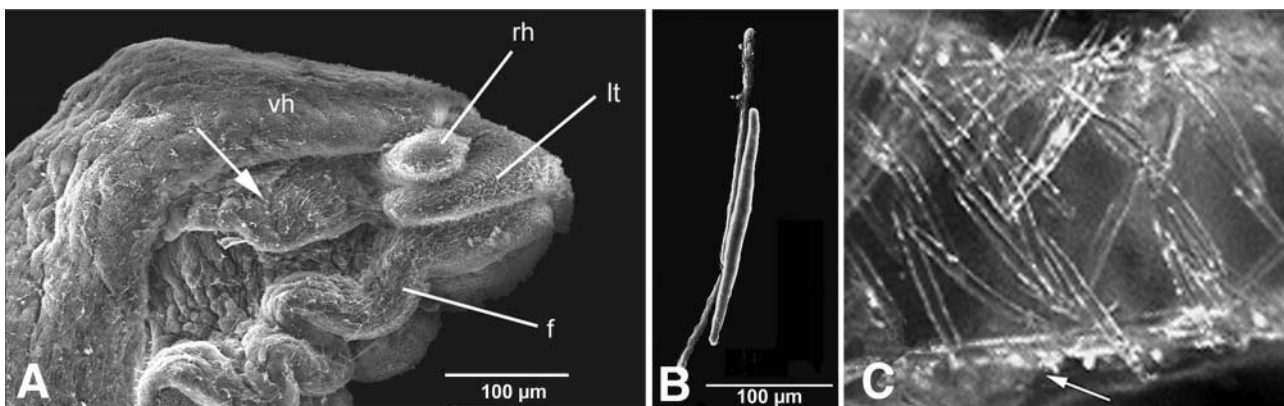


Figure 2. *Hedylopsis ballantinei* n. sp. **A.** SEM micrograph of the partly retracted anterior body (lateral view). **B.** SEM micrograph of spicules from the visceral hump. **C.** Macerated portion of the visceral hump with spicules (dorsal view). The arrow indicates the stiffened dorsal edge of a retracted specimen. Abbreviations: f, foot; lt, oral tentacles; rh, rhinophore; vh, anterior edge of visceral hump.

parallel. These bundles are orientated into both directions diagonal to the length axis, resulting in a crossed general arrangement of spicules (Fig. 2C).

General microanatomy (Fig. 1B): The anterior body cavity (head-foot complex) contains the central nervous system (CNS) and the anterior digestive organs such as oral tube, pharynx, voluminous salivary glands and oesophagus. The anterior portion of the visceral hump is filled with the reproductive organs (gonads and genital glands) on the left side, with the digestive gland, and with the circulatory and excretory system on the right side. The pericardium is situated anterodorsally, with the auricle posterior to the muscular ventricle. The kidney extends lateroventrally into the posterior visceral cavity that is otherwise filled with the digestive gland. Fahrner & Haszprunar (2002) described the circulatory and excretory systems of *Hedylopsis ballantinei* n. sp. (as *Hedylopsis* sp.) in detail. Anus, genital opening and nephropore open close together into a small but distinct mantle cavity that opens (ventro)laterally at the junction of the head-foot complex and the visceral hump.

Foot: The epidermis of the foot consists of polyciliary, light-blue staining epithelial cells. In addition there are epithelial and subepithelial gland cells with strongly staining, dark blue granules distributed over the entire length of the foot. Gland cells are especially concentrated at the anterior part of the foot, forming the foot gland. The connective tissue shows small, light blue-green muscle fibres crossing each other inside the foot.

Digestive system (Figs 1B, C, 3): The epithelium of the oral tube consists of cuboidal cells with numerous epithelial and subepithelial glandular cells; the latter are large and, as a whole, they build the oral gland. Jaws are absent. The oral tube connects to the bulbous, muscular pharynx. The radula is 80 µm long, narrow (15 µm) and U-shaped. The dorsal radula portion is nearly twice as long as the ventral one (Fig. 2C). The teeth of the ventral, older portion are considerably worn. Each of the four radulae examined shows 40–41 tooth rows, 15–16 in the older and 25–26 rows in the younger portion. The radula formula is: 40–41 × 1. 1. 2., i.e. the radula is asymmetric. The strong rhachidian tooth has a wide base and a prominent central cusp with 4–5 small lateral denticles on each side (Fig. 3A). On the left side there is just one lateral tooth, on the right side there are two laterals (or one lateral and one marginal tooth) in each row. The left lateral tooth is a rectangular plate with a slender, prominent central denticle that overlaps the lateral tooth of the next row (Fig. 3B). The first right lateral tooth resembles the left lateral in shape but lacks the denticle (Fig. 3C). The second right lateral tooth is awl-shaped (Fig. 3C). The paired salivary glands are well developed. They fill large parts of the dorsal portion of the

head-foot complex and extend somewhat into the visceral hump (Fig. 1B). The thin, tube-like oesophagus leaves the pharynx posterodorsally; it connects with the digestive gland and intestine without forming a morphologically or histologically detectable stomach. The digestive gland is a compact sac (i.e. not cladohepatic) filling large parts of the visceral cavity and extending to its posterior end. The intestine is a short tube that opens into the mantle cavity anterior to the nephropore and genital opening, ventrolaterally at the right side of the junction of the visceral hump and the head-foot complex.

Nervous system (Fig. 4): Reconstructed from serial histological sections, the central nervous system (CNS) of *Hedylopsis ballantinei* n.sp. consists of the paired cerebral, pleural, pedal and buccal ganglia, three distinct, asymmetric ganglia on the visceral nerve cord, an osphradial (?) ganglion, and a pair of rhinophoral ganglia (Fig. 4A, B). All ganglia except the buccal ganglia are situated pre-pharyngeally. All are intimately attached to each other, only the pedal commissure and the connective between the subintestinal/visceral ganglion and the right parietal/suprain-testinal ganglion are relatively long (Fig. 4A). All ganglia are surrounded by a layer of connective tissue and are divided inside into cortex and neuropile. The cortex is characterized by the presence of dark blue nuclei, which are not bigger than the nuclei of the surrounding tissue, and homogeneously light-blue stained nerve fibres. Inside the neuropile, the commissures and the connectives there are no detectable nuclei, and the tissue is as weakly stained as the nerve fibres within the cortex. All nerves, including the thick and heavily undulated optic, rhinophoral and labial nerves, lack any nuclei and are stained light blue.

The cerebral ganglia are the largest. They are connected by a strong cerebral commissure. A flattened elongate dorsal body sits dorsolaterally in a groove on each cerebral ganglion. In addition to the layer of connective tissue covering both cerebral ganglia and dorsal bodies, the latter are completely encapsulated by their own connective tissue. The dorsal bodies are thus discrete organs that are divided into a cortex with dark-staining cell bodies and a weakly staining medulla. Each cerebral ganglion bears a short connective to the large rhinophoral ganglion. From the latter, a thick nerve arises anteriorly then bifurcates into an undulated optic and rhinophoral nerve. The eyes are well developed, darkly pigmented and possess a weakly stained lens. Posteriorly, a thin nerve may connect with the statocyst. Anterolaterally, each cerebral ganglion bears a thickened, undulated labial nerve leading to the oral tentacles.

