COMPUTER-BASED 3-DIMENSIONAL RECONSTRUCTION OF MAJOR ORGAN SYSTEMS OF A NEW AEOLID NUDIBRANCH SUBSPECIES, *FLABELLINA ENGELI LUCIANAE*, FROM BRAZIL (GASTROPODA: OPISTHOBRANCHIA)

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ABSTRACT

Obtaining accurate and comprehensive anatomical information from small opisthobranch specimens has been a major problem. Computer-based 3-dimensional reconstruction from serial histological slides applying AMIRA (TGS Graphics) software is shown to be an efficient and fully reproducible way to analyse tiny and complex organ systems in their true relative positions and proportions; this method is herein applied to nudibranchs for the first time. We used *Flabellina engeli lucianae* n. subsp., a small aeolid (up to 8 mm body preserved length) from subtropical southern Brazil, to illustrate all major organ systems including nervous systems, and discuss them comparatively. *Flabellina engeli lucianae* differs externally from congeners by having a translucent body with opaque white and iridescent blue spots, orange ceratal bands, and by the special branching of cerata forming distinct groups on common peduncles. External and internal differences from the apparently geographically and hydrographically separated Caribbean specimens of *F. engeli engeli* Marcus & Marcus, 1968 are discussed in detail.

INTRODUCTION

Faunal studies on Brazilian opisthobranch gastropods began with Dunker (1875), von Ihering (1886, 1915) and MacFarland (1909). Between 1952 and 1985, Ernst and Eveline Marcus conducted extensive inventories, especially of intertidal, benthic opisthobranchs, increasing the number of opisthobranchs known from Brazil from ~30 species to ~170 species, many of them new (see Ev. Marcus, 1977; Rios, 1994). In numerous studies (e.g. Er. Marcus, 1955, 1957, 1958; Ev. Marcus, 1970, 1972, 1976, 1983; Ev. Marcus & Er. Marcus, 1952, 1960, 1967, 1969), they published morphological and anatomical descriptions that were excellent by contemporary standards regarding details given, and information on small species was often obtained or supplemented by histological work. However, subtidal communities were hardly sampled, and several descriptions suffered from the lack of sufficient material or from analytical restrictions of gross anatomical dissecting and paraffin-based histology. Later, original work on Brazilian benthic opisthobranchs is limited to a few studies: e.g. Ortea *et al*. (1994) redescribed the aeolid nudibranch *Nanuca sebastiani* Marcus, 1957; Troncoso *et al*. (1998) established a new subspecies *Hypselodoris picta lajensis* (Nudibranchia: Doridoidea); García *et al*. (2002); García & Troncoso (2003, 2004) reported several additional opisthobranchs from the northern Brazilian off-shore island Fernando de Noronha and described new species belonging to the genera *Aegires* Lovén, 1844, *Phidiana* Gray, 1850 and *Anetarca* Gosliner, 1991. Most recently, Padula & Absalão (2005) reported *Babakina festiva* (Roller, 1972) from southern Brazil, and Pola *et al*. (2005) described the bizarre *Tambja stegosauriformis* Pola, Cervera & Gosliner, 2005 from Cabo Frio, Rio de Janeiro State. These findings suggest that there may be several more macroscopic opisthobranch species to be discovered subtidally on the continental coast.

The aims of our own collecting in southern Brazil were to discover undescribed, previously unrecorded, or little known, species by using snorkeling and SCUBA-diving techniques, and to fix specimens appropriately for modern analytical methodology. Herein, we describe a small aeolid species, *Flabellina engeli lucianae* n. subsp., from southern Brazil, comparing it with the similar Caribbean *F. engeli* Marcus & Marcus, 1968 that was partially redescribed most recently by Calado *et al*. (2005). In addition to standard examinations, i.e. observing living specimens and anatomical study through dissecting and ultrastructural scanning techniques of hard parts, we performed histological examinations of semithin sections. As a novelty for nudibranchs, we reconstructed major organ systems 3-dimensionally from serial histological sections using AMIRA software (see e.g. Neusser *et al*., 2006; Ruthensteiner, 2006); advantages and limitations of this methodology are discussed.

