

Insemination by a kiss? Interactive 3D-microanatomy, biology and systematics of the mesopsammic cephalaspidean sea slug *Pluscula cuica* Marcus, 1953 from Brazil (Gastropoda: Euopisthobranchia: Philinoglossidae)

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Abstract Increasing molecular evidence suggests that the phylogeny of euthyneuran gastropods differs greatly from century textbook concepts. The presence, homology and evolution of characters in major subgroups thus need to be reinvestigated. Traditionally basal opisthobranch Cephalaspidea (“head-shield snails and slugs”) were pruned to a new taxon concept, with benthic euopisthobranch and tentacle-bearing cephalaspidean lineages basal to burrowing, head-shield bearing philinoidean species. Among the latter, mesopsammic “microslug” lineages evolved at least twice. Herein we explore in 3D micro-anatomical detail the putatively basal philinoglossan *Pluscula cuica* (Marcus, Boletim da Faculdade de Filosofia, Ciências e Letras. Universidade de São Paulo 164:165–203, 1953a) from its type locality in Brazil. The species possesses several “accessory” ganglia and a reduced posterior mantle cavity that retains some putative shell-building tissue and an osphradium. The hermaphroditic, monaulic genital system opens in a posterior position; it retains a bursa copulatrix but lacks a distinct

receptaculum seminis. Autosperm is transferred to the cephalic copulatory organ via an external sperm groove, not through the hemocoel, as suggested in the original description. The penis opens through the oral tube, sperm is transferred by a “kiss”. A conspicuous yellow gland is discussed as a modified Blochmann’s gland. Retaining several putative symplesiomorphies with philinoids, *Pluscula* is discussed as the most basal offshoot in meiofaunal Philinoglossidae. However, the supposed “primitiveness” of the fused rather than separate cerebropleural ganglia and the triganglionate rather than pentaganglionate visceral nerve cord was based on misobservations. Higher categories such as Philinoglossacea for Philinoglossidae, and a separate family Plusculidae for *P. cuica* are no longer warranted. Inner cephalaspidean relationships and a scenario of more or less successive philinoglossid adaptation to meiofaunal environments should be investigated by molecular studies with more comprehensive taxon sampling.

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Introduction

Gastropoda are renowned for their morphological, and therefore ecological, diversity (e.g., Beesley et al. 1998). In recent decades, phylogenetic studies have rapidly increased our understanding of their evolution. However, accumulating molecular evidence suggests that the topology of Heterobranchia — covering roughly half of gastropod diversity — differs greatly from traditional textbook concepts. The long held split of Euthyneura into monophyletic Opisthobranchia and Pulmonata has been challenged (e.g., Haszprunar 1985; Dayrat and Tillier 2002; Klussmann-Kolb et al. 2008; Dinapoli and Klussmann-Kolb 2010) and a “new euthyneuran tree” has emerged (Jörger

et al. 2010a; Schrödl et al. 2011a, b; Göbbeler and Klussmann-Kolb 2011), the backbone topology of which has been confirmed in phylogenomic approaches (Kocot et al. 2011; Smith et al. 2011). In the light of radically changing concepts and classifications, morphological characters, taxa and traits need to be reinvestigated (Schrödl et al. 2011a).

Among the most aberrant and problematic heterobranchs are several lineages of minute slugs that are specialized members of the meiofauna. Living in the marine interstitial or mesopsammon, i.e., the interstices between sand grains in well oxygenated sands (Swedmark 1964, 1968), all these taxa — most acochlians, rhodopemorphs, some Cephalaspidea, Sacoglossa and Nudibranchia (Arnaud et al. 1986)—exhibit characteristic morphologies. Convergent evolved characters are small sizes, vermiform bodies, losses of body appendages, eyes and pigmentation, development of adhesive abilities, spicules and additional ganglia, and unusual reproductive traits such as the production of spermatophores, hypodermal insemination, production of only few eggs, and loss of a free-floating larval stage (Swedmark 1968, 1971; Salvini-Plawen 1973; Schrödl and Neusser 2010; Neusser et al. 2011a; Schrödl et al. 2011a). Similar features and tendencies are also found in other groups of metazoans that inhabit the same habitat (Swedmark 1964; Higgins and Thiel 1988; Rundell and Leander 2010). In addition to showing reductions and convergent innovations, the reduced adult size common to these taxa is suggestive for progenetic processes (e.g., Hanken and Wake 1993). Retaining simple juvenile features means losing diagnostic apomorphies of higher clades and gaining pseudoarchaic ones; this may lead to entirely wrong classificatory conclusions (Martynov et al. 2011; Martynov and Schrödl 2011). Furthermore, minute specimen sizes have historically hampered both collecting efforts and structural analyses. Incongruities from previous descriptions were detected and corrected during 3D microanatomical reanalyses of meiofaunal sacoglossans (Rückert et al. 2008) and acochlians (e.g., Neusser et al. 2006, 2009a; Jörger et al. 2008, 2010b; Eder et al. 2011) that were originally examined using paraffin-based histology. Interstitial cephalaspideans have not yet been analyzed in such depth.

The Cephalaspidea or “bubble-shells” were long thought to be the most basal and conservative major opisthobranch clade, including several distinct taxa characterized by the name-giving head-shield, an organ used for infaunal digging (Gosliner 1994; Mikkelsen 1996; Burn and Thompson 1998). However, the inclusiveness of the taxon concept has decreased over time. Acteonoidea and Ringiculoidea were already excluded from Cephalaspidea on morphological grounds (Haszprunar 1985; Mikkelsen 1996, 2002); the former were placed at the base or outside Euthyneura by multi-locus analyses (Göbbeler and Klussmann-Kolb 2010, 2011; Dinapoli and Klussmann-Kolb 2010; Jörger et al. 2010a; Schrödl et al. 2011a, b). The previously disputed

cephalaspidean *Cylindrobulla* (Jensen 1996; Mikkelsen 1996, 1998) was confirmed as a “bubble-shelled” sacoglossan panpulmonate by molecular analyses (Händeler and Wägele 2007; Maeda et al. 2010; Neusser et al. 2011b). Finally, Malaquias et al. (2009) removed the small-sized benthic Runcinacea from Cephalaspidea; this has been confirmed by molecular studies using larger outgroup sets (Jörger et al. 2010b; Göbbeler and Klussmann-Kolb 2011). With the remaining Cephalaspidea now appearing as a non-basal taxon within so-called Euopisthobranchia (Jörger et al. 2010a), head-shield bearing lineages are scattered over the euthyneuran tree. This reclassification has important implications for the understanding of euthyneuran evolution. For example, euthyneuran head tentacles and head shields show essentially similar cerebral innervation patterns (Huber 1993; Faller et al. 2008; Staubach et al. 2008; Jörger et al. 2010b) and thus may simply transform according to habitats and life styles.

Within Cephalaspidea, morphology-based classifications are heterogeneous and authors claimed at least four ‘super-familial’ ranks. The most basal Cephalaspidea in all available multi-locus studies were the little-known Diaphanoidea (e.g., Malaquias et al. 2009; Jörger et al. 2010a; Göbbeler and Klussmann-Kolb 2011). Intriguingly, this paraphyletic group (Göbbeler and Klussmann-Kolb 2011) contains tentacle-bearing members such as benthic *Colpodaspis* and infaunal *Toledonia* (Brown 1979; Golding 2010) suggesting that there is no simple ecological rule. Therefore, one might suggest that diaphanoidean tentacles may be phylogenetic remainders of a benthic euopisthobranch ancestor, while higher cephalaspideans have evolved their eponymous head-shields de novo. Stable inner cephalaspidean topologies and detailed micro-anatomical data to test these hypotheses are not yet available. Albeit with varying topologies, members of at least four families of the carnivorous Philinoidea commonly cluster close together: *Scaphander* (Scaphandridae), *Philine* (Philinidae), Aglajidae and Gastropteridae (Malaquias et al. 2009; Göbbeler and Klussmann-Kolb 2011). These philinoid families contain slender carnivores with a reduced or internalized shell (save *Scaphander*) and a rearward displaced mantle cavity (Burn and Thompson 1998). Mesopsammic, at least externally shell-less philinoideans have evolved independently at least twice (Arnaud et al. 1986; Malaquias et al. 2009; Jörger et al. 2010a): within the burrowing Philinidae (*Philine exigua* Challis, 1969a and juveniles of other species), and with the entirely mesopsammic ‘Philinoglossacea’ Thiele, 1931 of still unknown affinities.

The philinoglossans are a small group containing four genera and seven described species (four of which belong to *Philinoglossa* Hertling, 1932). These miniaturized slugs (body length rarely exceeds 4 mm) show a ribbon-shaped body with posteriorly overhanging dorsum, lack a distinguishable head-shield (except for the Mediterranean *Abavopsis latosoleata* Salvini-Plawen, 1973), a gill, and have at best a vestigial shell.

These multiple reductions have significantly hampered phylogenetic studies based on morphological data: Wägele and Klussmann-Kolb (2005) recovered philinoglossans within a group containing the meiofaunal members from several traditional heterobranch ‘orders’. Molecular studies have shown to be better suited to solve similar tasks (e.g., Malaquias et al. 2009; Jörger et al. 2010a) but so far only a few have included philinoglossans in their sampling. Accordingly, their phylogenetic position within philinoid Cephalaspiidea is not known: Vonnemann et al. (2005) recovered *Philinoglossa praelongata* Salvini-Plawen, 1973 basal but inside a polytomy. Both Malaquias et al. (2009) and Göbbeler and Klussmann-Kolb (2011) identify a clade of *Philinoglossa* and Gastropteridae as sister to Aglajidae plus Philinidae, with Scaphandridae basal. Jörger et al. (2010a) recovered *Scaphander* as sister to *Philinoglossa*, but without covering the aforementioned families. So far, monophyly of ‘Philinoglossacea’ has never been tested by including more than single representatives into molecular analyses. Not much is known about the biology of the group.

The monotypic genus *Pluscula* is represented by the Brazilian *Pluscula cuica* Marcus, 1953a, the only philinoglossan species described from the Americas. It is potentially the most basal of philinoglossans, since it is described with characters that appear to be plesiomorphic and are not found in the other genera (Marcus 1953a). These characters are a thin internalized shell, the genital opening in a posterior position, still separate cerebral and pleural ganglia, and five distinguishable ganglia on the visceral nerve cord. On the other hand, the mode of autosperm transfer is suggested to be unique and peculiar: Marcus (1953a) observed numerous spermatozoa in the body cavity and concluded that autosperm move from the gonad directly to the copulatory organ—through the hemocoel, instead of being transported along the external ciliated groove running along the right body side, as in most other cephalaspiideans. Due to these peculiarities, some authors place *Pluscula cuica* in a family of its own (Plusculidae: Marcus 1959; Franc 1968; Bouchet and Rocroi 2005) or subfamily (Plusculinae: Salvini-Plawen 1973). Therefore, *Pluscula cuica* might be a key organism for the understanding of philinoglossan evolution and the internal phylogeny of philinoid groups, and interesting for its peculiar reproductive mode.

Within a framework of comparative morphological and evolutionary studies on mesopsammic heterobranchs, we analyzed the entire microanatomy of *Pluscula cuica* using computer-based 3D reconstruction from semi-thin histological sections. Our aims were to (1) check, correct, and supplement the original description; (2) elucidate the structure and function of the reproductive system, in particular with regard to the potentially highly peculiar modes of autosperm transport and transfer; and (3) evaluate potentially ancestral features in a phylogenetic context, reconsidering the familial status of the species, and the relationships of philinoglossans to other cephalaspiideans.

Materials and methods

Specimens of *Pluscula cuica* were extracted from bulk samples of coarse sand taken from the uppermost subtidal at low tide at Ilhabela, São Paulo, Brazil (type locality) in 2005 following the method described by Schrödl (2006). Specimens were relaxed in isotonic magnesium chloride solution, fixed in ethanol (75 % or 96 %) or, for histology, in 4 % glutaraldehyde (in 0.2 M cacodylate buffer, 0.1 M sodium chloride, 0.35 M sucrose buffered at pH 7.2). The latter specimens were further postfixed with 1 % osmium tetroxide in 0.2 M cacodylate buffer/0.3 M sodium chloride, then dehydrated over a graded acetone series and embedded in Spurr’s epoxy resin (Spurr 1969). Specimens are stored at the Bavarian State Collection of Zoology (ZSM), Department Mollusca, Munich, Germany, and in the malacological collection of Museu de Zoologia da Universidade de São Paulo (MZSP, vouchers 104098–104100), Brazil (C. M. Cunha, personal communication).