The pedal ganglia are slightly smaller than the cerebral ganglia. They are situated lateroventrally to the pharynx and are connected by a relatively thin and long commissure. Short connectives lead to the cerebral ganglia and even shorter ones to the pleural ganglia. Two nerves have been detected leaving

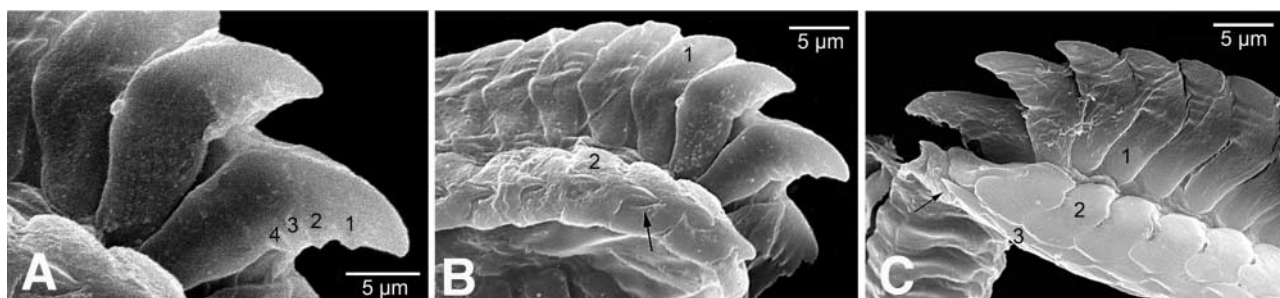


Figure 3. *Hedylopsis ballantinei* n. sp., SEM-micrographs of radular features. **A.** Rhachidian teeth with denticles (numbered). **B.** Rhachidian (1) and left lateral (2) teeth with long denticle (arrow). **C.** Rhachidian teeth (1), first right lateral teeth (2) lacking denticle and second right lateral teeth (3) that are slender, elongated and pointed (arrow).

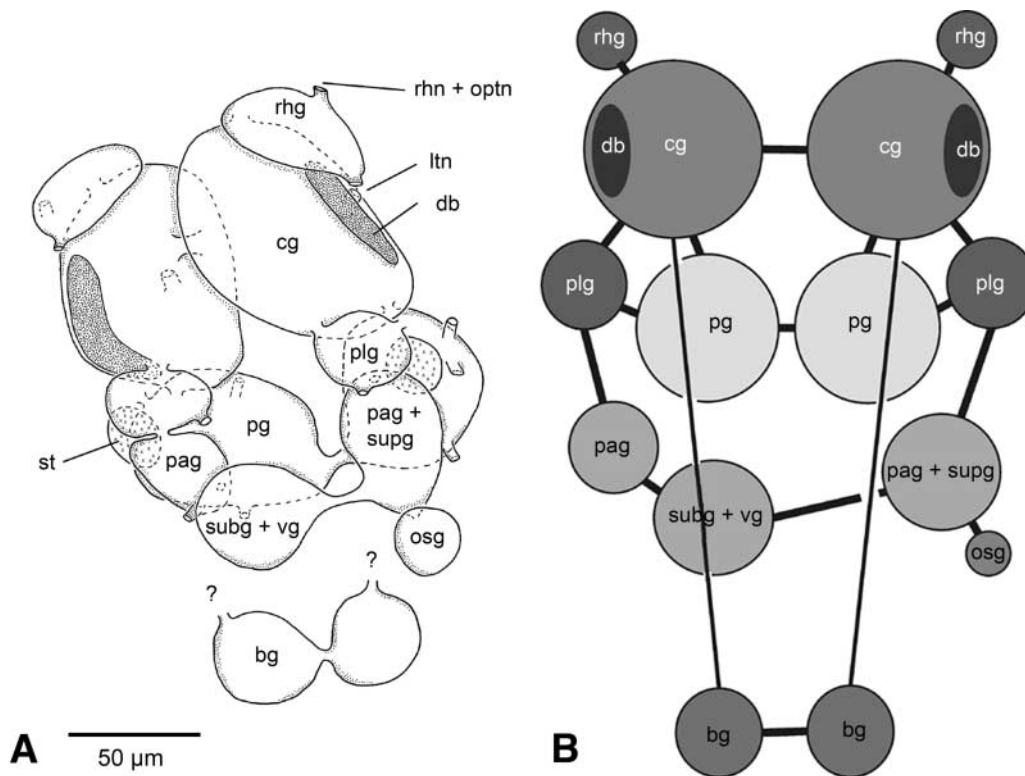


Figure 4. Central nervous system of *Hedylopsis ballantinei* n. sp. (terms according to Schmekel, 1985). **A.** Graphical reconstruction (dorsal view). **B.** Schematic view (pleuroparietal- connectives artificially elongated). Abbreviations: bg, buccal ganglion; cg, cerebral ganglion; db, dorsal body; ltn, oral tentacle nerve; optn, optic nerve; osg, osphradial ganglion; pag, parietal ganglion; pg, pedal ganglion; plg, pleural ganglion; rhg, rhinophoral ganglion; rhn, rhinophoral nerve; st, statocyst; subg, subintestinal ganglion; supg, supraintestinal ganglion; vg, visceral ganglion.

the left pedal ganglion, one towards the anterior, the other posterior. In addition, a third nerve arises anterodorsally from the right pedal ganglion and runs posteriorly. Statocysts nestle on both sides between the pedal, pleural and the first ganglion of the visceral nerve ring.

The pleural ganglia are much smaller than the pedal ganglia. One nerve arises dorsally from each pleural ganglion. The cerebro-pleural connectives and pleuro-pedal connectives are short. The pleural ganglia are connected to the visceral nerve ring through a short pleuro-parietal/supraintestinal connective on the right side and a short pleuro-parietal connective on the left. The left parietal ganglion is as small as the pleural ganglion. A very short connective leads towards the larger subintestinal/visceral ganglion. The latter bears a nerve posteriorly leading backwards. There is a relatively long connective between the subintestinal/visceral ganglion that is situated left of the midline and the similarly sized parietal/supraintestinal ganglion on the right. A small, presumably osphradial, ganglion is attached posteriorly to the parietal/supraintestinal ganglion; a distinct osphradium was not detected. The buccal ganglia are of similar size to the pleural ganglia. They are situated posterior to the pharynx and interconnected by a short, thin commissure ventral to the oesophagus. The nerve leaving each buccal ganglion in an anterior direction seems to be the long buccal-cerebral connective, but this could not be followed for its entire length.

These histological results are corroborated by immunocytochemical results with regard to the number and basic arrangement of major ganglia. In addition, the rhinophoral and labial nerves are shown to be considerably ramified distally. Several thin nerve cords run longitudinally into the foot and visceral hump. The resolution of tiny nerves relative to the strongly

fluorescing major ganglia of the central nervous system was not sufficient to determine their exact origin with certainty.

Reproductive system (Fig. 5): The gonad extends over two-thirds of the visceral hump. It consists of many follicles arranged like a chain of pearls. Every follicle contains sperm and egg cells that are not clearly separated into distinct areas. Both sperm and eggs show different stages of development. Egg stages observed reach *ca* 60 μm in diameter and lack any yolk. Mature sperm are spirally elongated with a slightly thickened, dark-stained head. The pre-ampullary gonoduct shows a sac-like widening before entering the anterior portion of the ampulla as a narrow duct. The ampulla is an elongate, blind sac. It is coated with a one-layered epithelium of large columnar cells. The lumen of the ampulla is weakly stained and does not show any sperm or other detectable structures. Anteriorly, the ampulla connects with the first of four sac-like genital glands. These glands are intimately attached to each other and are fused by broad connections. All glands show a similar histological structure. Anteriorly, the anteriormost gland narrows to a short duct leading to the genital opening. There are no traces of anterior male genitalia.