MATERIAL AND METHODS

The specimens were collected between November 2002 and December 2005 using SCUBA at 4–15 m depth. After observing the specimens *in situ* they were relaxed with a 10% $MgCl_2$ solution and preserved in 70% ethanol. One specimen was preserved in 96% ethanol without previous narcotization. Three specimens were dissected macroscopically (Table 1). SEM examinations of jaws and radulae were made using a Leo 1430 VP scanning electron microscope. Two specimens were embedded in Spurŕs low viscosity resin (Spurr, 1969), one of them was serially cross-sectioned (2 μ m) using a microtome with Ralph glass-knives. The sections were stained with methylene blue-azure II (Richardson *et al*., 1960) and examined microscopically. The anterior third of the body (mouth to posterior end of pericardium) was selected for 3-dimensional

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Table 1. Specimens of *Flabellina engeli lucianae* n. subsp. examined.

Museum No.	Preservation	Collecting site (Brazil)	Analytical methods applied	Body length, breadth, height (mm) (preserved)	Radula: length (mm); No. of rows
MZSP 48251 (holotype)	70% ethanol	Paraty	External morphology only	*c.* 10 mm	?
ZSM 20040127 (paratype)	70% ethanol	Trindade	Embedded in Spurŕs resin, serial semithin sectioned, 3-dimensional reconstruction	8; 2; 3	?
ZSM 20040128 (part of paratype 2004027)	96% ethanol	Trindade	–	Piece of the animal's foot	?
ZSM 20040130 (paratype)	70% ethanol	Trindade	Dissected, light microscopical pictures of the radula	10; 2; 3	0,65; 20
ZSM 20040129 (paratype)	70% ethanol	Parati Mirim – Ilha do Rato	–	7; 1,5; 2,3	?
ZSM 20040131 (paratype)	70% ethanol	Parati Mirim – Ilha do Rato	Dissected, SEM's of hard parts	8; 2; 3	0,52; 19
ZSM 20040133 (paratype)	70% ethanol	Búzios – Praia do Forno	Dissected, SEM's of hard parts	12; 2; 3,5	?
ZSM 20040134 (paratype)	70% ethanol	Búzios – Praia do Forno	Embedded in Spurr's resin; uncut	7; 2; 3	?
ZSM 20040132 (paratype)	96% ethanol	Búzios – Praia do Forno	External examination only	'1/2 animal'	?

reconstruction of relevant organ systems; the posterior body cavity contains parts of the digestive gland and gonad only. Every second histological section was used for reconstruction, i.e. it was photographed, the image processed digitally, imported into AMIRA 3.0 (TGS Template Graphics Software, Inc., USA), and aligned. The body surface and all detectable nervous, digestive, circulatory, excretory and genital tissues and organs were marked. After creating a 3-dimensional reconstruction, the organ systems were analysed separately and with regard to their relative positions from different angles of view. Anatomical, histological and 3-dimensional results were cross-checked, and schematic drawings were prepared.

SPECIES DESCRIPTION

Family Flabellinidae Voigt, 1834

Genus *Flabellina* Voigt, 1834

Flabellina engeli lucianae new subspecies

Type material: Holotype: MZSP 48251, Lula Beach, Paraty, Rio de Janeiro, Brazil 23°11′38.5″S 44°38′06.5″W, 4 m depth (Simone col. 10/xii/2005). Paratypes: Rio de Janeiro, Brazil; Trindade, 8 m depth, ZSM Moll 20040127, one specimen (series of semithin histological sections), ZSM 20040128, part of foot (S. DaCosta col. 22/xi/2002), 8 m depth, ZSM 20040132, half specimen (S. DaCosta col., 22/xi/2002); Buzios, Forno Beach, 12 m depth, ZSM 20040134, one specimen (in Spurr's resin) (S. DaCosta col., i/2003); Paraty-Mirim, Rato Is., ZSM, No. 20040129, one specimen (accidentally dried and rehydrated) (S. DaCosta col., xii/2003); Paraty-Mirim, Rato I. to Buzios, Forno Beach, 8–15 m depth, ZSM 20040130, 20040131, 20040133, three specimens (S. DaCosta col., xi/2002 to i/2003).