For 3D reconstruction, three specimens in epoxy blocks were trimmed and serially sectioned at 1.5 µm using either Ralph glass knives (specimens ZSM Mol-20070316, 20070323) or a HistoJumbo diamond knife (specimen ZSM Mol-20070317) (Diatome, Biel, Switzerland) with contact cement at the lower cutting edge, following the method described by Ruthensteiner (2008). Ribbons of sections were collected on microscope slides, stained with methylene blue/azure-II (Richardson et al. 1960) and sealed with araldite resin. Sections of the complete diamond-sectioned specimen—a moderately contracted adult specimen of approximately 1.7 mm length—and, separately, its central nervous system were photographed with a ProgRes C3 ccd camera (Jenoptik, Jena, Germany) mounted on a Leica DMB-RBE microscope (Leica Microsystems, Wetzlar, Germany). Photographs were stack processed (resized, changed to greyscale, unsharp masked) in Adobe Photoshop (Adobe Systems, Mountain View, CA) and imported into Amira 5.2 software (Visage Imaging, Berlin, Germany) with a resolution of 1,024×768 or 2,080×1542 pixels, respectively. After alignment of the photographs, organ systems were labeled manually onto the sections. Rendered 3D models of the organ systems were created for the complete specimen (based on 575 photographs, every second section was used). Details of the specimen’s nervous system were analyzed in a separate aligned stack (256 photos, every section used), but labeled in the complete body’s model. Anatomical features were compared among all three specimens (one juvenile, one functionally male, the other adult).

Further two specimens fixed in 75 % ethanol (lot: ZSM Mol-20070835) were photographed through a Leica dissection microscope and macerated in KOH solution for analysis of shell remnants and the radula. Radulae were viewed through above mentioned light microscope for counting of tooth rows and detection of denticulate tooth margins.

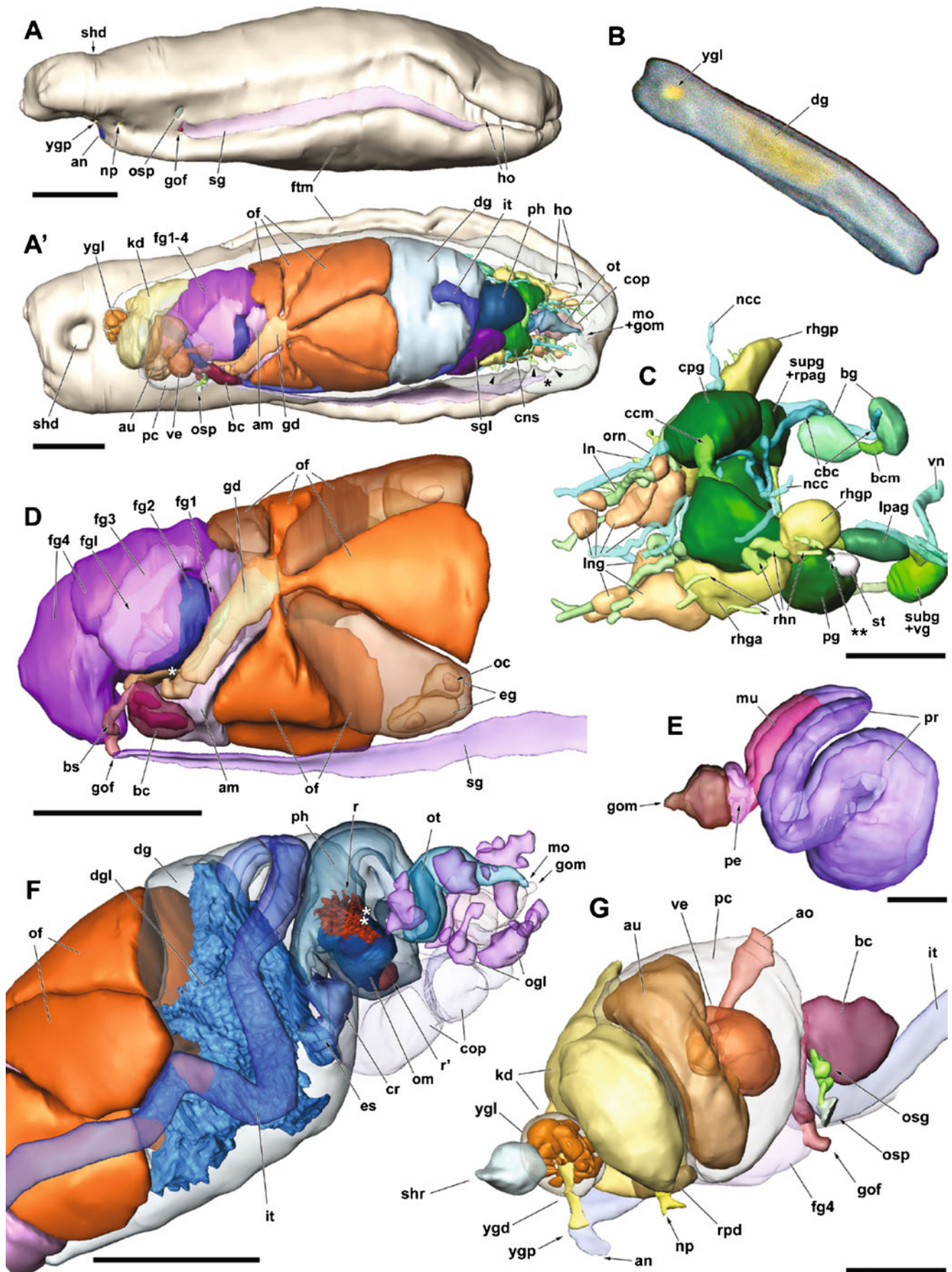


Fig. 1 a–g Three-dimensional reconstructions of *Pluscula cuica* microanatomy. **a** External aspect of body showing body openings, right view. **a'** Dorsal view of body with the dorsum above body cavity and head shown transparent, showing inner organ systems, *arrowheads* short nerves innervating Hancock's organs, *asterisk* anterior end of seminal groove. **b** Live specimen, ca. 2 mm total length, dorsal view. **c** Anterior left view of the central nervous system, pedal nerves omitted, *double asterisk*: large cell next to statocyst, **d** Posterior part of reproductive system, dorsolateral right view, *white asterisk* branching point of gonoduct to female glands and ampulla. **e** Copulatory apparatus, ventral view, anterior towards left. **f** Oblique right view of digestive system, salivary glands omitted, *double white asterisks* positions of salivary duct openings and small glandular field inside pharyngeal lumen. **g** Oblique dorsolateral right view of pericardial complex and surrounding organs. *am* ampulla, *an* anus, *ao* aorta, *au* auricle, *bc* bursa copulatrix, *bcm* buccal commissure, *bg* buccal ganglion, *bs* bursa stalk, *cbc* cerebro-buccal commissure, *ccm* cerebral commissure, *cns* central nervous system, *cpg* cerebropleural ganglion, *cop* copulatory apparatus, *cr* putative crop, *dg* digestive gland, *dgl* lumen of digestive gland, *eg* egg, *es* esophagus, *fg1–fg4* nidamental glands (proximal to distal), *fgl* lumen of nidamental glands, *gd* gonoduct, *gof* female genital opening, *gom* male genital opening, *ho* Hancock's organs, *it* intestine, *kd* kidney, *ln* labiotentacular nerve, *lng* accessory labiotentacular ganglia, *lpag* left parietal ganglion, *mo* mouth opening, *mu* muscular tube, *ncc* nervus clypei-capitis, *np* nephropore, *oc* oocyte, *of* ovarian follicles, *ogl* oral glands, *om* odontophore musculature, *orn* oral nerve, *osg* osphradial ganglion, *osp* osphradium, *ot* oral tube, *pc* pericardium, *pe* penis, *pg* pedal ganglion, *ph* pharynx, *pr* prostate, *r* distal part of radula, *r'* origin of radula, *rhga/rhgp* anterior/posterior accessory rhinophoral ganglion, *rhn* rhinophoral nerve, *rpd* renopericardial duct, *sg* seminal groove, *sgl* salivary gland, *shd* shell dimple, *shr* shell remnant, *st* statocyst, *subg+vg* combined subintestinal and visceral ganglion, *supg+rpag* combined suprainstestinal and right parietal ganglion, *ve* ventricle, *vn* visceral nerve, *ygd* duct of yellow gland, *ygl* yellow gland, *ygp* opening of yellow gland. *Bars a, a', d, f* 250 μ m; *c, e, g* 100 μ m. Interactive version of this figure is available in the supplementary online material.

The interactive model was prepared following the protocol of Ruthensteiner and Heß (2008), using Adobe Acrobat 9.0 Professional Extended software. The model can be accessed in the supplementary online interactive version of Fig. 1.

Results

Remarks on taxonomy

Euthyneura Spengel, 1881: Tectipleura Schrödl et al., 2011a: Euopisthobranchia Jörger et al., 2010a

Cephalaspidea P. Fischer, 1883: Philinoidea Gray, 1850: Philinoglossidae Hertling, 1932 (or Plusculidae Marcus, 1959) *Pluscula cuica* Marcus, 1953a (type by monotypy)

Marcus (1959) separated monotypic Plusculidae from the Philinoglossidae Hertling, 1932 (type species *P. praelongata* Hertling, 1932) based on *P. cuica* retaining a reduced circular shell, the separation of cerebral and pleural ganglia, and the posterior position of the genital opening. Other described distinguishing features include lack of eyes, presence of five distinguishable ganglia on the visceral loop and the derived mode of autosperm transport

from gonad to copulatory organ (via the hemocoel), among others. Bouchet and Rocroi (2005) used Plusculidae Franc, 1968. In contrast, Salvini-Plawen (1973) used a philinoglossid subfamily Plusculinae. Other authors included *Pluscula* and all other genera among Philinoglossidae (e.g., Arnaud et al. 1986).

While generally considered as part of the Philinoidea (e.g., Burn and Thompson 1998; Bouchet and Rocroi 2005), earlier authors commonly used the now obsolete 'order' Philinoglossacea sensu Thiele, 1931 of equal rank to Cephalaspidea (e.g., Marcus and Marcus 1954; Salvini-Plawen 1973). For practical reasons, we use the term 'philinoglossan' to address *Pluscula cuica* and the three other philinoglossid genera.

General anatomy and histology

Living specimens of *Pluscula cuica* are white, with externally visible yellowish digestive gland and the conspicuous 'yellow' gland in the caudal part (Fig. 1b). The body is approximately rectangular in dorsal aspect, and about 3.5 to 4.5 times longer than wide (ca. 1.7 mm \times 500 μ m in the reconstructed specimen), with a smooth epidermis. The dorsal side is slightly convex; head shield and notum are fused without a detectable groove. The head end is concave with rounded corners. The overhanging posterior end of the notum has a dimple on top under which where remnants of the shell-forming tissue are located; the depression appears to be more pronounced in fixed specimens. Slightly more anterior, the conspicuous spherical yellow gland may be visible, if filled (Fig. 1a',b). Four body openings that are usually found inside the mantle cavity are located underneath the right side of the posteriorly overhanging notum (Fig. 1a). Notum and foot are separated by wide longitudinal grooves along the circumference of the body; the grooves are widest on the sides of the head, thinnest along the anterior face of the body, left and right to where the mouth is situated. The foot is only slightly indented anteriorly, it is wider than the notum in the anterior half of the body; posteriorly, the foot is shorter than the notum with a slightly pointed, but not projecting end.

Notum and foot sole show a distinct margin of short motile cilia. Small intraepidermal, light pink glands can be found, especially close to the head; numerous larger pink-staining and fewer dark blue glands are located subepithelially and open to the outside via thin ducts (Fig. 4a). Within the lateral grooves, the epidermis is thinner and lacks glands and contingent ciliation except for interspersed multiciliated cells and the motile cilia of the seminal groove. Left and right of the head, the Hancock's organs are three shallow depressions with dense microvillous border (Fig. 1a,a'; 4d).

Below the epidermis there is loose connective tissue (formed by round cells that contain an unstained vacuole) that is intersected by muscle fibers, especially in the foot.

Instead of the previously described shell, the decalcified examined specimens show only a dense batch of blue-staining, irregularly sorted fibrous material located within the connective tissue of the overhanging notum end, just below the dorsal depression (Fig. 5g). This circular shell organ/vestige (80 μm diameter, 55 μm thick; Fig. 1g) lacks any trace of a dissolved shell.

The main body cavity is round in cross-section along most of the body's length and separated from the outer connective tissue by a strong layer of mostly longitudinal muscle fibers. All major organ systems reconstructed herein are situated within this body cavity (Fig. 1a). A diaphragm is not detectable.