Sperm ultrastructure (Fig. 6): Within the specimens examined, sperm was only found within the gonads. Ultra-thin cross sections (Fig. 6A) show at least five different areas of the sperm midpiece and tail with a central axoneme ($2 \times 9 + 2$ microtubules). In the midpiece, the axoneme is surrounded by a dark-stained mitochondrial derivative showing 3, 2 or 1 glycogen helices that are indicated by more or less weakly stained oval areas.

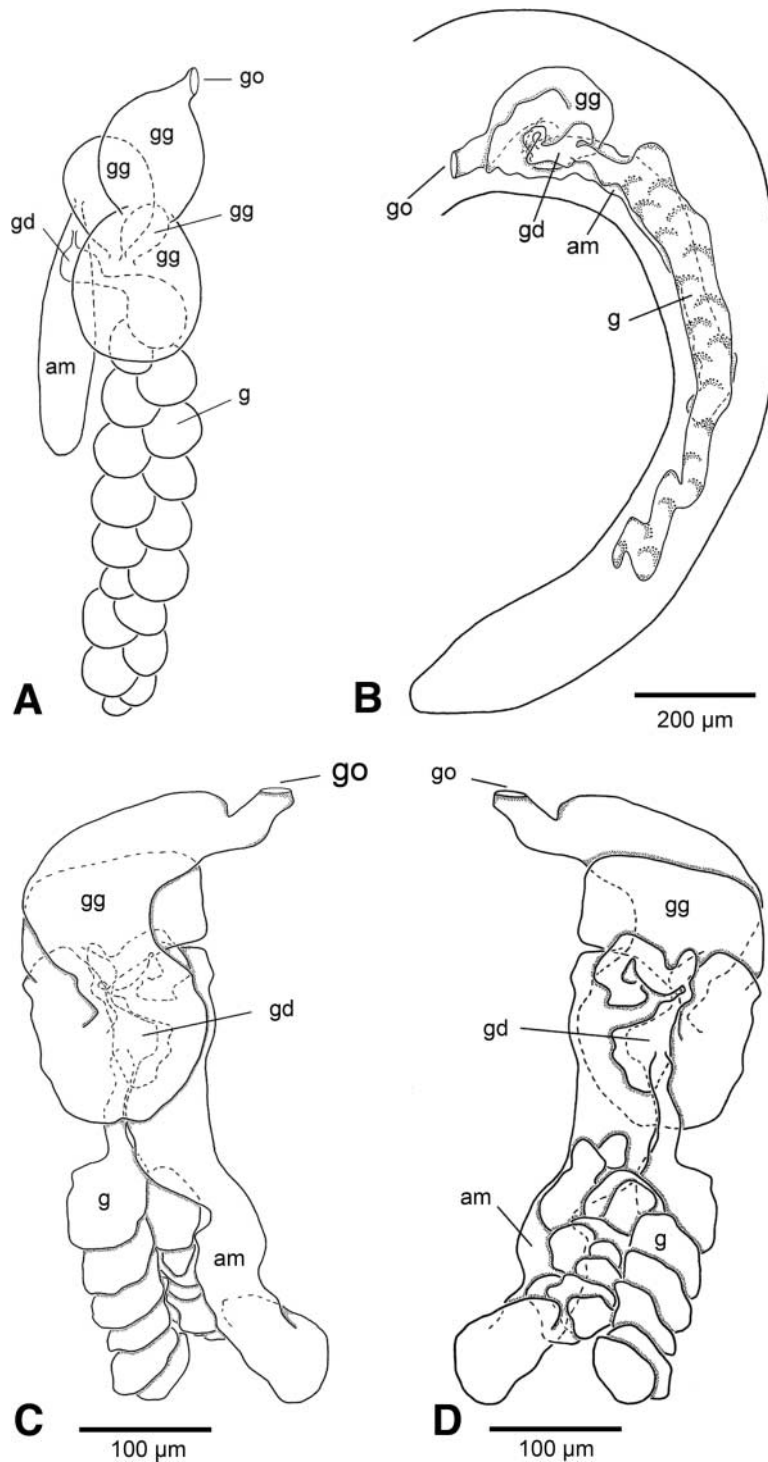


Figure 5. Reproductive system of *Hedylopsis ballantinei* n. sp. **A.** Schematic drawing (dorsal view). **B.** Graphical reconstruction, lateral view. **C.** Graphical reconstruction, dorsal view. **D.** Graphical reconstruction, ventral view. Abbreviations: am, ampulla; g, gonad; gd, gonoduct; gg, genital gland; go, genital opening.

Many cross sections show the flagellum surrounded by a layer of darkly stained granules (probably glycogen according to Thompson, 1973), that may relate to a thickened posterior tail portion. A few cross sections show only the flagellum without surrounding mitochondria or glycogen layer. Longitudinal sections (Fig. 6B) indicate the dense spiral arrangement of the three glycogen helices of the anterior part of the sperm midpiece, completing one whorl at ca 2 μm.

DISCUSSION

Morphology

External morphology: External features are fairly well known among acochlidian taxa. Externally, *Hedylopsis ballantinei* n. sp. conforms with other known *Hedylopsis* species, i.e. the European *Hedylopsis spiculifera* and its probable synonym *H. suecica*. They all have two

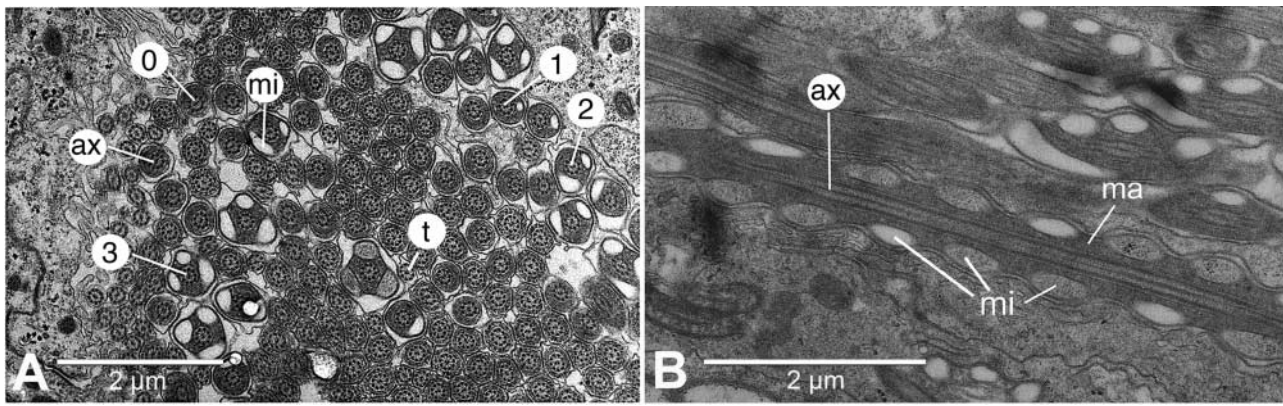


Figure 6. Sperm ultrastructure of *Hedylopsis ballantinei* n. sp. **A.** Cross section through different regions of sperm midpieces and tails. **B.** Longitudinal section through sperm midpieces with three glycogen helices around central axoneme. Abbreviations: ax, axoneme; ma, matrix; mi, sperm midpiece with mitochondrial derivatives showing 0, 1, 2, 3, glycogen helices; t, sperm tail with axoneme.

pairs of head appendages, one pair of cylindrical rhinophores and one pair of flattened oral tentacles. Other genera such as *Acochlidium*, *Strubellia*, *Pseudunela*, *Microhedyle* and *Tantulum* are characterized by cylindrical rhinophores and cylindrical oral tentacles. *Asperspina* shows broadened rhinophores and oral tentacles, *Palliohedyle* cylindrical rhinophores and reduced oral tentacles, *Paraganitus* small rhinophores, *Pontohedyle* and *Ganitus* completely reduced rhinophores (see Rankin, 1979; Wawra, 1987). The foot extends beyond the visceral hump in *Acochlidium*, reaches nearly the same length as the visceral sac in (crawling) *Hedylopsis spiculifera*, *H. suecica* and *H. ballantinei* n. sp., and is shorter in other Acochlidia.