Additional material: photographs of specimens from Laje Santos and Isla Arvoredo, Brazil.

Etymology: This subspecies is dedicated to two Lucianas; the daughter of the first author, and the wife of the second author.

External morphology (Figs 1A–D, 2A–C): crawling animals reach a maximum length of 20 mm (preserved animals up to 8 mm). Body elongate and slender, tail pointed. Propodial tentacles well-developed, recurved and pointed. Oral tentacles long (up

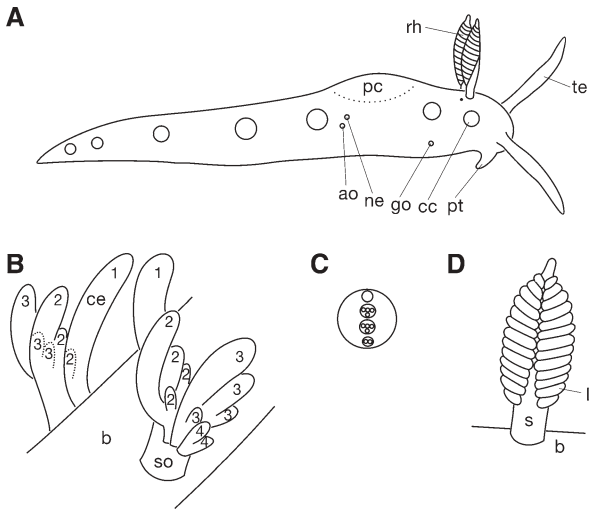

Figure 1. *Flabellina engeli lucianae* n. subsp., (semi)schematic drawings of a preserved specimen. **A.** Entire specimen, lateral (right) view. **B.** Ceratal arrangement on peduncles; cerata having joined bases are numbered equally. **C.** Schematic arrangement of cerata; view from above on peduncle (outer circle) with particular or groups (middle-sized circles) of cerata (small circles). **D.** Rhinophore, frontal view. Abbreviations: ao, anal opening; b, body; cc, ceratal cluster; ce, ceras; go, genital opening; l, lamella; ne, nephroproct; pc, pericardial hump; pt, propodial tentacle; rh, rhinophore; s, stalk; so, peduncle; te, oral tentacle.

to 5 mm) and round in cross-section. Rhinophores slender, club-shaped, reaching 4 mm in length; stalked; club slender and perfoliated with 13 relatively broad lamellae; tip elongate (Fig. 1D). Seven pairs of ceratal clusters on distinct peduncles (Fig. 1A), two of them anterior to pericardium. Cerata slender, maximum length of 4 mm; tip pointed. Second to sixth clusters with up to 11 cerata, anterior and posterior clusters with reduced numbers. Fully developed clusters showing a characteristic arrangement (Fig. 1B, C) bearing one long inner ceras, four cerata with a common base, another group of four joined cerata, and an outer group of two joined cerata. Gonopore, nephropore and anus located on right side of body, between second and third ceratal clusters. Gonopore ventral to second ceratal cluster. Pleuroproctic anus situated in interhepatic space, slightly anterior to third ceratal cluster. Nephropore opening immediately anterodorsal to anus.

Colour (Fig. 2A–C): body translucent bluish; pink or orange foregut and gonad shining through tissue. Oral tentacles opaque white, but bases translucent orange and tips translucent. Opaque white spot anterior between oral tentacles; elongated white spot posterior to base of each tentacle. Rhinophores translucent with opaque white lamellae. Opaque white spot between rhinophores. Propodial tentacles opaque white except for translucent base. Cerata opaque white, with translucent bases and tips; an orange band at half length with diffuse borders. No distinct notal border present, but position indicated by an opaque white line, or more or less irregular blotches. Tail with opaque white median line. Broken row of seven opaque white spots along sides of body; below, on sides of foot, additional row of *c*. 27 small iridescent blue dots.