In the most posterior end of the body cavity lies a conspicuous gland which is visible in living specimens as a bright orange-yellow spot (Fig. 1a',b). The gland is roughly spherical (diameter 100 μm) and surrounded by a thin sheath of muscle fibers. It comprises large, columnar cells with a vacuole that in most cells contains remnants of a grey-staining liquid. The cells are of apparently holocrinous nature and discharge into a central epithelial duct (Fig. 5f); the duct opens to the outside just dorsal of the anus (Fig. 1g).

Digestive system

The mouth opening is located medially within the transversal groove separating notum and foot (Fig. 1a'). The oral tube is thin-walled, surrounded by irregular arrangements of pink-staining, single-celled glands (Fig. 4a). Approximately 50 μm from the outside, the copulatory organ branches from the ventral side of the tube. Following this split, the oral tube becomes wider, its inner wall with numerous longitudinal folds, indicating strong extendibility of this part (Fig. 4b,c). There are approximately ten elongate to egg-shaped, light pink-staining oral glands or various sizes situated around the oral tube (Fig. 1f, 4a); a connection to the tube's lumen is, however, detectable only in some.

The pharynx is elongate and curved (Fig. 1f). Its anterior part curves upward, is spacious and comparably thin-walled; in KOH-macerated specimens the pharynx reveals a thin cuticular covering. The posterior part of the pharynx curves downward, is more muscular and contains the odontophore in its ventral portion (Fig. 1f). There are small patches of violet-staining glandular cells to the left and right of the open radula (Fig. 4e). Inside the odontophore, thick longitudinal muscle fibers run parallel to the posterior two thirds of the still folded radula; only the anterodorsal part of the radula is spread open, underlain by paired fluid-filled lacunae. The radula itself has no distinct descending limb and lacks rhachidian teeth; there are approximately 16–20 rows of curved, pointed lateral teeth (six per row). The inner laterals are the largest and are widest at one-quarter of their height (masticatory border); the second and third laterals are smaller and grow continuously thinner towards the tip

(Fig. 4e). Neither serial sections nor light microscopic observation of the radula showed serration of the first laterals (not shown).

The salivary glands are voluminous tubes, their cells filled with comparatively few droplets of dark-blue staining secretion. In the reconstructed individual, the right salivary gland is situated ventrally and appears considerably larger; its ciliated salivary duct can be traced to the right intersection of the thin-walled and muscular walls of the pharynx (white asterisks in Fig. 1f). The left salivary gland is situated dextrodorsally and appears much smaller (Fig. 1a'). The ciliated esophagus exits the pharynx posteriorly and curves downward where it forms a spherical chamber (a vestigial crop?; Fig. 1f); esophagus and putative crop show the longitudinal folds also found in the oral tube. From there a thinner part connects to the stomach dextroventrally. A histologically distinct stomach is not detectable; the presumed stomach lumen appears to extend dorsally, towards the intestine. The digestive gland—pale yellow in living specimens, Fig. 1b—is an externally smooth sac, its outer wall is covered by a mesh of criss-crossing muscle fibers. The digestive gland's rounded anterior face fills much of the body cavity, its posterior face slopes downward (also visible in living specimens) and ends in an elongate tip at about two thirds of the body's length (Fig. 1f). The digestive lumen is outlined irregularly by an epithelium formed mainly by high columnar cells that are rounded apically (surface shown in Fig. 1f) and filled with blue-staining droplets (Fig. 4g, 5c).

The origin of the ciliated intestine is pushed into the digestive lumen in an about 70 μm long trunk-like extension at the anterodorsal side (Fig. 4g); its connection to the stomach is unclear. From there, the intestine curves to the right and runs backwards along the body side to the end of the body, where the anus is situated medially, just dorsal of the foot sole's posterior tip (Fig. 5f).

Central nervous system

The cerebral nerve ring is situated prepharyngeally and most of its ganglia adhere closely to the dorsal and lateral sides of the pharynx (Fig. 1a'). In all ganglia, neurons are situated peripherally just underneath a blue-staining fibrous layer, with central fibrous neuropil extending to the outside as nerves. Accessory ganglia can be distinguished histologically by their distinctly smaller neurons and less obvious separation into cortex and neuropil (Fig. 4b–d).

The paired cerebropleural ganglia are the largest ganglia and are connected by the thick cerebral commissure; each ganglion is hemispherical anteriorly and oblong posteriorly. The cerebropedal and pleuropedal connectives connect each cerebropleural ganglion to the pedal ganglia. The connectives to the ganglia on the visceral loop (pleuroparietal c.) are short (left side) and very short (right side). The

cerebrobuccal connectives are long and slightly undulated; they emerge from the medioventral side of each cerebropleural ganglion and run along the sides of the pharynx. Only the right cerebro-buccal connective could be traced along its entire length.

From each cerebropleural ganglion, four nerves emerge and run laterally and frontally. The anterior and median oral nerve is of medium thickness and appears to innervate the oral tube and mouth opening; on the left side, this nerve shows a distal bifurcation. Slightly more laterally, the very thick labiotentacular nerve emerges; this nerve shows two branches that are equipped with several accessory ganglia: the lateral branch innervates a large ganglion ($70 \times 50 \mu\text{m}$), the median branch shows along its length four smaller ganglia ($25\text{--}40 \mu\text{m}$) that are closer to the digestive tract. On the left side, the first of the small ganglia and the large ganglion are partially fused. The large ganglion emits several short nerves innervating the most anterior epidermal pit in position of the Hancock's organ, while the smaller ganglia show nerves running medially, towards the oral tube and mouth opening.

Two further nerves emerge from the sides of each cerebropleural ganglion. One is thin and extends dorsolaterally (headshield nerve; Fig. 1c). The rhinophoral nerve is very thick (diameter $20 \mu\text{m}$) and emerges laterally; it shows a rather wide connection to the cerebropleural ganglion with possibly two separate roots in the cerebro-pleural ganglion. The rhinophoral nerve splits close to its base, each part supplying two large accessory ganglia: the anterior one is elongate and about $100 \mu\text{m}$ long, the posterior one is situated more posterodorsal and oval ($70 \times 50 \mu\text{m}$). Again, each ganglion innervates sensory cells in pits of the Hancock's organs via at least two to three short nerves (Fig. 4d). A fifth cerebral nerve, thin and running to the oral tube, was detected only on the left side, emerging anterior of the left cerebrobuccal connective. *Pluscula cuica* lacks eyes.

The paired buccal ganglia are of medium diameter and situated at the posterior side of the pharynx just below the origin of the esophagus, under which the buccal commissure passes. Buccal nerves could not be detected.

The paired pedal ganglia are almost spherical and connected by the long pedal commissure. Several nerves of different diameter originate from each ganglion, in general running to the body sides and into the foot. One anterior-running nerve emerges just next to the cerebropedal connective, two nerves emerge close by on the anteroventral face of the pedal ganglion and run anteriorly, and a very thick posterior nerve exits from the posteroventral side. A further posterior-running nerve was found only on the left side, while a dorsolateral nerve emerging just anterior to the statocyst was detected only on the right.

The spherical statocysts are located on the posterodorsal side of each pedal ganglion; each statocyst is of

approximately $30 \mu\text{m}$ diameter and contains a single statolith (Fig. 4f). The static nerve could not be detected. Just anterodorsally to the statocysts of both sides there is a conspicuous 'blister'-like cell containing a large unstained vesicle or vacuole (Figs. 1c, 4f).

There are three medium-sized ganglia on the euthyneurous visceral loop; two are close together on the left side (1, the left parietal and 2, the combined subintestinal and visceral ganglion; terminology after Haszprunar 1985), the third (combined suprainstestinal and right parietal ganglion) being situated just behind the right cerebropleural ganglion. Ganglia two and three are connected by a very long connective passing below the pharynx close to the pedal commissure. The left parietal ganglion is elongate and shows a single nerve curving to the left body side. Ganglion number two (medium-sized, rounded) shows two nerves: the left one thin, the right one (visceral nerve) very thick. Both nerves run posterior inside the body cavity. Ganglion number three (medium-sized) shows another very thick nerve running posterior along the right side of the body cavity.

An additional ganglion, consisting of two to three small lobes, can be found between the female genital opening and the sac of the bursa copulatrix (Fig. 1g). The connection to the central nervous system (CNS) could not be clarified, but there is a short nerve running to a small ciliated pit located inside the right lateral groove just dorsal to the genital opening. This pit consists of higher cells than the surrounding epidermis and might represent a small osphradium (Fig. 5e); we therefore regard the associated ganglion to be an osphradial ganglion.

Pericardial complex

The pericardial complex comprises the main parts of the circulatory and the excretory systems and fills the posterior end of the body cavity.

The circulatory system consists of the thin-walled pericardium, broad auricle and oval ventricle and is located at the posterior right of the body cavity (Fig. 1a'). The auricle is almost as wide as the posterior end of the pericardium and curves around the more anterior ventricle (Fig. 1g). The proximal end of the ventricle is equally thin-walled but shows a transversal, valve-like septum separating left and right (Fig. 5d); the ventricle's distal tip points marginally to the left and has a slightly thicker, muscular wall from which the aorta emerges. The aorta exits the pericardium at its anterior tip; it runs along the upper right of the body wall, parallel to the intestine. Right of the pharynx it splits into two thin-walled hemolymph vessels (Fig. 4c,f); one turns left, runs below the pedal commissure and then anteriorly, the other passes the CNS on the right and terminates close to the oral tube (not shown).

The horseshoe-shaped kidney broadly touches the posterior wall of the pericardium and expands to the left; it is characterized by the typical vacuolate, unstained epithelium. The ciliated renopericardial duct exits from the posterior right end of the pericardium and curves to the left, leading into the thinner limb of the kidney. This runs into the larger part of the kidney at the left body side, which then curves to the front and right again. A very short and thin nephroduct connects to the renal pore located inside the longitudinal groove just right of the foot's tip (Fig. 1g).

Reproductive system

Pluscula cuica is a monaulic hermaphrodite with follicular gonad, posterior right genital opening, ciliated sperm groove on the right body side and copulatory organ opening through the mouth. The posterior part of the reproductive system fills about half of the body cavity.

In the reconstructed specimen, the gonad (ovotestis) consists of six thin-walled, cone-shaped follicles that radiate from a common mid-dorsal position in the gonoduct (Fig. 1a',d). The follicles are widest at the base where they touch the lateral and ventral body wall or the sloping posterior part of the digestive gland (Fig. 1f). Spermatozoa with screw-shaped head fill most of the follicles' volume (Fig. 5c) and are arranged around large nursing cells. Except for the most dorsal, each follicle also contains two to three oocytes in various stages of development (bright nucleus and blue-staining nucleolus without surrounding yolk, or with various amounts of blue-staining yolk droplets) in its ventral portion. Other cell types—gamete precursors or types of nursing cells—are loosely arranged around the periphery of the follicles.