The homology of cephalic appendages in lower heterobranchs, pulmonates and basal opisthobranchs is problematic. According to Huber (1993), architectibranchs possess rhinophoral and labial nerves leading to Hancock's organ, while one or two pairs of sensory head tentacles innervated by the rhinophoral and labial nerves are present in many gymnosomes, sacoglossans, nudipleurans and acochlidians that all lack Hancock's organs. In contrast, head shields of Cephalaspidea s.s. (except for the interstitial *Philinoglossa*) and head shield tentacles of Architectibranchia are innervated by the nervus clypei-capitis that is absent in Acochlidia and Nudibranchia. Assuming convergent evolution of philinoglossan, nudibranch and acochlidian head tentacles, the reduction of the nervus clypei-capitis would be an autapomorphy of Acochlidia. The reduction of the Hancock's organ, the formation of oral tentacles innervated by the labial nerve and of rhinophores innervated by the rhinophoral nerve may also be autapomorphies, with secondary reduction of rhinophores in certain members of subgroups.

Fusiform spicules are embedded within the tissue of the visceral hump of the marine hedylopsacean *Hedylopsis spiculifera*, *H. suecica* and *H. ballantinei* n. sp., and in *Asperspina* where they form a dense, tough, roof-like aggregation. The freshwater acochlidiid *Acochlidium amboinense* (Strubell, 1892) possesses elongate but blunt spicules (Bayer & Fehlmann, 1960), while irregularly shaped or rounded calcareous spicules are present in several marine Microhedylidae species. In opisthobranchs other than Acochlidia, Nudipleura, *Rhodope* and *Platyhedyle*, spicules are generally absent (see Gosliner, 1994). The evolutionary development of spicules in the certainly marine acochlidian ancestor and their secondary loss in several species of different subgroups appears more likely than assuming their development several times independently.

The presence of a small but distinct mantle cavity was first mentioned for the northern Russian *Hedylopsis murmanica* Kudinskaja & Minichev, 1978 (belonging to *Asperspina* according

to Wawra, 1987), and then reported from *Hedylopsis ballantinei* n. sp. (as *Hedylopsis* sp.) by Fahrner & Haszprunar (2002). Also the 'vestibulum' of the marine hedylopsid *Pseudunela cornuta* (Challis, 1970) may well refer to a mantle cavity (see Rankin, 1979). In addition, Challis (1968) described both kidney and gonoduct of *Paraganitus ellynae* opening into a small, ciliary cavity that she called 'cloaca'. The anus could not be found but, as usual in acochlidians, it is likely to be associated with the nephropore and gonopore. Since the mantle cavity of *Hedylopsis ballantinei* n. sp. also has cilia at its opening to the exterior (Fahrner & Haszprunar, 2002), the 'cloaca' of the ganitid microhedylacean *P. ellynae* probably represents the remnants of a true mantle cavity as well. Most other acochlidian species have not yet been investigated in sufficient histological detail. A mantle cavity thus occurs at least in certain members of Hedylopsidae, Asperspinidae and, probably, in Ganitidae. Since lower heterobranchs, pulmonates and basal opisthobranchs possess a mantle cavity, its presence is considered to be plesiomorphic for Acochlidia; its reduction in size may be an autapomorphy of Acochlidia.

Digestive system: The digestive tract of *Hedylopsis ballantinei* n. sp. comprises a glandular oral tube, a muscular pharynx with a pair of large salivary glands, and an oesophagus connecting to the digestive gland and short intestine without forming a distinct stomach. The radula of *Hedylopsis ballantinei* n. sp. is unusual among opisthobranchs in being asymmetrical, with one denticulate and one undenticulate first lateral and an additional, slender, lateral tooth on the right side of a strong, denticulate central tooth. Very similar radulae have been reported for *Hedylopsis spiculifera* and *H. suecica* by Marcus (1953) and Cobo-Gradin (1984). *Asperspina rhopalotecta* (Salvini-Plawen, 1973) and some other acochlidians, including the freshwater species *Strubellia paradoxa*, *Acochlidium sutteri*, *A. bayerfehlmanni* and *Tantulum elegans* Rankin, 1979 also possesses only one left but two right laterals (see Wawra, 1980, 1987). Other acochlidian species have symmetrical radulae with either two, one or no lateral teeth on each side.

The salivary glands of *Hedylopsis ballantinei* n. sp. are voluminous. Other species such as *Asperspina riseri* (Morse, 1976) and *Acochlidium amboinense* Strubell, 1892 have also been reported to show such large glands (Bücking, 1933; Morse, 1976). The 'salivary' gland of *Hedylopsis spiculifera* indicated by Odhner (1937) was considered to be in fact the prostate by Wawra (1989). The specimen of *H. suecica* (det. Odhner) reconstructed herein shows a well developed prostate anterior to the large, paired salivary glands. The salivary ducts of *Hedylopsis ballantinei*

could not be reconstructed to their insertion into the pharynx due to the dark staining properties of these glands. However, their paired structure, position posterior to the central nervous system, and the lack of other glandular structures that might be salivary glands disproves them to be a prostate.

The digestive gland of *Palliohedyle weberi* (Bergh, 1895) is 'cladohepatic', consisting of a short left and a large right sac entering separately into the stomach via two or three openings (Bergh, 1895). *Acochlidium amboinense* shows a digestive gland separated into several diverticula that fuse before entering the stomach by a common opening (Bücking, 1933). *Hedylopsis ballantinei* n. sp., *H. spiculifera* and *H. suecica* studied herein, as well as supposedly most other acochlidian species, possess a holohepatic digestive gland that is connected with the oesophagus and intestine in a histologically indistinct stomach area.

Generally, cuticular structures other than the radula have not been mentioned for any acochlidian digestive system, except for a pair of cuticular plates ('jaws') found in *Ganitus evelinae* by Marcus (1953), in *Paraganitus ellymnae* by Challis (1968), and jaw-like cuticular structures in *Microhedyle glandulifera* (Kowalevsky, 1901) by Wawra (1978).

Since all acochlidian species appear to possess a simple digestive system (as described above) with a narrow radula, these conditions are regarded to be plesiomorphic within Acochlidia. Almost all gastropods including many acochlidians have a symmetrical radula, thus a symmetrical state is considered plesiomorphic for acochlidians. Lacking detailed structural information on most acochlidian species, no phylogenetic assumptions are made here on the absence or presence of jaws. According to Wägele & Willan (2000), a holohepatic digestive gland is plesiomorphic within opisthobranchs and nudibranchs; thus, we consider the unbranched digestive gland of most acochlidian species to be plesiomorphic and the cladohepatic state of a few freshwater Acochliidae as apomorphic.