Epidermis (Fig. 3D): histologically, epidermis consisting of cylindrical cells, staining bright blue; in between many violet-stained secretory cells.

Foot (Fig. 3A): foot sole densely ciliated. Subepidermal, violet staining cells of foot gland filling anteroventral body cavity while being limited to lateral bands posterior to pharynx. Some weakly staining connective tissue within foot gland layer; some blue-stained, diagonal, crossing muscle fibres detectable.

Central nervous system (Figs 2D–G, 4): comprising paired cerebropleural, pedal, buccal, gastrooesophageal and rhinophoral ganglia, which surround anterior oesophagus. All these ganglia showing an outer cortex and an inner medulla. Cortex of all but rhinophoral ganglia characterized by giant neurons with large, dark-blue staining nuclei. Ganglionic neuropile, commissures, connectives and other nerves uniformly blue-grey stained and lacking any nuclei.

Cerebral and pleural ganglia completely fused; single, short, thick commissure. Cerebropleural ganglia slightly larger than pedal ganglia. Cerebropleural-pedal connectives short. Large rhinophoral ganglia anterodorsally attached to cerebropleurals, connectives very short. Cortex of rhinophoral ganglia showing numerous small cells with dark-staining nuclei. Two rhinophoral nerves leaving each rhinophoral ganglion and leading into rhinophores. Rhinophoral nerves thick, convoluted basally, lacking nuclei.

First cerebropleural nerve (nervus labiotentacularis according to Huber, 1993) leading anteriorly into oral tentacles. Second nerve (nervus oralis) following pharynx anteriorly; at level of oral tube ramifying into several thin nerves innervating buccal area. Third nerve thicker and directed posteriorly, passing statocyst, entering salivary gland and branching into three nerves; one of these dividing again shortly after leaving salivary gland. These tiny nerves could not been followed but their posterior direction indicates them to be parts of posterior pallial nerve (see Hoffmann, 1939).

Well-developed lens eyes situated laterally at cerebropleural ganglia, close to pedal ganglia; eye nerves short. Statocysts closely posterior to eyes, static nerve short. Statocysts hollow spheres containing 15–20 ovoid, dark-brown staining, crystalline statoconia. In addition to cerebropleural-buccal connectives, left cerebropleural ganglion bearing three, right one four further thin nerves with unclear identity.

Pedal ganglia situated ventrally to cerebropleural ganglia and ventrally to anterior oesophagus. Pedal commissure short; a parapedal commissure could not be distinguished. Each pedal ganglion bearing eight nerves: five thicker nerves running to foot and ramifying considerably, three thinner nerves could not be followed to their destination.

Buccal ganglia attached to posterior pharynx, and situated close to pedal ganglia. Cerebropleural-buccal connectives and buccal-gastro-oesophageal connectives leaving each buccal ganglion. Buccal commissure very short, bearing a single buccal nerve. Small ovoid gastro-oesophageal ganglia lying anterodorsally to buccal ganglia. Buccal-gastro-oesophageal connectives moderately short. Each gastro-oesophageal ganglion bearing a nerve that could not been followed.