All follicles discharge via short stalks into the dorsally situated gonoduct, a ciliated tube that runs posteriorly, then curves downward. A short stalk (white asterisk in Fig. 1d) leads downward and connects via a small pore to the very large ampulla—a thin-walled sac filled densely with spermatozoa and extending anterior between the gonad's follicles. Unusually for this organ, the walls of the ampulla are irregularly covered with large cells filled with up to ten very large blue-staining droplets (lipids?) (Fig. 5a,b). The postampullary gonoduct curves to the left, forming the nidamental gland mass with a thick and strongly glandular wall and irregularly shaped ciliated lumen. The entire gland mass consists of three, possibly four histologically different parts, three of which form the more convoluted but thinner part running to the left. The first gland (albumen gland) is a short tube characterized by rounded, light blue/pinkish staining cells with gaps between them (Fig. 5b); the second gland (membrane gland) is equally short and has more columnar cells filled with dark blue-staining small droplets (Fig. 5a); the third gland (mucus gland, proximal limb) is an elongate tube and shows columnar, pink-staining cells. Between glands one and two, the

gonoduct wall forms a thin-walled pouch expanding dorsally (another connection could not be found); this pouch is filled densely with spermatozoa (Fig. 5b). It is not clear whether these are auto- or allosperm. The third nidamental gland turns downward. From the turning point on, a uniform part of the gland mass (mucus gland, distal limb) crosses the entire body cavity in a wide curve; its wall resembles that of gland three in histology but is much thicker (cells are at least twice as high and stain slightly darker pink) (Fig. 5a,b). Close to the right body wall, the distal gonoduct becomes non-glandular again for a short distance before opening to the outside; in this part a thin duct splits off and runs straight dorsally (Fig. 5e). Near the end of this duct a spherical pouch (bursa copulatrix) is located at the right body wall (Fig. 1d,g); the bulb is smooth on the outside and shows a more irregular inner surface, its

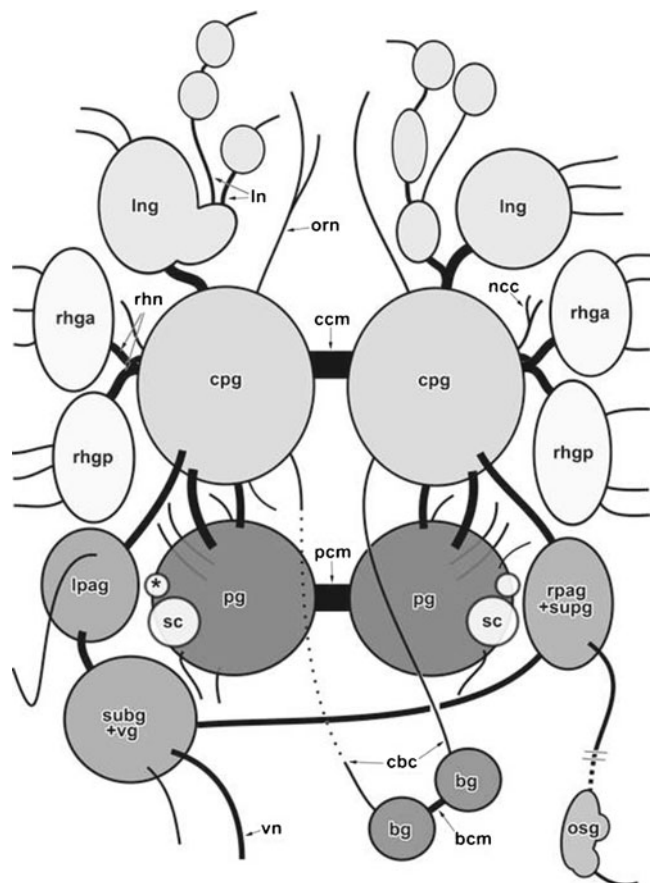


Fig. 2 Schematic dorsal view of the central nervous system (CNS) and nerves, anterior at top. Roughly to scale except for length of pleuro-parietal connectives. *bg* buccal ganglion, *bcm* buccal commissure, *cbc* cerebro-buccal connective, *ccm* cerebral commissure, *cpg* cerebropleural ganglion, *ln* labiotentacular nerve, *lhga* accessory labial nerve ganglion, *lpag* left parietal ganglion, *ncc* nervus clypei-capitis, *osg* osphradial ganglion, *orn* oral nerve, *pcm* pedal commissure, *pg* parietal ganglion, *rhga* anterior accessory rhinophoral ganglion, *rhgp* posterior accessory rhinophoral ganglion, *rhn* rhinophoral nerve, *rpag+supg* combined suprainintestinal and right parietal ganglion, *sc* statocyst, *subg+vg* combined subintestine and visceral ganglion, *vn* visceral nerve, *asterisk* large 'blister' cell next to statocyst

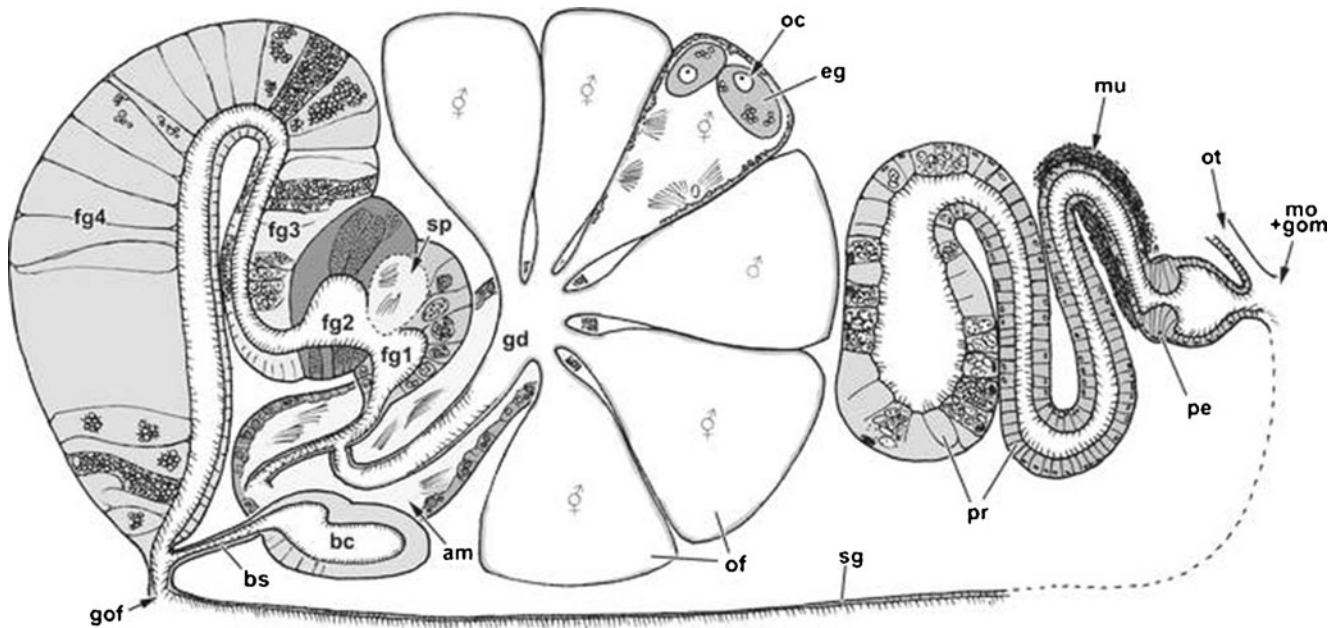


Fig. 3 Schematic dorsal view of the reproductive system, anterior at right. *am* Ampulla, *bc* bursa copulatrix, *bs* bursa stalk, *eg* egg, *fg1* albumen gland, *fg2* membrane gland, *fg3* thin portion of mucus gland, *fg4* large portion of mucus gland, *gd* gonoduct, *gof* female genital

opening, *gom* male genital opening, *mo* mouth opening, *mu* muscular tube, *oc* oocyte, *of* ovarian follicles, *ot* oral tube, *pe* penis, *pr* prostate, *sp* sperm package, *sg* seminal groove

lumen is filled with a homogeneous pink-stained fluid (Fig. 5a). The genital opening is a small pore located ventrally in the right lateral groove (Fig. 1a). From the genital opening, a wide ciliated ribbon runs along the ventral portion of the right lateral groove (Figs. 1a,d,g, 5a). The ciliated strip (or sperm “groove”) disappears approximately at the level of the pharynx (asterisk in Fig. 1a’), so that there appears to be no further specialized structure for sperm transport to the opening of the copulatory organ within the oral tube. In the foot margin below the end of the sperm groove, there is a group of additional glandular cells that open below the sperm groove.

The copulatory organ opens together with the mouth (Fig. 4a). It is a convoluted, blind-ending tube and extends ventrally in the body cavity as far back as the pharynx (Fig. 1a’,f). It connects to the outside via a ciliated duct lined with a regular epithelium of light blue-staining cells with basal nuclei. At first the duct expands slowly before forming an almost spherical pouch, its lumen containing few spermatozoa (Figs. 1f, 2 and 3). The posterior wall of this hollow structure is considerably thicker and forms a circular rim projecting into the lumen, likely forming a penial papilla when everted to the outside (Figs. 1e, 4b). Pouch and papilla are followed by an elongate tube curving to the left; this tube shows only thin epithelial lining but is surrounded by a conspicuous mantle of thick, circular muscle fibers (Fig. 4c). The following prostate is the largest part of the copulatory organ and forms three loops before ending blindly. Its walls are thick, ciliated and glandular; the cells are

filled with unstained vacuoles and mostly apically distributed blue-staining droplets (Fig. 4c,f).

The smaller examined specimen proved to be functionally male. Its gonad consists of six follicles (two large, four smaller) that contain only spermatogenesis. The gonoduct is long, sinuous and non-glandular. There is a comparatively small ampulla with characteristic histology (blue vacuoles in epithelium). The bursa is small and empty. The copulatory organ is small but shows all elements found in the larger specimen.

Discussion

As expected, histological examination of semithin sections and 3D models generated a detailed dataset of microanatomical information with the potential to correct and/or supplement the original description of *Pluscula cuica* Marcus, 1953a. We compare these data to those available in other philinoglossans, with focus on their relationship to other cephalaspideans and in the light of new euthyneuran systematics that were established by recent molecular approaches.

External morphology and habit

Pluscula cuica can be identified by its typical philinoglossan streamlined habit without an external shell or distinct head-shield. The body is ribbon-shaped and elongate, although less than in *Philinoglossa praelongata* (see Arnaud et al. 1986). The cephalaspidean head-shield is either absent

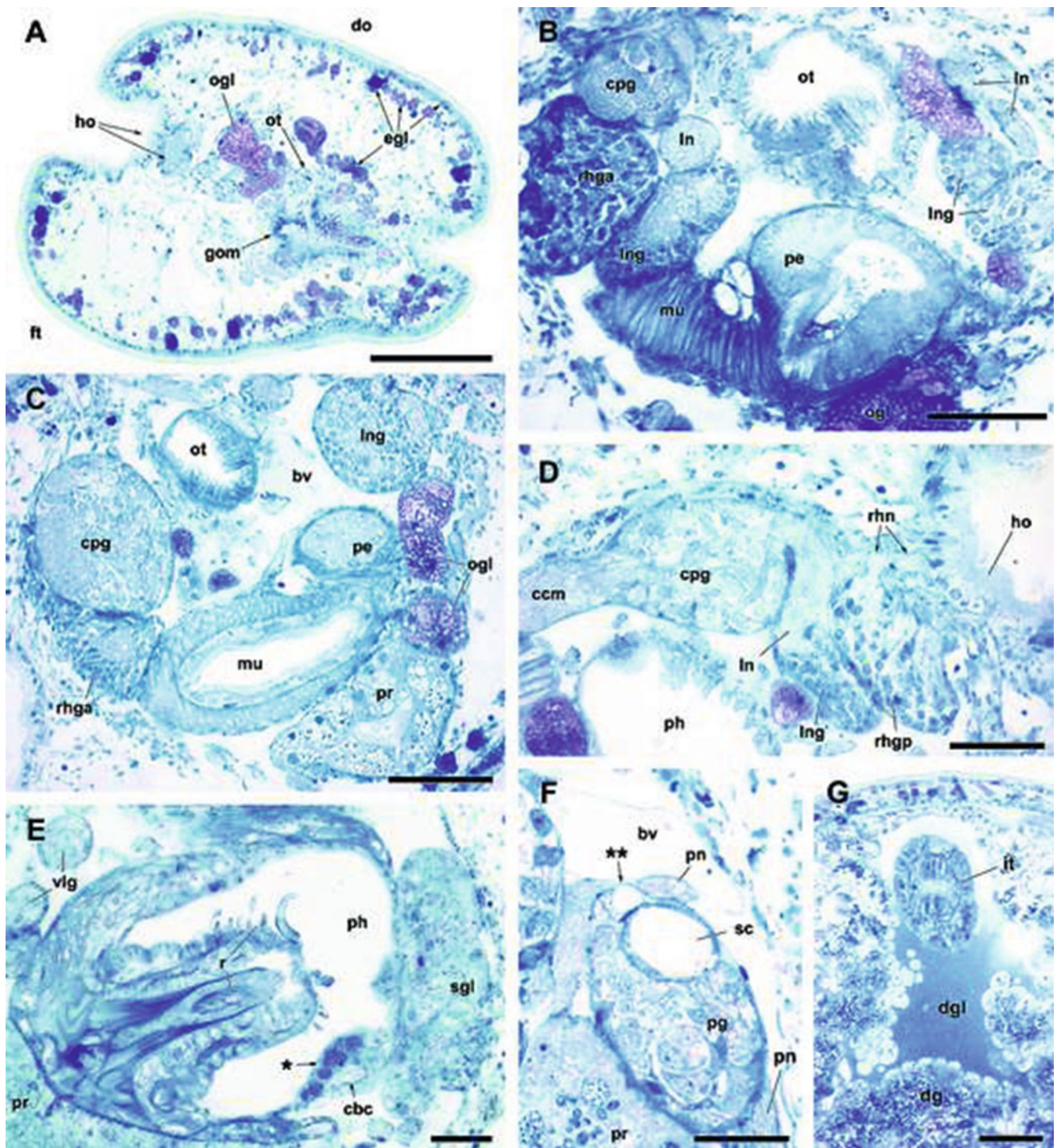


Fig. 4 a–g Semithin histological cross-sections of anterior body half. Dorsal side at *top*, in *e*: at right. **a** Level of mouth opening, showing lateral grooves. **b** Anterior part of CNS and copulatory organ. **c** Section of CNS and copulatory organ posterior to **b**. **d** Detail of right Hancock's organ and its innervation. **e** Pharynx with muscular odontophore and spread radula; *asterisk* patch of glandular cells. **f** Detail of pedal ganglion with statocyst and 'blister' cell (*double asterisk*). **g** Trunk-like anterior end of intestine inside digestive gland lumen. *bv* blood vessel, *cbc* cerebro-buccal connective, *ccm* cerebral commissure, *cpg* cerebro-pleural ganglion, *dg* digestive gland, *dgl* lumen of digestive gland, *do*