Central nervous system (CNS): The CNS of *Hedylopsis ballantinei* n. sp. is prepharyngeal as in most lower heterobranchs and architectibranchs (Gosliner, 1994) and, thus, the plesiomorphic condition. In *Hedylopsis ballantinei* n. sp. the CNS is highly concentrated with most ganglia intimately attached to one another. The cerebral, pedal and pleural ganglia are, however, separated in all Acochlidia investigated so far (see Huber, 1993). This anterior part of the CNS of *Hedylopsis ballantinei* n. sp. conforms well to that described for *H. spiculifera* by Wawra (1989) and Huber (1993). Re-examination of serial sections of *H. spiculifera* and *H. suecica* shows the 'Procerebrum' mentioned by Wawra (1989) to be the ganglion herein called the rhinophoral ganglion; his 'Tentakelnerv' is the cerebro-rhinophoral connective. The rhinophoral ganglion of *Hedylopsis ballantinei* n. sp. is, however, considerably larger than that of *H. spiculifera*. Dorsal bodies encapsulated by connective tissue and showing distinct cortex and medulla layers are present in *H. ballantinei* n. sp., *H. spiculifera* and *H. suecica*, and are reported for the first time for acochlidian species. Identical organs have been described from basommatomorph pulmonates (see Switzer-Dunlap, 1987) and are commonly regarded as an autapomorphy for pulmonates (see Huber, 1993). However, their presence seems plesiomorphic not only for euthyneurans; similar, but less concentrated neurosecretory tissues associated with the cerebral ganglia ('juxtaganglionar organ') have also been detected in aplysioid opisthobranchs, the gymnosome *Hydromeles*, and several prosobranchs such as *Patella*, *Trochocochlea*, *Diodora* and *Haliotis*, especially during the period of female gonad maturation (Martoja, 1965a, b; Switzer-Dunlap, 1987). Careful studies will ultimately reveal dorsal bodies to be present in other acochlidian species and other opisthobranch groups as well, and their special structure and functions might have some phylogenetic significance.

Studying *H. spiculifera* and *Microhedyle glandulifera*, Huber (1993) considered two cerebral nerves, the joint oral/rhinophoral nerve and the labial tentacle nerve, plus the thin nerve leading to the statocyst, as being characteristic of Acochlidia. From histological reconstructions of several specimens of *Hedylopsis ballantinei* n. sp. it appears that the optic nerve arises jointly with the rhinophoral nerve from the rhinophoral ganglion (that was overlooked in *H. spiculifera* by Huber, 1993), while the labial tentacle nerve connects directly with the cerebral ganglion.

From histological results, the thick and highly convoluted rhinophoral, optic and labial nerves of *H. ballantinei* n. sp. do not possess nuclei and, thus, are true nerves rather than associations of small ganglia. The lack of such 'accessory ganglia' was considered to be diagnostic for Hedylopsacea by Wawra (1987). Accessory ganglia are further present in several interstitial, comparatively small and usually aberrant members of different opisthobranch groups, such as *Platyhedyle* (Sacoglossa), *Philinoglossa* (Philinoidea), *Pseudovermis* (Nudibranchia), and the enigmatic epibenthic *Rhodope* (see Huber, 1993). Accessory ganglia, thus, seem to be special adaptations of either small opisthobranchs (Huber, 1993) or especially of those adapted to an interstitial environment, that evolved convergently.

Gastroesophageal ganglia, as reported for *H. spiculifera* by Wawra (1989), have not been found in *Hedylopsis ballantinei* n. sp. The buccal nerves are thick and convoluted close to the buccal ganglia, however, without detectable nuclei.

Hedylopsis ballantinei n. sp., *H. spiculifera* and *H. suecica* identically show three separate ganglia on the visceral loop and an additional associated ganglion on the right side. The identity of these ganglia, however, is problematic. According to the hypothesis of the nervous systems of opisthobranchs by Schmekel (1985), and of euthyneurans by Haszprunar & Huber (1990), the basal condition shows five separate ganglia on the visceral loop (pentaganglionate condition), i.e. the left and right parietal, subintestinal, suprainestinal and visceral ganglion. Thus, two of the visceral loop ganglia of *Hedylopsis ballantinei* n. sp., *H. spiculifera* and *H. suecica* are supposedly fused with others. The first ganglion on the left side of the visceral loop is as small as the pleural ganglion; it is considered to be the left parietal ganglion [while regarded as the subintestinal or suboesophageal ganglion in *H. spiculifera* by Huber (1993) and Wawra (1989), respectively]. The following ganglion is also situated on the left side of the body; its diameter exceeds that of the left parietal ganglion by about one-third, so that it has about 60% more volume; it is regarded as the fused subintestinal/visceral ganglion, with one large nerve running posteriorly. The comparably large ganglion on the right side of the visceral loop is considered to be the fused right parietal/suprainestinal ganglion. The very small additional ganglion connected with the suprainestinal ganglion, according to Huber (1993) and due to its position and arrangement, is herein called an osphradial ganglion, although no distinct osphradium could be detected by histological means.

According to literature data, the structure of the central nervous systems of other taxa of Acochlidia is heterogeneous with regard to the number and arrangement of major ganglia, and may differ significantly from the results presented herein for *Hedylopsis ballantinei* n. sp., *H. spiculifera* and *H. suecica*. Older studies such as the description of *H. suecica* by Odhner (1937) suffered from inadequate techniques; the absence of rhinophoral ganglia (that may be the 'Tentakeldrüsen' associated with the 'olfactorischen Nerv') and the finding of only two ganglia on the visceral loop (instead of three plus an associated one) was due to oversight. Cobo-Gradin (1984) apparently followed Odhner uncritically in describing the same arrangement for *H. spiculifera*. A major problem was the comparative but severely flawed literature revision of acochlidian organ systems by Rankin (1979). Regarding *H. suecica*, Rankin illustrated all usual commissures and connectives between the ganglia that were

mentioned by Odhner (1937), but forgot to include the pleuro-pedal connectives. As one of several further examples, in the schematic drawing of the CNS of *Pseudunela cornuta* (Challis, 1970), Rankin (1979) missed the left pleuro-pedal connective and misinterpreted the suprainestinal, visceral and subintestinal ganglia (Challis, 1970) as parietal, visceral and parietal plus visceral. Interestingly, the CNS described for *Tantulum elegans* by Rankin (1979) is in fact almost identical to the CNS described for *H. spiculifera* and *Microhedyle glandulifera* (as *Unela*) by Huber (1993). Rankin's cerebral 'lobes' are certainly rhinophoral ganglia, and assuming that the buccal ganglia are not integrative parts of the visceral loop as obviously misinterpreted by Rankin, the visceral loop shows three separate ganglia. The right 'parietal-buccal-visceral' seems to include the supraoesophageal ganglion with an associated small osphradial ganglion ('accessory visceral ganglion') on the right side with a nerve ('nephridial nerve') running posteriorly to the nephridial duct.