Digestive system (Figs 5–7): oral tube surrounded by a layer of subepidermal oral glands. Glandular cells ovoid, with variable sizes. No deferent ducts detected. Pharynx muscular, epidermis covered by thin cuticle. Anterolateral pair of thin and ovoid cuticular jaws. Masticatory border partly free from jaw plate, containing up to four denticle rows; marginal row with elongate conical denticles, inner rows with small and depressed bumps (Fig. 7C). Radula (Fig. 7A, B) triseriate, with formulae of $20 \times 1.1.1$ (650 μm length; specimen ZSM 20040130) and $19 \times 1.1.1$ (522 μm length; specimen ZSM 20040131). Rachidian teeth triangle-shaped. Functional rachidian measuring 62 μm from base to tip. Central cusp prominent, 12 μm long, tip slightly recurved; base slightly elevated or at same level as 6–8 shorter and thinner lateral denticles on each side. Lateral teeth elongate triangular, with extended primary cusp; 6–7 well-developed, irregular denticles on inner side, outer side smooth. A pair of tubular salivary glands extending from pharynx to stomach. Salivary gland cells large and dark-stained. Oesophagus short and thin-walled, not distinguishable from stomach histologically. Stomach large and swollen, bearing three major digestive gland ducts. Two anterolateral branches exit at level of second ceratal cluster, inserting left and right precardial cerata, respectively. Third one leaving stomach posteroventrally as a straight duct. Short lateral branches inserting all cerata of precardial rows. Digestive gland cells weakly staining, with numerous blue-stained granules. Cerata each bearing apical, unstalked cnidosac. Cnidosacs ovoid, muscular, showing an apical constriction with a pore. Dark-blue staining cnidocysts attached to inner cnidosac wall. Short intestine leaving stomach posteriorly to lateral right digestive gland duct. Anal opening located slightly anteriorly to third ceratal cluster. Intestine thin-walled and longitudinally folded proximally; epithelium ciliated.

Circulatory and excretory systems (Fig. 8): pericardial hump externally visible between second and third ceratal clusters. Voluminous pericardium lying posteriorly to stomach and dorsally to anterior part of kidney; epithelium thin. Heart two-chambered, with trapezoid, thin-walled auricle situated left-posteriorly to ventricle. Only one vessel could be detected entering auricle posteriorly. Ventricle pear-shaped, wall thick and muscular. Main aorta exits anteriorly. Renopericardial duct leaving pericardium right-anteriorly; duct narrow and ciliated along its entire length, entering kidney anterodorsally. Kidney very elongated, folded sac extending from stomach to tail,