dorsum, *egl* different types of epidermal glands, *ft* foot, *gom* male genital opening, *ho* Hancock's organ, *it* intestine, *ln* labiotentacular nerve, *lng* accessory labiotentacular ganglion, *mu* strong muscular lining / muscular tube of copulatory organ, *ogl* oral gland, *ot* oral tube, *pe* penis, *ph* pharynx, *pn* pedal nerves, *pr* prostate, *rhga* anterior accessory rhinophoral ganglion, *rhgp* posterior accessory rhinophoral ganglion, *rhn* rhinophoral nerve, *sc* statocyst, *sgl* salivary gland, *vlg* visceral loop ganglia (sectioned at margins). *Bars* **a** 100 μ m; **b–e**, **g** 50 μ m; **f** 25 μ m

or modified into a shield confluent with the rest of the notum. We prefer the second interpretation, since the anterior part of the *Pluscula* shield is cerebrally innervated. Also, a vestigial separation of the head and body shields by a transversal groove in the first quarter of the body is present in another philinoglossid, *Abavopsis latosoleata* (Salvini-Plawen 1973, own observations). As in most other philinoglossans, the broad dorsum and foot are separated by lateral grooves that create a more or less x-shaped aspect in cross-section (an exception is *Sapha* Marcus, 1959, which is more or less round). Histological similarity of notum and foot surfaces (ciliated epithelium, epidermal glands) might be associated with the ability to crawl on either body side (observed by Hughes 1991), since all-around ciliation is present in many small-sized interstitial heterobranchs and facilitates movement between sand grains (Swedmark 1968). The foot of *Pluscula* is slightly wider than the notum and might reflect vestigial cephalaspidean parapodia. These lateral foot extensions are more pronounced in *Abavopsis*, which shows foot margins that curve upward (Salvini-Plawen 1973). This is slightly less the case in *Philinoglossa*, and *Sapha* shows only indistinct foot margins (Marcus 1959). Parapodia are a feature found in most philinoids (Burn and Thompson 1998), so the presence of a widened foot in *Abavopsis* and *Pluscula* might reflect the ancestral condition.

Pluscula shows the typical caudal overhang of the notum, underneath which the body openings are located in the body wall (see below). The caudal overhang of *Pluscula* is broad and fin-like as in *Abavopsis* and the *Philinoglossa* species; where it was observed to form a bilateral symmetric cavity if the overhang is bent downwards (Salvini-Plawen 1973). In *Sapha*, the overhang is pictured as short and pointed (Marcus 1959); an undescribed '*Philinoglossa*' from Fiji (Morse 1987) resembles this species in that aspect.

In their elongate habit and reduced shell, philinoglossans resemble most the aglajid genera *Philinopsis* or *Nakamigawaia* of which some are infaunal burrowers (Gosliner 1980). These taxa however have a fairly long head-shield (half of body length or longer) in contrast to the vestigial head-shield found in *Abavopsis*, which is rather short as in Gastropteridae (Salvini-Plawen 1973; Gosliner 1989).

Shell remnants and mantle cavity associated organs

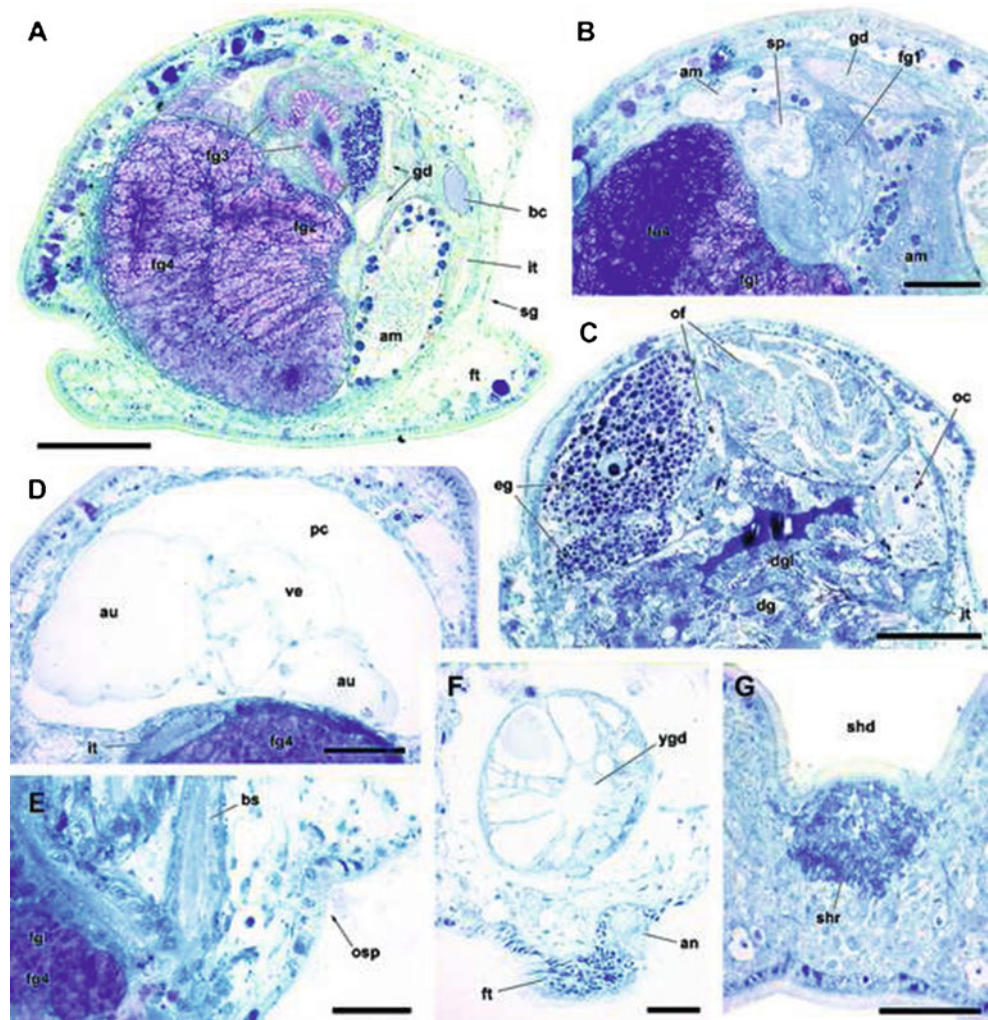
Pluscula cuica and all other philinoglossans are externally shell-less and show a reduced mantle cavity that is roofed by the caudal overhang of the mantle. In *Pluscula*, within a short stretch of epidermis on the right body side there is the anus, the yellow gland opening, the nephropore, the genital opening and the osphradium. *Pluscula cuica* was described to possess a small internalized circular shell below a dorsal depression in the caudal end (Marcus 1953a), neither of which is present in any other philinoglossan. While not easily visible in live

Pluscula, the dimple is quite distinct in the preserved ones examined in this study. However, no remainders of a decalcified shell in macerated specimens, or remnants of an organic matrix or empty spaces in histological sections were observed. Still, the presence of putative vestigial shell-forming tissue just underneath the dimple was confirmed herein, and this is interpreted as an ancestral feature that apparently was lost in (all?) other philinoglossans. Other features present in the putatively ancestral philinoidean mantle cavity and known only for *Pluscula* but no other philinoglossan are the osphradium (detected herein for the first time) and the genital opening associated with the mantle cavity (see the respective chapters).

The spherical yellow gland found in the caudal overhang of philinoglossans is a conspicuous histological feature and visible in many live specimens. In *Abavopsis* and *Philinoglossa* (except *P. marculsi* Challis, 1969b) it is described as an externally visible bright orange spot (Salvini-Plawen 1973, 1984), implying a strongly yellow secretion. In *Sapha*, the gland is located in the pointed tail end (Marcus 1959). Since filled glands apparently turn black by certain preserving agents, Salvini-Plawen (1984) considered them to be homologous with the 'black larval kidney' found in some other heterobranchs, implying paedomorphism (see Haszprunar 1985). These organs are in fact described to be present in larval *Philinoglossa* (Swedmark 1968), but are reduced during metamorphosis—otherwise they would be visible as conspicuous black bodies, as observable, e.g., in some post-metamorphic *Philine* (Horikoshi 1967). Alternatively, Salvini-Plawen (1973) suggested the gland to be part of an adhesive mechanism that he observed in *P. praelongata*: this species supposedly attaches to sand grains by its tail end, aided by 'glands of the epidermis and the pallial gland'. This was not observed for other philinoglossans yet but might well represent an adaptation similar to other members of the meiofauna. Some of these show localized adhesive mechanisms, e.g., rhodopemorphs that possess a caudal adhesive gland that are likely derived from glands of the foot sole (Brenzinger et al. 2011b), and thus not homologous to the gland in philinoglossans.

The nomenclature of glands located in the floor or roof of the mantle cavity in traditional opisthobranch taxa is confusing (see e.g., Wägele and Klussmann-Kolb 2005; Wägele et al. 2006 for review), therefore homologies are difficult to establish. With respect to the yellow gland of *Pluscula*, position and histology—large unstaining vacuoles in the spherical gland surrounded by muscle fibers, with epithelial duct opening ventrally, yellow secretion—were already described by Marcus (1953a). He noted similarities to the 'Blochmann's' gland in *Aplysia* but followed Guiart (1901) in simply naming it a 'pallial' gland. Salvini-Plawen (1973) highlighted the similarities to the Runcinacean 'pallial' or 'suprabranchial' gland; we confirmed this observation. In histological aspects, the gland of *Pluscula* resembles most the 'yellow' gland of

Fig. 5 a–f Semithin histological cross-sections of posterior body half. Dorsal side at top. **a** Overview at level of nidamental glands. **b** Detail of nidamental glands with interjected sperm package. **c** Ovarial follicles. **e** Most distal gonoduct and osphradium. **f** Yellow gland. **g** Caudal dorsal depression with shell ‘remnant’, insert: complete cross-section. *am* Ampulla, *an* anus, *au* auricle, *bc* bursa copulatrix, *bs* bursa stalk, *dg* digestive gland, *dgl* digestive gland lumen, *eg* egg, *fg1* albumen gland, *fg2* membrane gland, *fg3* short limb of mucus gland, *fg4* large limb of mucus gland, *fgl* female gland lumen, *ft* foot, *gd* gonoduct, *it* intestine, *oc* oocyte, *of* ovarian follicle, *osp* osphradium, *pc* pericardium, *shd* shell ‘dimple’, *shr* shell ‘remnant’, *sg* seminal groove, *sp* interjected sperm package, *ve* ventricle, *ygd* duct of yellow gland. Bars **a**, **c** 100 μ m; **b**, **d**, **g** 50 μ m; **e**, **f** 25 μ m



aglaeids which Rudman (1972a, 1978) considered unique for that family. Dayrat and Tillier (2002) rejected the homology of yellow and purple/Blochmann's glands, and Blochmann's glands were coded as absent for aglaeids by Wägele et al. (2006). Most other philinoids also show glands in the mantle cavity, but these are often groups of single subepithelial cells that do not open through a common epithelial duct and are therefore difficult to homologize. For example, the 'pallial' glands of some *Philine* species consist of a patch of cells that open separately into the mantle cavity (e.g., Challis 1969a; Rudman 1972b: 'posterior' gland; Guiart 1901: 'fossette glandulaire'). Nevertheless, a conspicuous yellow secretion was reported for *P. trapezia* Hedley, 1902 (Rudman 1998) and *P. caledonica* Risbec, 1951 (Risbec 1951; which might be the same species according to Rudman, 1998). Members of the Gastropteridae sometimes show a patch of dark-staining glandular cells surrounding the anus (Brodie et al. 2001; Klussmann-Kolb and Klussmann 2003); their additional large 'posterior pedal gland' is different in structure or in position (e.g., Gosliner 1994). Lemche (1956) reported the unicellular or multicellular 'Blochmann's' glands of *Cylichna*, positioned

dorsally in the mantle cavity roof and with an epithelial duct, to contain a secretion that is yellow in life but does not stain with methylene blue. *Scaphander lignarius* L., 1758, a species that produces a thick yellow fluid when disturbed (Guiart 1901), possesses single-celled Blochmann's glands that open through an epithelial duct (Perrier and Fischer 1911). Therefore, it seems that glands situated dorsally in the mantle cavity (or what is left of it) are present plesiomorphically in most philinoids, and persist in many or most other cephalaspideans and Euopisthobranchia. The aforementioned histological staining properties and position have also been reported for the Blochmann's gland of *Haminoea* by Wägele and Klussmann-Kolb (2005: Fig. 5d). Therefore, we regard the yellow gland of philinoglossids and aglaeids to be a derived multicelled Blochmann's gland. The specific configuration may represent a synapomorphy of these two families. However, since the most recent molecular phylogenies never found a sistergroup relationship between the two families, the yellow gland might have been lost or modified in other philinoidean lineages, or may be a product of convergent evolution in philinoglossids and aglaeids. Regarding the function of the aglaeid gland,

Rudman (2001) assumed either an excretory or defensive function and observed the secreted substance to be toxic for annelids. Sleeper et al. (1980) identified the gland's secretions in *Navanax* as 'alarm pheromones', Cruz-Rivera (2011) observed an 'amber-coloured' secretion to repel potential fish predators.