The presence and arrangement of visceral loop ganglia is quite variable between and within different lower heterobranch and basal opisthobranch taxa (see e.g. Huber, 1993; Gosliner, 1994), and the identity of specific ganglia cannot be easily resolved. The condition in *H. ballantinei* n. sp. reflects that of *Rissoella* sp., *Pyramidelloidea* spp. and *Diaphana* spp. investigated by Huber (1993); there is, however, no more indication of streptoneury. All careful original studies on other species from different acochlidian families such as on *Hedylopsis spiculifera* by Huber (1993) and *Pseudunela cornuta* by Challis (1970) (both Hedylopsidae), on *Tantulum elegans* by Rankin (1979) (Tantulidae), on *Microhedyle glandulifera* by Huber (1993); as *Unela*) and *M. remanei* by Marcus (1953; as *Unela*) (both Microhedylidae), and on *Ganitus evelinae* by Marcus (1953) (Ganitidae), indicate a basically similar arrangement of the central nervous system to that found herein for *H. ballantinei* n. sp. This arrangement thus appears to reflect the usual and, presumably, plesiomorphic condition within Acochlidia.

Reproductive system: The posterior reproductive system of *Hedylopsis ballantinei* n. sp. is similar to that described for *H. spiculifera* (female phase) by Wawra (1989). Differences refer to the sperm structure, the arrangement of the ampulla, and the arrangement of genital glands. The specimens of *Hedylopsis ballantinei* n. sp. examined show a hermaphroditic gonad with sperm and eggs in different stages of development as in other *Hedylopsis* species. In a comprehensive electron-microscopical review, Thompson (1973) described elongate spiral sperm from all major euthyneuran taxa studied, although acochlidians were not included. Light-microscopically, sperm of *H. ballantinei* n. sp. is usually elongate and spiral, with a slightly thickened but elongate head. Thus, sperm of *H. ballantinei* n. sp. resemble the 'tyrtovii-type' that Wawra (1987) supposed to be characteristic for Microhedylacea *sensu* Wawra (= Asperspinidae, Microhedylidae, Ganitidae). A few spermatids of *H. ballantinei* n. sp. show an inflated, 'pear-shaped' apical region as first reported from *H. spiculifera* by Kowalevsky (1901). Odhner (1937) described sperm of *H. suecica* to possess an inflated head region, and a pear-shape was also mentioned for sperm of *H. spiculifera* by Cobo-Gradin (1984) and Wawra (1989). Wawra (1987) even supposed the pear-shaped sperm type to be characteristic ('*spiculifera*-type') for all Hedylopsacea (Hedylopsidae, Acochliidae, Tantulidae). However, Franzén (1955) described sperm of *H. suecica* as spiral. A re-examination of specimens from Livorno identified as being *H. spiculifera* by Wawra (label) also revealed exclusively elongate spiral sperm. The occasional occurrence of sperm with pear-shaped, inflated heads in some specimens of *Hedylopsis ballantinei* n. sp. and other *Hedylopsis* species might refer to developing stages, as suggested

for pear-shaped sperm of the nudibranch *Hypselodoris tricolor* by Medina, Moreno & López-Campos (1986).

While all acochlidian species are likely to possess the usual euthyneuran spiral sperm type, there might be some variation regarding structural details of the head that should be subject to future ultrastructural analysis. The first, and still preliminary, electron microscopical results on sperm of an acochlidian species herein show *Hedylopsis ballantinei* n. sp. to possess sperm with a long central flagellum surrounded by mitochondrial derivatives with densely coiled, apically three, then two, and only one spiral glycogen helices with different lengths. The sperm tail consists of the central flagellum surrounded by a granular layer, the glycogen piece. Due to the many cross sections showing this structure, the thickened tail portion appears to be considerably long. The few cross sections showing only the flagellum without any mitochondrial or glycogen layer may be interpreted either to represent a thin posteriormost tail portion, or developing stages. No distinct nuclear region could be observed on the cross-sections studied. That might indicate a short head region in relation to a very long midpiece and tail.

Disregarding the still lacking knowledge on the nuclear portion, the sperm midpiece and tail of *Hedylopsis ballantinei* n. sp. resembles that of *Acteon tornatilis* described by Thompson (1973). In contrast to any other opisthobranch known to us (see e.g. Healy & Willan, 1991; Healy, 1996), sperm of *Hedylopsis ballantinei* n. sp. has three spiral helices, while *A. tornatilis* and some pulmonates have four, *Aplysia* spp. have two, and most Cephalaspidea s.s. and nudipleurans have just one helix (see Thompson, 1973). Comparative investigations on sperm of other acochlidian species are badly needed.

The proximal gonoduct of *Hedylopsis ballantinei* n. sp. inserts into an elongate sac, called 'ampulla' in *H. spiculifera* by Wawra (1989), close to the terminal connection with the postampullary gonoduct. In the lectotype of *H. suecica* and in specimens of *H. spiculifera* from Rovinj and Livorno, the pre-ampullary duct enters the ampulla at about half its length (Wawra, 1989). The distinction of sac-like albumen and mucous glands joining with the postampullary duct close to the genital opening also differs from the situation found in *Hedylopsis ballantinei* n. sp. Here, the postampullary gonoduct enters proximally into a couple of more or less serially arranged, not clearly distinguishable glandular sacs and folds. A completely different posterior genital system was described for *H. spiculifera* from Galicia, northern Spain, by Cobo-Gradin (1984). A long gonad connects with the stalk of an ovoid ampulla (as bursa copulatrix) and with the stalk of a rounded, follicular albumen gland close to the genital opening. Since this arrangement resembles that originally described for *H. suecica* by Odhner (1937), Wawra (1989) suspected that Cobo-Gradin (1984) erroneously followed Odhner's misinterpretation.

Within the genus *Hedylopsis* there has also been considerable confusion regarding the anterior reproductive system. Kowalevsky (1901) was not able to find any penial papilla or prostate in *H. spiculifera*. Odhner (1937) described *H. suecica* as having an external sperm groove and, except for a small specimen, a quite large, muscular penial papilla with a penial cuticular spine and an internal vas deferens. However, no prostate was mentioned for *H. suecica*. According to Wawra (1989), Odhner (1937) misinterpreted the large prostate to be (part of) the salivary glands. *Hedylopsis suecica* (det. Odhner) studied herein possesses a large, muscular penial papilla with a cuticular spine, an internal vas deferens and both a prostate and large paired salivary glands. Specimens from Rovinj and Livorno identified as *H. spiculifera* by Wawra (1989) possess an external sperm groove, a penial papilla with cuticular spine and a prostate 'in the male phase'. According to Wawra (1989), 'specimens in the female phase' have a ciliary band (instead of the external sperm groove), yolky oocytes, an ampulla and female glands.

Even though not explicitly stated, his diagnosis may be interpreted as the male copulatory organs being (completely?) reduced. On the other hand, Wawra (1989) mentioned that every specimen with more or less developed sperm also had male copulatory organs. A large penial papilla was also present in all specimens of *H. spiculifera* re-examined by ourselves. In contrast, all specimens of *Hedyloopsis ballantinei* n. sp., even smaller ones only 2–3 mm in body length, show well developed sperm and well developed female genital organs indicating active spermiogenesis and oogenesis at the same time. Since no trace of either a penial papilla, or a prostate, or an external ciliary groove (but a ciliary field), or a vas deferens could be found, we conclude that *H. ballantinei* n. sp. most probably lacks any male copulatory organs during its whole ontogeny. Sperm transfer may occur via spermatophores and cutaneous fertilization as described from the hermaphroditic *Asperspina brambelli* and from males of the gonochoric *P. milaschewitchii* and *Ganitus evelinae* by Swedmark (1968), which also lack any anterior male genital organs. In this case, the ‘ampulla’ of *H. ballantinei* n. sp. may function not only as a place for autosperm storage, but also for forming a large spermatophore. Previous donation of spermatophores may also explain that none of the specimens studied had sperm in the ampulla.