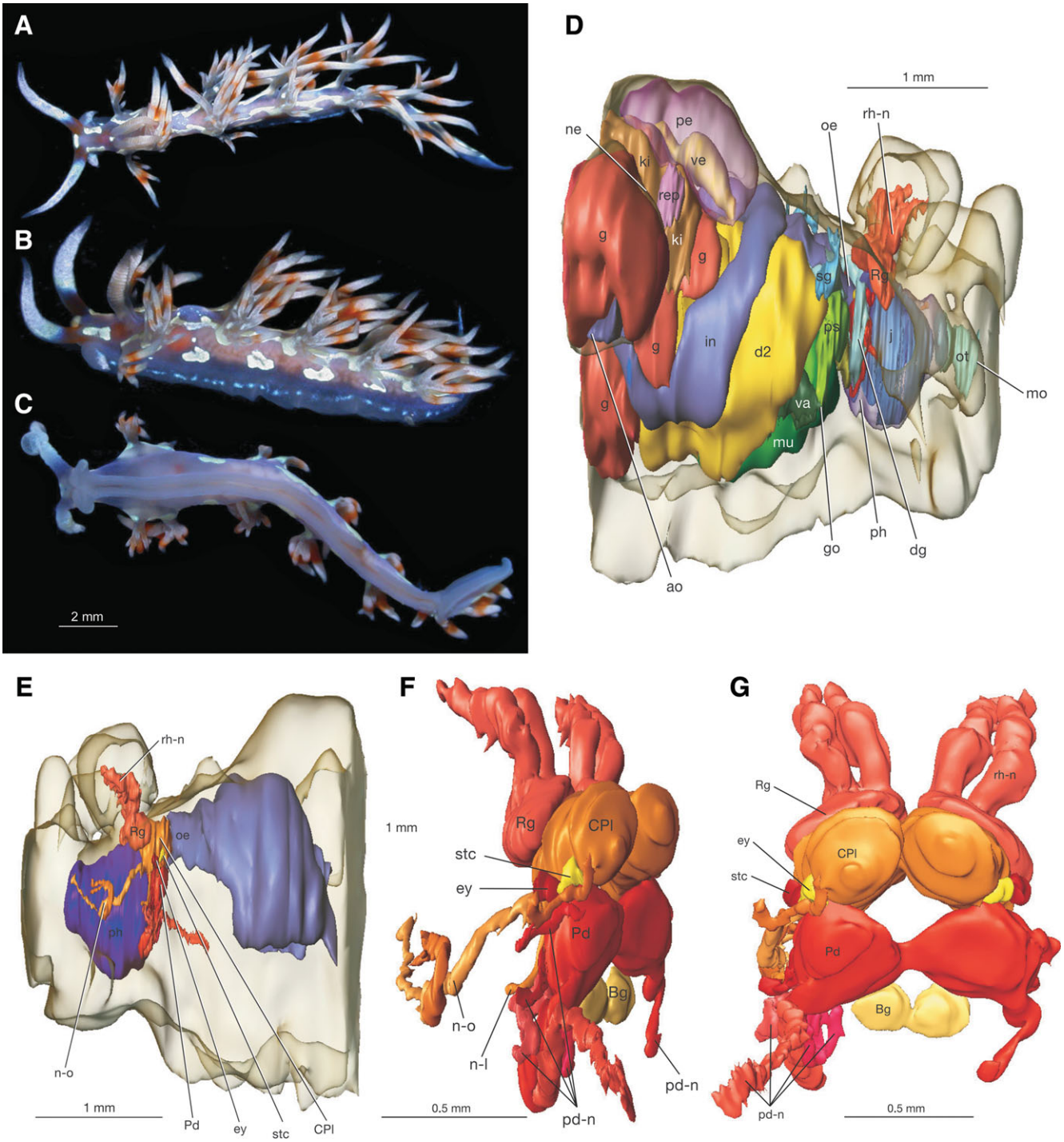

Figure 2. *Flabellina engeli lucianae* n. subsp. **A–C.** Living animals. A. Dorsal view. B. Lateral view. C. Ventral view. **D.** Lateral (right) view on all reconstructed organs (anterior third of body). **E–G.** 3-dimensional reconstruction of the central nervous system (CNS); on the right side, most nerves were omitted. E. CNS in its natural position (anterior third of body, body surface transparent, lateral view from the left). F. CNS in lateral (left) view. G. CNS in dorsal view. Abbreviations: Bg, buccal ganglion; CPl, cerebro-pleural ganglion; d2, genital gland 2; dg, digestive gland; ey, eye; g, gonads; go, genital opening; in, intestine; j, jaw; ki, kidney; mo, mouth; mu, mucous gland; n-l, nervus labiotentacularis; n-o, nervus oralis; ne, nephroproct; oe, oesophagus; ot, oral tube; Pd, pedal ganglion; pe, pericardium; ph, pharynx; pd-n, pedal nerves; ps, penial bulb; rep, renopericardioduct; Rg, rhinophoral ganglion; rh-n, rhinophoral nerve; sg, salivary gland; stc, statocyst; va, vagina; ve, ventricle.

covering large parts of gonad dorsally. Kidney epithelium thin and vacuolated, with lumen histologically empty. Short nephridial duct leaving kidney closely posterior to insertion of renopericardial duct. Nephropore located slightly anterior to anus.

Reproductive system (Fig. 9): gonad follicles filling body cavity posterior to stomach, covering posterior digestive gland duct and partly covered by kidney. Male and female gametes within same follicles, male portions central. Anterior reproductive organs androdiaulic. Preampullary hermaphroditic duct short