Pluscula cuica thus matches other philinoglossans in the reduction of a distinct shell, although associated tissues are still present. The mantle cavity is also lost, but most organs and body openings found within the ancestral cephalaspidean mantle cavity are still present underneath the caudal overhang. Only the gill and current-inducing ciliated strips—typical for philinoidean mantle cavities (e.g., Rudman 1972b)—are lost completely, as is the case in all other meiofaunal slug lineages (Swedmark 1968; Arnaud et al. 1986). This loss of course indicates that respiration has to take place entirely through the body wall, as is supported by Bartolomaeus' (1997) observation of numerous subepidermal blood sinuses in *Philinoglossa helgolandica* Hertling, 1932.

Circulatory and excretory systems

Our findings on the circulatory and excretory systems of *Pluscula cuica* correspond well to the original description (Marcus 1953a). The heart is located slightly right of the midline, and consists of a wide auricle posterior and slightly left of the ventricle, indicating that *Pluscula* is almost completely detorted. This organization is in general agreement with Bartolomaeus' (1997) ultrastructural study on the heart and kidney of *P. helgolandica* which showed that the valve is described to consist of only a single, flattened cell. Judging from our histological sections, there appear to be more nuclei in the valve of *Pluscula*. As described by Marcus (1953a), the kidney is largely horseshoe- or 'u'-shaped and consists of a slim part running from the pericardium to the left, and a more voluminous part curving back to the nephropore at the right body side. The parts of the kidney appear very similar in histology; we were not able to detect ciliation in the proximal part described for *P. helgolandica* by Bartolomaeus (1997).

Digestive system

The digestive system of *Pluscula* conforms well to the original description and the general philinoglossan organization. Described differences among the genera can be found in the presence of denticles on the first lateral teeth, possibly the presence of a vestigial crop in *Pluscula* and in the form and dimensions of the digestive gland.

Nearly all philinoglossids are described with a long and curved pharynx similar to that of *Pluscula*, with the radula situated far posterior (Hertling 1932; Marcus and Marcus 1954; Marcus 1959). Our material suggests that the anterior part of the pharynx and especially the posterior oral tube are rather expandable due to the presence of longitudinal folds.

Chitinous jaw plates present in Euopisthobranchia are secondarily lost in many Philinoideans (Burn and Thompson 1998), including philinoglossids. Jaws are present only in some philinids and allgastropterids (Rudman 1972b; Gosliner 1980, 1989), therefore jaws were lost multiple times convergently. All philinoglossans possess a radula (formula given as $n \times 3.0.3$ or $2.1.0.1.2$) that especially resemble philinids and gastropterids in tooth form (see Gosliner 1994). Since reduction of the rhachidian tooth row has occurred separately in all other philinoid families, it is therefore hardly useful for phylogenetic comparison with philinoglossans (see Gosliner 1980; Rudman 1972b). The first lateral teeth of *Pluscula*, *Abavopsis*, *Philinoglossa praelongata* and *P. marcus* are described without smaller denticles along the masticatory border; however, denticles of this size might be hard to detect without SEM studies and their number also depends on the size of specimens (see Salvini-Plawen 1973; Challis 1969b). Therefore, 'absence of denticles' in the literature might not always be a useful taxonomic character in philinoglossans, as is exemplified by *Pluscula*: neither Marcus (1953a) nor our light microscopical examination of sectioned material and separated radulae revealed denticles, but Marcus and Marcus (1954) mention about 20 denticles per tooth in later collected material. Comparative re-examination using scanning electron microscopy might be needed to reveal if denticulate teeth occur consistently in any philinoglossan, or if intraspecific plasticity reduces the taxonomic value of this character, as is known for some other marine gastropods (e.g., Padilla 1998; Reid and Mak 1999). Following the pharynx, *Pluscula cuica* shows a slightly dilated esophagus where most other philinoideans have an unarmed crop (Aglajidae, e.g., Rudman 1974, Gastropteridae: Gosliner 1989) or a gizzard armed with cuticular plates to grind up hard-shelled food (many Philinidae: Rudman 1972b). Neither crop or gizzard are described for other philinoglossans, but the structure found in *Pluscula* may represent vestiges of the ancestral condition, if not an artifact. A gizzard armed by cuticle was regarded as a synapomorphy of Euopisthobranchia (Jörger et al. 2010a), but spines or calcareous plates are reduced secondarily in many philinoideans (Burn and Thompson 1998).

Pluscula (and *Sapha*) do not possess a histologically distinct stomach between esophagus and digestive gland, in contrast to *Abavopsis* and *Philinoglossa*, which are described with a small and smooth-walled stomach (Salvini-Plawen 1973). In *Pluscula*, the pale yellow digestive gland is a single sac and located anterior to the gonad in mature specimens. The sloping rear face of the digestive gland—visible in living specimens—might be a useful diagnostic character for *Pluscula*, and was also observed in an undescribed species from Belize (K.M. Jörger, Munich, personal observation). In all other species the digestive gland extends almost to the end of the body cavity. *Sapha* and *Abavopsis* possess a single digestive gland (Marcus 1959; Salvini-

Plawen 1973); in *Philinoglossa* there are two tubular branches, one of which is long, coiling, and ventral to the gonad (Hertling 1932; Marcus and Marcus 1954; Salvini-Plawen 1973). The latter case resembles other philinoids that possess more than one digestive gland, e.g., *Philine exigua* (Challis 1969a; Martínez et al. 1993). In all philinoglossans, the intestine emerges from the stomach/ digestive gland anterodorsally and curves along the right body side; the anus is posteriomedian. Only in *Abavopsis* the intestine is described to emerge more on the left, running underneath (!) the digestive gland for much of its course (Salvini-Plawen 1973). The funnel-like extension of the proximal intestine into the digestive gland lumen was found only in the reconstructed specimen and may be an artifact, since it is not reported for other philinoglossans species.

There are no reports of philinoglossan food sources, although Marcus and Marcus (1954) mention ‘a large diatom’ in the intestine of *P. remanei* Marcus and Marcus, 1958. The lack of distinct cuticular armament in the gut implies that food is not hard-shelled. Radular morphology, coupled with the thin pharyngeal cuticle and infolding of the (?dilatible) preradular digestive tract, may hint at a carnivorous habit of philinoglossans on soft-bodied prey. Although predation was not observed directly, co-occurring acochlidians extracted from sand samples disappeared from Petri dishes when kept with philinoglossans over night and thus may be a possible food source, at least under lab conditions (own observations). Carnivory would be consistent with the general condition in Philinoidea.

Central nervous system

One reason to argue for a basal phylogenetic position of *Pluscula cuica* within Philinoglossidae, or for separation from the latter in its own family, was the supposed “primitiveness” of the cerebral nerve ring and the visceral nerve cord. This was based on the supposed separation of cerebral and pleural ganglia (Marcus 1953a, 1959; Salvini-Plawen 1973) and also the presence of five distinguishable ganglia on the visceral nerve cord (albeit four of them closely allied, forming two pairs; Marcus 1953a). Reexamination of the nervous system, however, shows that neither is the case. Free pleural ganglia in *Pluscula* were identified originally by Marcus (1953a) lateral to the cerebral ganglia, with connectives to the latter and the pedal ganglia. This is a misobservation, since cerebral and pleural ganglia form fused cerebropleural ganglia as is evident from semithin histological sections and visible on the 3D model. As other philinoglossans, *Pluscula* has cerebropleural ganglia showing characteristic double connectives to the pedal ganglia. Marcus’ laterally situated ‘pleural’ ganglion therefore is most likely the (posterior) rhinophoral accessory ganglion; however, the reported connective of these laterally situated ganglia to the pedal ganglion does not exist. This unusual

lateral-pedal connective was also described for the ‘lateral’ ganglia of *Philinoglossa remanei* and *P. praelongata* (Marcus and Marcus 1954; Salvini-Plawen 1973). It should be critically reinvestigated whether this connective presents a genuine structure.

The presence of five ganglia on the visceral cord has been proposed as a synapomorphy of Euthyneura (=Pentaganglionata, Haszprunar 1985, 1988), although most taxa possess a lower number of separate ganglia that have been interpreted as the result of various stages of ontogenetic fusion. Dayrat and Tillier (2000) challenged such a scenario claiming that there are very few reliable examples of euthyneurans showing a pentaganglionate condition, i.e., just six genera, of which two belong to basal heterobranchs according to molecular data (see Schrödl et al. 2011a). *Pluscula* was overlooked as a pentaganglionate candidate; if confirmed, it would be the only cephalaspidean reliably showing five ganglia on the visceral loop. Our results, however, demonstrate that mature *Pluscula* possess only three ganglia on the visceral nerve cord. These three ganglia correspond well to the single ganglion and two closely aligned pairs mentioned by Marcus (1953a), although our material shows more than superficial fusion. The visceral nerve cord of *Pluscula* is not fundamentally different from that of other philinoglossans, since all other species are described with three ganglia, except for *P. praelongata* which Huber (1993) reinvestigated and reported four (although his Fig. 10 shows only three).

Cerebral nerves and sensory organs

Pluscula cuica possesses a set of four paired cerebral nerves (plus a single nerve on the left side) that correspond well to the nerves found in previous investigations of other cephalaspidean species (Faller et al. 2008; Staubach et al. 2008). Following the nomenclature of nerves identified by the previous authors and Huber (1993) in other heterobranchs, we identified an oral nerve (anteromedian), the labiotentacular nerve (basally branched, with one large and several small extra ganglia), the rhinophoral nerve (possibly with a double root, basally branched with two large extra ganglia), and a small nervus clypei-capitis (head-shield nerve). The single median nerve extending from the left cerebral ganglion could not be identified, and a corresponding nerve on the right side was not detected either. The finding of a vestigial head-shield nerve (n. clypei-capitis) in *Pluscula* is important since it suggests an ancestral presence and secondary reduction of a functional cephalaspidean headshield in philinoglossans. Most cephalaspideans possess an elaborate nervus clypei-capitis that innervates the posterior part of the head-shield (e.g., Staubach et al. 2008); this nerve is less branched in other heterobranchs, if identified at all (Huber 1993). Reduction of an externally discernible head-shield is thus confirmed as one of the synapomorphies of philinoglossans (Arnaud et al.

1986). Only *Abavopsis latosoleata* shows a slight transversal groove indicating remainders of a separate head-shield (Salvini-Plawen 1973), and previously only this genus was shown to possess a thin nervus clypei-capitis branching from the base of the rhinophoral nerve (Huber 1993). If confirmed, a loss of the headshield nerve in *Philinoglossa* (shown by Huber 1993) and *Sapha* might represent a synapomorphy uniting these genera.

Pluscula cuica is unusual among philinoglossans in that it lacks eyes, which appears to be an apomorphy of the species. In *Abavopsis* and *P. praelongata*, the eyes are innervated through a branch of the large labiotentacular accessory ganglia (Salvini-Plawen 1973). Among meiofaunal slugs, loss of eyes is found convergently among several taxa (Swedmark 1971), e.g., among rhodopemorphs (own observation), pseudovermids (see Urgorri et al. 1991) and some acochlidians (Marcus 1953a).