The genital system of the Acochlidia is generally monaulic as in most basal Cephalaspidea s.l. (except for Acteonidae and at least one *Ringicula* species; see Gosliner, 1994), Cephalaspidea s.s., and most basal members of other opisthobranch clades; therefore, this most likely represents the plesiomorphic condition for Acochlidia. Hedylopsidae, Acochliidae, Tantulidae and Asperspinidae are hermaphroditic (Wawra, 1987) as usual for opisthobranchs (plesiomorphic condition for Acochlidia). Members of the Microhedyliidae and Ganitidae have separate sexes; this is unique within opisthobranchs and, thus, a potential synapomorphy of these groups. The vast majority of opisthobranchs copulate via a penial papilla and possess at least one allosperm receptacle (see e.g. Gosliner, 1994); these are regarded as plesiomorphic conditions for Opisthobranchia. According to Wawra (1987), all Acochlidia but *Strubellia paradoxa* (the ‘bursa copulatrix’ of which needs to be re-examined) appear to lack any allosperm receptacles (apomorphic condition). However, the reproductive systems of hedylopsacean freshwater species are poorly known (see Haase & Wawra, 1996) and the homology of special organs remains to be clarified by histological analysis. All hedylopsacean species but *Hedyloopsis ballantinei* n. sp. possess a protrusible penial papilla (plesiomorphic condition) as present in most other opisthobranchs (except for Acteonidae and *Umbraculum*; see Gosliner, 1994; Wägele & Willan, 2000). Like many other basal opisthobranchs, *Hedyloopsis spiculifera*, *Strubellia* and Asperspinidae possess an open ciliary sperm groove leading from the genital opening towards the base of the right rhinophore (plesiomorphic condition for Acochlidia). Other hedylopsacean species (but not *Hedyloopsis ballantinei* n. sp.) possess an ‘intraepidermal’ or subepidermal vas deferens (derived condition). According to Wawra (1987), sperm transfer in *Hedyloopsis spiculifera* probably functions through hypodermic injection by a hollow penial stylet (apomorphic condition); this fertilization mode may also be suspected for members of *Pseudumela* and *Acochlidium* having a hollow penial stylet. *Hedyloopsis ballantinei* n. sp. and all Asperspinidae, Microhedyliidae and Ganitidae appear to lack any penial papilla (see Wawra, 1987). The loss of the penial papilla, and the very unusual sperm transfer via spermatophores, that was mentioned for Asperspinidae, Microhedyliidae and Ganitidae by Swedmark (1968) and Wawra (1987) and is assumed for *H. ballantinei* n. sp., are derived conditions within Acochlidia and may be synapomorphies of the respective taxa.

Taxonomy

In the absence of any significant distinguishing features, we conclude that *H. suecica* is a junior synonym of *H. spiculifera*.

Hedyloopsis ballantinei n. sp. is very similar to *H. spiculifera* regarding external, digestive and, apart from the considerably larger rhinophoral ganglia, central nervous features. As discussed above, it differs with regard to the arrangement of ampulla and genital glands, and in the apparent lack of male copulatory organs that suggests sperm transfer via spermatophores rather than copulation by hypodermic injection as in *H. spiculifera*. We conclude it is a new species.

Phylogeny

Acochlidia are extraordinary and enigmatic opisthobranchs. While most species are small and obviously highly specialized to interstitial life in marine sands, they also comprise the only known opisthobranch species that have succeeded in colonizing freshwater systems, presumably at least three times independently (see Wawra, 1987). This is interesting from both a functional and an evolutionary point of view. Several marine acochlidians are monosexual, which is also unique amongst opisthobranchs, and some use spermatophores. Several species show an equally unusual radular asymmetry. Analysis of the systematic position of acochlidians within the Opisthobranchia has not only been hindered by many such mosaic-distributed aberrant features, organ reductions, and generally insufficient morphological descriptions, but also by problems of interpreting potentially significant character conditions such as the prepharyngeal CNS and the monaulic reproductive system as either plesiomorphic or secondarily derived (e.g. due to paedomorphism and/or specialization for the interstitial habitat). These problems cannot be entirely resolved herein. However, new morphological results together with a critical reinterpretation of literature data may allow reconstruction of an acochlidian ground plan and, from that, conclusions about their systematic position.

Monophyly of Acochlidia: The monophyly of the Acochlidia has not so far been disputed, although Gosliner (1994) discussed the Ganitidae as possible paedomorphic sacoglossans. All acochlidians show a unique, very uniform external organization with (1) external symmetry; (2) the absence of a shell; (3) a well-developed, elongate visceral hump that is separated from the rest of the body; and (4) a head-foot complex that can be at least partly retracted into the visceral hump. Together with the marine interstitial habitat, these are the most obvious autapomorphies of the group. Further potential autapomorphies, such as the presence of calcareous spicules, the reduced size of the mantle cavity with loss of ctenidia, the loss of tentacle nerves (nervus clypei-capitis *sensu* Huber, 1993), the presence of oral tentacles innervated by the nervus labialis and of rhinophores innervated by rhinophoral nerves, the loss of the Hancock’s organ, and the longitudinally arranged pericardial complex (the transverse orientation was considered as the plesiomorphic condition for opisthobranchs by Wägele & Willan, 2000), and the potential loss of allosperm receptacles, may be tested by future analyses. The first molecular systematic study including acochlidian taxa, i.e. *Hedyloopsis ballantinei* n. sp. (as *Hedyloopsis* sp.) and two Microhedyliidae spp., by Vonnemann *et al.* (2005) also supports the monophyly of Acochlidia.

Classification: The relationship between the different acochlidian groups is widely unresolved, and existing hypotheses are conflicting. Largely modifying the classification of Rankin (1979), Wawra (1987) proposed that the Acochlidia consisted of two major branches, Hedylopsacea (Hedylopsidae, Acochliidae, Tantulidae) and Microhedyllacea (Asperspinidae,

Microhedyllidae, Ganitidae). According to Wawra (1987), Hedylopsacea is characterized by (1) two pairs of cephalic tentacles; (2) eyes; (3) the absence of accessory ganglia at the anterior cerebral nerves; (4) a hermaphroditic reproductive system with a ciliary groove or vas deferens, and a cuticular armed, protrusible penial papilla; and (5) pear-shaped sperm (head). In contrast, Microhedyllacea were considered to show a tendency to reduce cephalic tentacles and eyes, to have many accessory ganglia, to be gonochoristic with a tendency to reduce anterior male genitalia and forming spermatophores, and to have spiral sperm. In addition, the foot is successively reduced to a narrow strip.