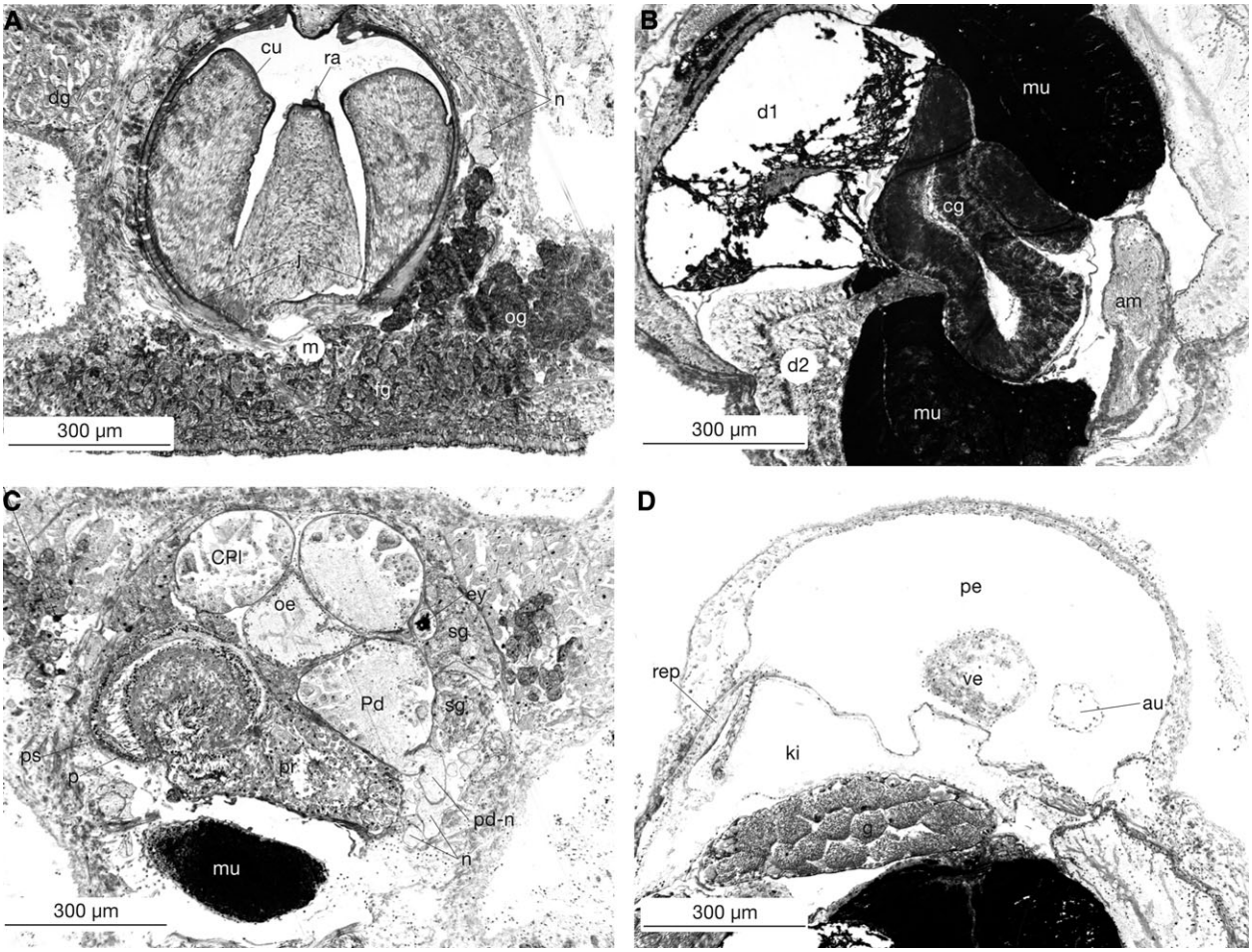

Figure 3. *Flabellina engeli lucianae* n. subsp., selected histological cross-sections. **A.** Pharynx. **B.** Female gland mass and ampulla. **C.** CNS. **D.** Pericardium and kidney. Abbreviations: am, ampulla; au, auricle; cg, capsule gland; CPl, cerebro-pleural ganglion; cu, cuticula; d1, genital gland 1; d2, genital gland 2; dg, digestive gland; ey, eye; fg, foot gland; g, gonad; ki, kidney; m, diagonal muscle fibres; mu, mucous gland; n, nerves; oe, oesophagus; og, oral glands; p, penis; Pd, pedal ganglion; pd-n, pedal nerve; pe, pericardium; pr, prostatic vas deferens; ps, penial bulb; ra, radula; rep, renopericardioduct; sg, salivary gland; ve, ventricle.