We are not aware of literature mentioning the paired ‘blisters’ embedded in the pedal ganglia next to the statocysts. They are not present in *Philinoglossa praelongata* (own observation). The structures might represent single specialized cells. If not for their position next to the statocysts, one might confuse the structures with the vestigial, unpigmented eyes found, e.g., in some acochlidians (see Challis 1968; Neusser et al. 2011a).

Accessory ganglia

Accessory ganglia anterior and lateral to the cerebropleural ganglia are described for all philinoglossans examined in detail, but nomenclature and proposed innervation patterns differ considerably in the descriptions (e.g., Marcus 1953a, 1959; Salvini-Plawen 1973; Huber 1993). In all cases there appear to be large ganglia (lateral and anterolateral to the cerebropleural ganglia) and distinctly smaller ones (mostly anterior and more median). In *Pluscula*, one large rhinophoral ganglion was identified originally as the pleural ganglion (see above); five further ‘precerebral’ ganglia were described on both branches of the labial nerve (Marcus 1953a). In *Sapha*, there are paired large ‘Hancock’s’ and ‘olfactory’ ganglia, and pairs of small ‘labial’ and ‘prepedal’ ganglia (Marcus 1959); innervation of these ganglia was not described. *Abavopsis* was originally described without accessory ganglia (Salvini-Plawen 1973), but Huber (1993) showed that there are two large ganglia on each rhinophoral nerve and one large and one small on each labiotentacular nerve, similar to the condition found in *Pluscula*. *Philinoglossa praelongata* was described originally with small anterior ‘accessory’ ganglia and two large ganglia innervating the Hancock’s organs: one ‘olfactory’ ganglion (with the two connectives to the cerebropleural and pedal ganglia as originally and falsely described for *Pluscula*; = accessory rhinophoral ganglion?) and one ‘labial’ ganglion (also innervating the eye; = large labiotentacular

ganglion?) (Salvini-Plawen 1973). Except for the double connective, this configuration largely agrees with Huber’s (1993) examination of the same species. A connective between the pedal and a large ‘precerebral’ ganglion was again described for *Philinoglossa remanei* by Marcus and Marcus (1954); this ganglion also innervates the Hancock’s organ together with two ‘olfactory’ ganglia, besides smaller ‘labial’ ganglia. The number of large ganglia in *P. remanei* (two or three) is not entirely clear. Summarizing the literature and homologizing with the ganglia found in *Pluscula*, the following general pattern of innervation of the accessory ganglia appears to be present in all philinoglossans: there is one accessory rhinophoral ganglion in *Sapha* and *Philinoglossa praelongata*, and two in *Pluscula* and *Abavopsis*. These and the large accessory labiotentacular ganglion innervate the posterior and anterior parts of the Hancock’s organ, as is postulated or observed for numerous cephalaspideans (e.g., Huber 1993; Mikkelsen 1996; Staubach et al. 2008). A variable number of smaller accessory labiotentacular ganglia innervate the lip and/or oral tube.

Additional, accessory ganglia innervated by cerebral nerves are characteristic features of meiofaunal slugs. These structures are described for rhodopemorphs (Salvini-Plawen 1991), pseudovermid nudibranchs (Ev. Marcus 1953a; Huber 1993), the sacoglossan *Platyhedyle* (Rückert et al. 2008), microhedylocean acochlidians (e.g., Neusser et al. 2006) and the limnic hedylopsacean *Tantulum* (Neusser and Schrödl 2007). Among Cephalaspidea, only philinoglossans and *Philine exigua* (Challis 1969a) show accessory ganglia. Wherever examined, these accessory ganglia are innervated by the rhinophoral and labiotentacular nerves (as in *Pluscula*). Accessory ganglia are often histologically distinct in lacking a separation into cortex and medulla (Neusser et al. 2006). Marcus (1953a) specifically states that this is not the case in *Pluscula cuica* (in contrast to the acochlidian *Ganitus evelinae* described in the same paper). Our material shows that the neurons in the accessory ganglia of *Pluscula* are considerably smaller than those in the other ganglia, making identification on histological sections possible at a glance. This is in contrast to the accessory ganglia of acochlidians that differ in overall organization but not in neuron size (as mentioned above). The function of the conspicuous accessory ganglia of meiofaunal heterobranchs has so far been a matter of speculation. Haszprunar and Huber (1990) argued that additional neurons were needed in small-sized ganglia to help mediating ‘essential activities’. However, they also noted that miniaturized slugs that are not meiofaunal, e.g., runcinids or the nudibranch *Vayssierea*, do not show these accessory ganglia (e.g., Huber 1993; Baba 1937) and that the evolution of accessory ganglia is therefore linked to the mesopsammic habitat. Since the accessory ganglia are invariably found associated with sensory nerves, they might rather reflect the need of additional nervous capacity in this three-dimensional interstitial living space, as was argued by Jörger et al. (2008). The development

of large accessory ganglia innervating the Hancock's organs may imply comparatively enhanced chemosensory or tactile capabilities, involved in trailing chemical cues or for simply finding the easiest way to push through the complex three dimensional pore-spaces of the interstitial habitat.

Osphradium

Pluscula cuica is the so far only meiofaunal slug demonstrated to possess an osphradium with an associated ganglion. Originally, a posterior 'genital' ganglion close to the female genital opening was described for *Pluscula* and *Sapha* (Marcus 1953a, 1959), but innervation patterns were not observed. In *P. remanei*, Marcus and Marcus (1954) assumed innervation by the visceral nerve. In *Abavopsis*, a possibly similar ganglion is located at the posterior end of the copulatory organ (Salvini-Plawen 1973). Our material of *Pluscula* confirms the presence of the ganglion next to the genital opening and also shows innervation of a small pit resembling a small osphradium in histology (ciliated pit with higher, unstained, columnar cells; see Edlinger 1980) and position (right body side, close to organs and body openings plesiomorphically situated in a mantle cavity). We therefore regard this posterior ganglion to be homologous to the osphradial ganglion of other heterobranchs. In this case the ganglion should be innervated by the nerve extending from the combined right parietal and suprainestinal ganglion (e.g., Haszprunar 1988) and not the visceral nerve which leads into the same general direction. A chemosensory osphradium has not been reported for any other meiofaunal slug. Many acochlidians possess an osphradial ganglion, but an osphradium was detected only in the secondarily large-bodied *Strubellia* and *Acochlidium* (Brenzinger et al. 2011a). Osphradia are likely present in many meiofaunal slugs with an associated ganglion, but in these cases the sensory epithelium has been reduced to only few sensory cells. Presence of sensory areas in other species bearing osphradial ganglia needs reinvestigation.

Reproductive system

The reproductive system of *Pluscula cuica* unites usual and thus plesiomorphic philinoid cephalaspidean features with those that appear highly derived but typical for meiofaunal slugs. The hermaphroditic gonad of adult *Pluscula* is not divided into distinct female and male follicles save for the medial and strictly male follicle. The latter was also described by Marcus (1953a), but interpreted as an autosperm ampulla rather than part of the gonad. Contrary to *Pluscula*, *Sapha* and *Philinoglossa remanei* have strictly female acini located either at the left side or ventral of the strictly male ones, respectively (Marcus and Marcus 1954; Marcus 1959). Data on *Abavopsis* are not conclusive. Spatial separation of gamete production is a feature commonly found in meiofaunal slugs (Swedmark

1968): *Rhodope* shows a consecutive separation of male and female ovotestis follicles (Brenzinger et al. 2011b), some meiofaunal acochlidians have separate ovaries and testes (Morse 1976) or are completely gonochoric (Challis 1968; Schrödl and Neusser 2010). The meiofaunal *Philine exigua* has some follicles that produce either only one type of gamete besides follicles that produce both (Challis 1969a).

Philinoideans generally possess three different sperm storing structures (besides one associated with the copulatory organ): a proximal ampulla for autosperm, a receptaculum seminis for long term storage of allosperm, and a distal bursa copulatrix for allosperm storage and/or lysis (e.g., Gosliner 1994; Mikkelsen 1996). Identification of these structures according to their relative positions rather than histology or a combined approach is advocated (Gosliner 1994; Valdés et al. 2010), but may be a preconception that misses actual structure, homology and function (e.g., Mikkelsen 1996; Wägele and Willan 2000). Our histological data suggests that *Pluscula* possesses a stalked, sac-like ampulla that is unusual in several aspects: first, it is extremely large and splits off an unusually long part of gonoduct that is located between gonad and nidamental glands (instead of being a widening close to the gonad). The ampulla reaches far anterior, but it opens to the gonoduct at its posterior end. Second, the ampulla shows an unusual but distinct histology with large (?lipid) droplets covering the wall, instead of being a thin-walled sac conforming to the gonoduct in histology (see Gosliner 1994). A proximal ampulla is described for all philinoglossan genera; it is also sac-like but smaller in *P. remanei* (Marcus 1953a; Marcus and Marcus 1954), but tubular in *Sapha* (Marcus 1959).

Pluscula does not show a distinct receptaculum seminis: this organ usually follows the ampulla closely and would be identifiable by spermatozoa embedded into the muscularly lined wall with their heads (e.g., Beeman 1977). No such structure is found in the material examined herein, and no receptaculum is described for any other philinoglossans. Loss of a distinct proximal receptaculum seminis may represent a synapomorphy of philinoglossans, since it is present in other philinoidean groups (e.g., Rudman 1972a, b; Gosliner 1980, 1989).

We interpret the distal stalked sac, filled with pink secretion and branching from the gonoduct close to the genital opening, to be a bursa copulatrix. Marcus originally described this structure in *Pluscula* as a 'spermatheca or receptaculum seminis that contains spermatozoa' (1953: p 180); he also describes a 'red and blue' staining secretion. This histological character is typical for the allosperm-digesting bursae, but not for a receptaculum according to newer terminology (Beeman 1977; Valdés et al. 2010). No other philinoglossan is described with a similar structure, but a bursa with at least temporary gametolytic function is present in most other philinoids and may represent a plesiomorphic character in *Pluscula*.

The pocket containing spermatozoa between the membrane and mucous glands in one examined specimen is most likely not a permanent feature. It may be a received package of allosperm or a spermatophore, a temporary fertilization chamber, or a package of autosperm on its way out.

The three parts of the female gland mass of *Pluscula* correspond well to the albumen, membrane and large mucous glands of most other ‘opisthobranchs’ (Gosliner 1994; Klussmann-Kolb 2001), but comparison to other philinoglossans is not straightforward due to ambiguous literature. *Philinoglossa remanei* has a ‘protein’ gland and sac-like mucous glands (Marcus and Marcus 1954); the nidamental glands of other species are not described in further detail. In *Abavopsis*, the nidamental glands are situated posteriorly as in *Pluscula*, but are apparently followed by a long distal gonoduct part leading to the anteriorly shifted genital opening (Salvini-Plawen 1973). In *Philinoglossa* and *Sapha*, the distal gonoduct is short since the female glands are also shifted towards the genital opening (Marcus and Marcus 1954; Marcus 1959). This situation differs from that of *Pluscula* and other philinoideans and may be a synapomorphy of a *Philinoglossa/Sapha* clade.

The female genital opening in *Pluscula* is close to the posterior end of the body—as in other philinoids—showing its affiliation with the ancestral mantle cavity (Burn and Thompson 1998). In the remaining philinoglossans the opening is in the anterior right third, e.g., at the posterior border of the head-shield in *Abavopsis* (Salvini-Plawen 1973); therefore, the seminal groove that is present in philinoglossans is generally short compared to that of *Pluscula*. At least in *Pluscula*, there is a gap between the seminal groove and the male genital opening. Marcus (1953a) identified acidophilous glands along the rim of the anterior sperm groove in mature individuals, and assumed a role in guiding spermatozoa. We were able to identify additional glands in the foot at this position, although they seem to open through the foot sole and not the sperm groove.