However, some of these criteria refer to hypothetical reduction series (foot) or are inconsistent: the microhedyllacean Asperspinidae possess two pairs of well developed cephalic tentacles, and *Parhedyle tyrtowii* and *Pontohedyle milaschewitschii* possess eyes, while eyes are lacking in the hedylopsacean Tantulidae. It is therefore suspected that a well-developed foot, two pairs of cephalic tentacles, and eyes were present plesiomorphically in Acochlidia, and that these features have been reduced in several subgroups independently. This study on *Hedylopsis ballantinei* n. sp. discovered large rhinophoral ganglia separated from the cerebral ones, and somewhat smaller rhinophoral ganglia are also present in *H. spiculifera* and *Tantum elegans*. The rhinophoral ganglia may be homologous to the (few) accessory ganglia present in the hedylopsacean *Pseudumela cornuta* and to the many accessory ganglia on the rhinophoral nerves of microhedyllacean species that would then be a derived condition. However, the thick and undulating rhinophoral, optic and labial nerves as also present in *Hedylopsis ballantinei* n. sp. may easily be misinterpreted as 'associated ganglia', thus this character should be reinvestigated carefully for other acochlidian species. The microhedyllacean Asperspinidae are hermaphroditic like Hedylopsacea (plesiomorphic for Acochlidia), and the gonochoristic condition of other microhedyllaceans is certainly a derived condition. While cephalic anterior male genital organs are present in most hedylopsaceans, in *H. spiculifera* and *Strubellia* they are reduced during ontogeny (Wawra, 1989). Anterior male genitalia are absent in *Hedylopsis ballantinei* n. sp. and all microhedyllacean species. The present study suggests that Wawra's last character, the light-microscopical shape of sperm, is inapplicable for separating major acochlidian clades. The Acochlidia thus cannot be justifiably divided into the two major clades Hedylopsacea and Microhedyllacea as defined by Wawra (1987). It appears that extant, marine and hermaphroditic hedylopsaceans and Asperspinidae form a plesiomorphic grade giving rise to ancestors of freshwater hedylopsaceans, i.e. *Strubellia*, Acochlididae and Tantulidae, and to gonochoristic microhedyllaceans (Microhedyllidae and Ganitidae). Consequently, at least Hedylopsacea, Hedylopsidae and Asperspinidae *sensu* Wawra (1987) are paraphyletic. This classification certainly needs refinement that may be achieved through careful reinvestigation of poorly known species and subsequent cladistic analysis.

Acochlidian ground plan: In addition to the autapomorphies of Acochlidia mentioned above, several character conditions such as the (postmetamorphic) lack of an operculum and a narrow, symmetrical radula are obviously plesiomorphic for acochlidian taxa and belong to the acochlidian ground plan. *Hedylopsis ballantinei* n. sp. may play an important role in understanding acochlidian phylogeny since it combines an array of further, probably plesiomorphic, features for acochlidians. Plesiomorphic conditions in *Hedylopsis ballantinei* n. sp. include its marine interstitial habitat and small body size, as well as morphological characters of all major organ systems, i.e. it has a detorted, 'slug-like' external shape with (1) a well-developed foot; (2) two pairs of well-developed cephalic tentacles; (3) a pair of well-developed

eyes; (4) a rudimentary mantle cavity; (5) an auricular excretory system plesiomorphic for opisthobranchs (Fahrner & Haszprunar, 2002); (6) a simple digestive system with an unidirectional stomach lacking any cuticular elements; (7) a tube-like, holohepatic digestive gland; a (8) prepharyngeal; (9) epiathroid central nervous system with (10) separate cerebral; pleural and pedal ganglia; (11) dorsal bodies associated with the cerebral ganglia; (12) a visceral loop with three separate ganglia, and an associated (presumably osphradial) ganglion on the right side of the supraoesophageal ganglion; (13) a monaulic; (14) and hermaphroditic reproductive system, like other opisthobranchs; and (15) elongate spiral sperm with, however, an aberrant number of three glycogen helices. An open sperm groove and a protrusible cephalic penial papilla as present in e.g. *H. spiculifera* can also be considered plesiomorphic for Acochlidia due to outgroup comparison with basal opisthobranchs.

Systematic position: Like several other major traditional opisthobranch groups (Cephalaspidea s.s., Anaspidea, Sacoglossa, Thecosomata, Gymnosomata, Nudibranchia plus Pleurobranchoidea; see Mikkelsen, 1996; Wägele & Willan, 2000), the Acochlidia appear morphologically to be well characterized as a monophyletic group (Odhner, 1937; Wawra, 1987; this study), but their phylogenetic relationships remain unclear (see e.g. Dayrat & Tillier, 2002). Reconstructing the phylogeny of the Opisthobranchia by means of 18S and 28S rDNA sequences reveals the Acochlidia as one of several basal opisthobranch offshoots with a poorly supported topology (Vonnemann *et al.*, 2005).

Transferring morphological information on basal Acochlidia (as elaborated above) into the cladogram on Cephalaspidea s.l. by Mikkelsen (1996), the Acochlidia share some symplesiomorphies with basal 'acteonoidean' clades, such as the retention of a (considerably reduced) mantle cavity, of a prepharyngeal CNS with still separate cerebral, pedal and pleural ganglia, and several separate visceral loop ganglia. Unlike Acteonoidea, Mikkelsen's most basal offshoot, the stemline taxa and Acochlidia lack an operculum and are monaulic (*vs* diaulic). In contrast to *Hydatina*, all other groups on the stemline and Acochlidia have a protrusible cephalic penis. After the Ringiculidae branch off, the remaining groups on the stemline and acochlidians share several synapomorphies, i.e. an external sperm groove, a mantle cavity opening laterally, and a simple unidirectional stomach. At least the latter synapomorphy is also shared by the acteonoidean Diaphanidae that were not included in the analysis by Mikkelsen (1996). All further stemline taxa including anaspideans and cephalaspideans s.s. show the unique possession of a gizzard with cuticular plates that are absent within Acochlidia.

The narrow radula of diaphanids, as well as the special structure of broad, thin laterals and triangular, massive central teeth of the members of the diaphanid genus *Toledonia*, resembles exactly that of basal acochlidians. Thus, Gosliner (1994) suspected that Acochlidia may have been derived from *Toledonia*-like ancestors, prior to sacoglossans that lack any lateral teeth and may have split off from *Newnesia*-like ancestors. A descent of Sacoglossa from Diaphanidae was, however, doubted by Jensen (1996) who regarded pharyngeal and radular similarities to be convergences due to similar piercing and suctional feeding modes. A further potential synapomorphy of diaphanids, acochlidians and sacoglossans may be the presence of rhinophores, but their homology may be doubted due to their different innervation (see Huber, 1993). *Hedylopsis ballantinei* n. sp. shows three separate ganglia (although much more concentrated) with a presumably free left parietal ganglion. Similarly, the left parietal ganglion was described as free in the diaphanid *Newnesia antarctica* by Odhner (1926), as well as in basal, shelled sacoglossans (Mikkelsen, 1996). Diaphanids and acochlidians

still show a prepharyngeal CNS and separate cerebral and pleural ganglia (symplesiomorphies). In contrast, all sacoglossans including the interstitial *Platyhedyle* show a derived postpharyngeal CNS with fused cerebropleural ganglia; Wawra (1988) and Huber (1993) corrected earlier misinterpretations (e.g. Wawra, 1979) of apparently separate cerebral and pleural ganglia in *Platyhedyle*. The reproductive system is plesiomorphically monaulic in diaphanids and Acochlidia, while diaulic in all known Sacoglossa. So far, no significant synapomorphy of basal Acochlidia and basal Sacoglossa is evident, and each group shows a number of autapomorphies (see e.g. Jensen, 1996; this study). Thus, if Sacoglossa was derived from diaphanid-like ancestors, it is very likely that this occurred independently from Acochlidia. In conclusion, Acochlidia are herein considered to be monophyletic, having an independent origin from Sacoglossa, presumably from *Toledonia*-like diaphanid ancestors.

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