Pluscula cuica possesses a sac-like cephalic copulatory organ that contains several histologically separable parts. Marcus (1953a) originally identified the following elements (from anterior to posterior): an epidermal pouch, a narrow and tubular penis, followed by a short tubular prostate, and a bulbous ‘seminal vesicle’. Our material shows that the penis consists of a rather short ring-like structure at the base of the epidermal pouch which is followed by a tube with strong subepidermal circular muscles. The posterior part is histologically uniform because the prostate and its autosperm-storing end are confluent, instead of forming a distinct ‘seminal vesicle’. In *Sapha*, the copulatory organ was also described to consist of four parts (Marcus 1959), but with a different order: following a distinct penial papilla, there is a long prostate and then a sphincter-like muscle (and not vice versa), the muscle closing the large spherical seminal vesicle. Marcus

and Marcus (1958) show a similar configuration in *P. helgolandica*, but mention the short part anterior to the ‘seminal vesicle’ to be of glandular nature, not muscular. In *P. remanei* and *Abavopsis*, the copulatory organ is described as a simple, bag-like structure with variable orientation, even looping around the oral tube (Marcus and Marcus 1954; Salvini-Plawen 1973). It remains unclear whether the copulatory organ is truly less elaborate in the latter taxa compared to the condition found in *Pluscula*. Nevertheless, the sac-like copulatory organ of philinoglossans in general appears to differ from that of other philinoideans in being less elaborate, probably due to size constraints. Judging from histological examinations, there is no true eversible papilla (perhaps excepting *Sapha*) but only a slightly prominent ring, and there never is the cuticular armament found at least in some groups, e.g., Gastropteridae (Anthes and Michiels 2007a, b). Functionally more important, in philinoglossans there is no separate posterior-leading vas deferens (“ejaculatory duct” according to Mikkelsen 1996) leading directly to the prostate as e.g., in *Philine* species (Rudman 1972b); therefore, autosperm have to enter and exit the copulatory organ via the same opening. This two-way configuration is more similar to what is found, e.g., in the spermatophore-producing *Runcina* species (Kress 1985).

Sperm transfer by a “kiss”?

Rather than anterodextrally as in most cephalaspideans, the male genital opening of *Pluscula* is situated frontally at the head. It is joined to the anterior oral tube, as was also observed by Marcus (1953a). The same condition is reported for *P. helgolandica* (Marcus and Marcus 1958), *Sapha* (Marcus 1959) and *Abavopsis* (Salvini-Plawen 1973). This means that the copulatory organ of philinoglossans has to be everted through the mouth during copulation. It seems that philinoglossans have taken to the extreme a trend that is found in Aglajidae and Gastropteridae (see Anthes and Michiels 2007a, b), where the male genital opening is shifted to underneath the anterior side of the headshield. This is in contrast to other philinoideans that have it located more on the right side of the head (e.g., Rudman 1972b), as is the plesiomorphic condition for cephalaspideans. More specifically, the male genital opening inside the mouth is also found in the meiofaunal acochlidian *Pontohedyle milaschewitchii* Kowalevsky, 1901; this aphyllid species glues spermatophores indiscriminately onto a partner’s epidermis (Jörger et al. 2008, 2009). In the meiofaunal *Philine exigua*, the opening appears also to be more anterior than in other, burrowing or benthic members of the genus (Challis 1969a). The extreme anterior shift may therefore be another adaptation particular of meiofaunal groups, facilitating sperm transfer within the limited space and dynamics of sand interstices (Swedmark 1964): in an animal

moving between sand grains, it is the anterior face that touches a partner most readily. Sperm transfer would be possible by a simple “kiss” on a quickly passing partner’s epidermis (in the case of hypodermal injection or dermal insemination), or on the genital opening in species that copulate. This latter head-to-tail mode of copulation can be suggested for *Pluscula* because of the opposite positions of the male and female genital openings, and because sperm transfer by a trailing ‘male’ is known to take place in a number of other philinoideans (e.g., Rudman 1972a). However, since the other philinoglossan genera have also shifted the female genital opening anteriorly, copulation in these genera could more be bilateral or sequential, but also more head-to-head and thus again less space-consuming.

Autosperm transport through the hemocoel?

Marcus’ (1953a) original description of *Pluscula cuica* suggests a highly peculiar mode of autosperm transport, probably unique among gastropods: on their way between the gonad’s follicles and the sperm-storing part of the copulatory organ, sperm were hypothesized to move directly through the hemocoel, and not along the gonoduct and external ciliated groove. This was concluded because (1) apparently all ‘mature’ specimens examined by Marcus showed numerous spermatozoa free in the body cavity, with the highest density between gonad and copulatory organ, and (2), the external ciliated groove was found to disappear before connecting to the copulatory organ, implying that its original function as a conveyor of autosperm was lost.

We can confirm the peculiar lack of a continuous sperm groove in *Pluscula*, although the gap could be explained by the presence of sensory epithelium (Hancock’s organs) in this place (Fig. 1a). Since the lateral furrow itself is quite narrow, it might still have sufficient capability in guiding sperm towards the mouth. Furthermore, there are additional glands below the end of the sperm groove which Marcus (1953a) hypothesized to facilitate a further passage of sperm by producing ‘protective secretions’ (1953: p 181). The lack of a continuous sperm groove might be a consequence of an overall beneficial apomorphic anterior shift of the copulatory organ. A gap in ciliation may not be much of a hindrance to sperm transport: spermatozoa are known to be capable of moving along the epidermis of species without such a groove [Karlsson and Haase 2002 in the nudibranch gastropod *Aeolidiella*; Brown (1979) on *Colpodaspis thompsoni*]. Since our specimens examined were mature hermaphrodites and none of them contained free spermatozoa in the hemocoel (as would be expected assuming internal autosperm transport) we conclude that sperm is conveyed externally via the sperm groove, as usual.

How then to explain Marcus’ observation of hemocoelic spermatozoa in *Pluscula*? If autosperm, it could be squeezing

or fixation artifact, or it could have been allosperm. In other meiofaunal slugs, a proportionally common mode of sperm transfer is by hypodermal injection or dermal insemination: it was suggested for species of *Rhodope* (Brenzinger et al. 2011b) and was observed in the microhedylacean acochlidians *Pontohedyle* and *Ganitus evelinae* Marcus, 1953a (Jörger et al. 2009; Marcus and Marcus 1954). In these generally aphyallic species, sperm are transferred through the epidermis; at least in *Pontohedyle* this happens by lysis of epidermal cells induced by the dermally applied spermatophore (Jörger et al. 2009). After dermal insemination, the spermatozoa move through the body cavity and fertilization supposedly takes place somewhere inside the gonoduct or directly in the gonad. Explaining Marcus’ (1953a) observation of hemocoelic sperm in *Pluscula cuica* in a similar way is, however, inconsistent with the presence of a distal bursa copulatrix in the species. Such an allosperm storage organ is usually present only in copulating species, or in non-copulating species that may inject spermatozoa directly into the (large) bursa using a copulatory stylet (e.g., the acochlidian *Pseudunela*; Neusser et al. 2009b). Since hemocoelic spermatozoa have never been reported in other philinoglossans, their occurrence should be critically reinvestigated in other species.

Origin of the Philinoglossidae

The advent of molecular systematics cast doubt on long-held beliefs in euthyneuran topologies, and studies using multi-locus markers started to change our concepts of their evolution (e.g., Göbbeler and Klussmann-Kolb 2011; Jörger et al. 2010a). The backbone topology of a “new euthyneuran tree”, with Nudipleura basal to the common clade of Euopisthobranchia and panpulmonates—as summarized by Schrödl et al. (2011a)—was supported by recent phylogenomic data (Kocot et al. 2011; Smith et al. 2011), and is also compatible with a recent molluscan phylogenetic study based on housekeeping genes (Vinther et al. 2011). In contrast, the traditional concept of monophyletic Opisthobranchia and Pulmonata is contradicted by all phylogenomic and other approaches that include nuclear rather than mitochondrial genes.

Rather than being basal opisthobranchs, the Cephalaspidea in a modern sense (sensu Malaquias et al. 2009) form one of several clades of the so called Euopisthobranchia (Jörger et al. 2010a) among tectipleuran Euthyneura (Schrödl et al. 2011a). Philinoglossans lack the major euopisthobranch synapomorphy, a cuticularized gizzard. Having a large body-shield rather than a head-shield, a posterior mantle cavity, and a simple, frontal copulatory organ they somewhat resemble similarly small-sized runcinids. However, molecular data clearly indicate that philinoglossans are cephalaspideans in the strict sense (Jörger et al. 2010a; Göbbeler and Klussmann-Kolb 2011). The prepharyngeal nerve ring combined with monaulic genital system qualifies Philinoglossidae as Cephalaspidea

sensu Malaquias et al. (2009), and the presence of a secondarily modified head-shield innervated by the nervus clypei-capitis fits with the placement into a higher, non-diaphanoidean clade. Having a narrow radula, a carnivorous gut type without cuticle and gizzard plates and a slender, at least externally shell-less body points towards a placement among philinoidean lineages. In fact, both multi-locus analyses with broader taxon sampling (Malaquias et al. 2009; Göbbeler and Klussmann-Kolb 2011) identify a philinoidean clade of *Philinoglossa* and Gastropteridae as sister to Aglajidae plus Philinidae, with Scaphandridae as outgroup. At the current state of knowledge, possible shared characters of a gastropterid/philinoglossid clade may be a comparatively short headshield and the anterior shift of the copulatory organ. A philinoglossid/aglajid clade on the other hand would be supported by the presence of a spherical yellow gland and the loss of jaws. Molecular hypotheses on the origin of Philinoglossidae within Philinoidea thus are consistent with morphological evidence discussed herein and by Salvini-Plawen (1973), although the exact position remains unclear. Nevertheless, a previously proposed higher category, i.e., ordinal Philinoglossacea Thiele, 1931, is no longer required.

We show that previously discussed “primitive”, potentially progenetic or at least aberrant features such as separate pleural and cerebral ganglia, a pentaganglionate visceral loop, and hemocoelic autospERM transfer in *Pluscula* were due to misobservations or artifacts. A gizzard with three plates that is characteristic of ancestral, non-carnivorous cephalaspideans including philinoidean Scaphandridae and Philinidae is absent in most Aglajidae, Gastropteridae (Rudman 1978; Gosliner 1989), and likely carnivorous philinoglossans. This supports their independent origin from mesopsammic *Philine exigua* as indicated by molecular analysis (Jörger et al. 2010a). We propose that philinoglossans are small-sized, though not obviously pedomorphic invaders of mesopsammic spaces, evolving a detorted streamlined body, precerebral accessory ganglia, a frontal, potentially unilateral mode of sperm transfer, losing and modifying allosperm receptacles, reducing the ancestral shell, and reducing and modifying the mantle cavity and associated organs. All these traits are adaptive and synapomorphic for Philinoglossidae, but have evolved convergently in interstitial members of other heterobranch lineages. The conspicuous yellow gland found in *Pluscula* and other philinoglossans can be roughly homologized with similar glands in other philinoidean lineages (especially Aglajidae), but limited comparative histological knowledge inhibits definite conclusions.

Within Philinoglossidae, the case of *Pluscula cuica* showing a number of morphological plesiomorphies that support its basal position among philinoglossans is weakened. We could not find any shell, but putative vestiges of shell-forming tissue at most. An osphradium, the vestigial crop and the comparatively elaborate copulatory organ described herein might be

further plesiomorphies but need comparative reinvestigation in the other genera. Stronger evidence supporting a basal position are the retained posterior position of the female genital opening and the presence of a putative bursa copulatrix. None of these features was described from other philinoglossans. If confirmed, their apparent absence might be a synapomorphic loss, indicating that *Pluscula* is the most basal branch of Philinoglossidae, as had been assumed by previous authors (Marcus 1953a; Salvini-Plawen 1973). In conflict with this scenario are the putative retention of parapodia and an at least temporarily detectable separation of the head-shield from the rest of the notum in *Abavopsis*, and presence of two digestive gland branches in *Philinoglossa* species. Both parapodia and nervus clypei-capitis are more developed in *Abavopsis*, but remainders are still detectable in *Pluscula*. We suggest that a separate family Plusculidae for *Pluscula cuica* as established in the literature (e.g., Bouchet and Rocroi 2005) is no longer warranted.

Pluscula cuica can be distinguished externally from all other known philinoglossans by the lack of eyes, the dimple in the dorsal side of the caudal overhang, the presence of only a single digestive gland with a sloping posterior face. So far identified internal features include aforementioned plesiomorphies, and possibly the presence of the paired ‘blister’ cells next to the statocysts.

The remaining Philinoglossidae are united by further reductions (shell-associated tissue, bursa copulatrix) and shared characters (anterior shift of the female genital opening). *Philinoglossa* appears to be most derived (vermiform, tail-end glueing, simple copulatory organ, lateral separation of ovotestis follicles; Salvini-Plawen 1973) but shows two digestive gland lobes (cephalaspidean plesiomorphy). This highlights the continuing lack of comparable microanatomical data on the philinoglossans. Some current datasets, such as the denticulation of the lateral radula teeth as a criterion for species delimitation, should be reviewed (Salvini-Plawen 1973). The origin of monophyletic Philinoglossidae from a presumed gastropterid—or aglajid-like ancestor—and the evolutionary scenario proposed herein with more or less successive adaptation to meiofaunal environment, should be further investigated. An integrative approach combining more comprehensive molecular datasets with additional morphological data seems most promising to evaluate proposed homologies and traits of evolution.

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