and narrow. Ampulla short, tubular, wider than preampullary duct. Epithelium of ampulla showing medium-sized, elongated and blue–gray staining cells. Ampulla completely filled with sperm. Postampullary duct long and relatively wide, thin-walled and filled with unordered sperm; dividing into short oviduct and vas deferens soon becoming wider and prostatic. Secretory cells big and blue-stained, lumen filled with amorphous secretions. Penial sheath ciliated in its proximal portion but surrounded by a glandular layer distally. Muscular penial papilla withdrawn and partially invaginated, ciliated outside and inside. Penis, vagina and nidamental duct having a single, common ciliated gonopore. Vagina wide, flattened, intimately attached to and not fully separated from nidamental duct. Vagina bifurcating into slightly widened fertilization chamber connecting to capsule gland, and short duct entering receptaculum seminis close to oviduct. Receptacle relatively large, ovoid and embedded within female gland mass. Epithelium of receptacle thin, covered by layer of blue-stained muscle fibers with some elongated blue–gray staining secretory cells with dark-blue nuclei. Receptacle containing sperm with heads directed towards epithelium. Short oviduct filled with unordered sperm. Female gland mass comprising several, histologically distinct portions herein classified according to Klussmann-Kolb (2001a, b). Proximal, winding capsule gland characterized by cells filled with dark-blue staining granules. Membrane gland less winding, bearing cuboid cells with blue coloured walls. Mucous gland large, bilobed, with large loops, entirely stained dark-violet hiding exact cellular structure. Mucous gland distally narrowing into short nidamental duct. There are two further glandular portions with unclear identity: gland one located on right side of distal reproductive system, between vagina and capsule gland and covered by gland two. Gland one appearing winding, irregulary sponge-like, with violet staining cells with large empty spaces; it thus may be part of mucous gland (see Schulze & Wägele, 1998). Gland two large, forming a wide loop posterolaterally attached to female gland; more homogenous, light-blue staining vacuolated mass, probably with constricted central lumen.

Distribution: southwestern Brazil from Búzios, Rio de Janeiro, to Arvoredo Island, Santa Catarina (Fig. 10).

DISCUSSION

Methodology

The taxonomy of opisthobranch species is usually based on a combination of external and internal features. The latter at

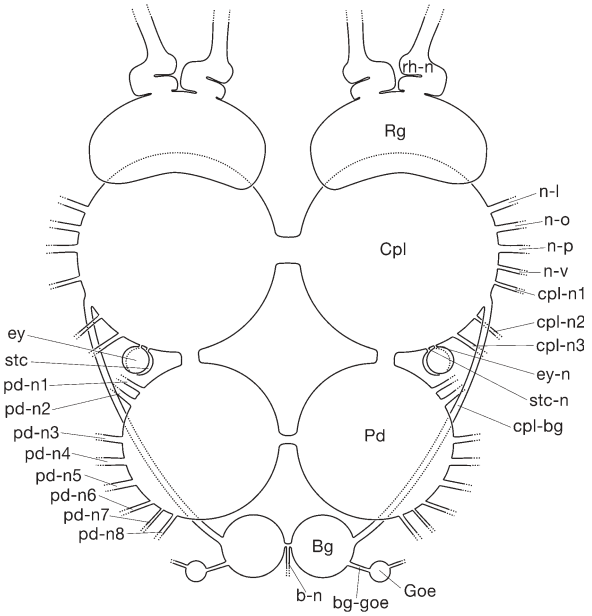

Figure 4. *Flabellina engeli lucianae* n. subsp. Schematic drawing of the central nervous system (dorsal view). Abbreviations: Bg, buccal ganglion; bg-goe, buccal-gastrooesophageal connective; b-n, buccal nerve; cpl-n 1-4, cerebropleural nerves 1-4; CPl, cerebropleural ganglion; cpl-bg, cerebropleural-buccal connective; ey, eye; ey-n, eye nerve; Goe, gastrooesophageal ganglion; n-l, nervus labiotentacularis; n-o, nervus oralis; n-p, posterior pallial nerve; n-v, visceral loop; Pd, pedal ganglion; pd-n1–8, pedal nerves 1–8; Rg, rhinophoral ganglion; rh-n, rhinophoral nerve; stc, statocyst; stc-n, static nerve.

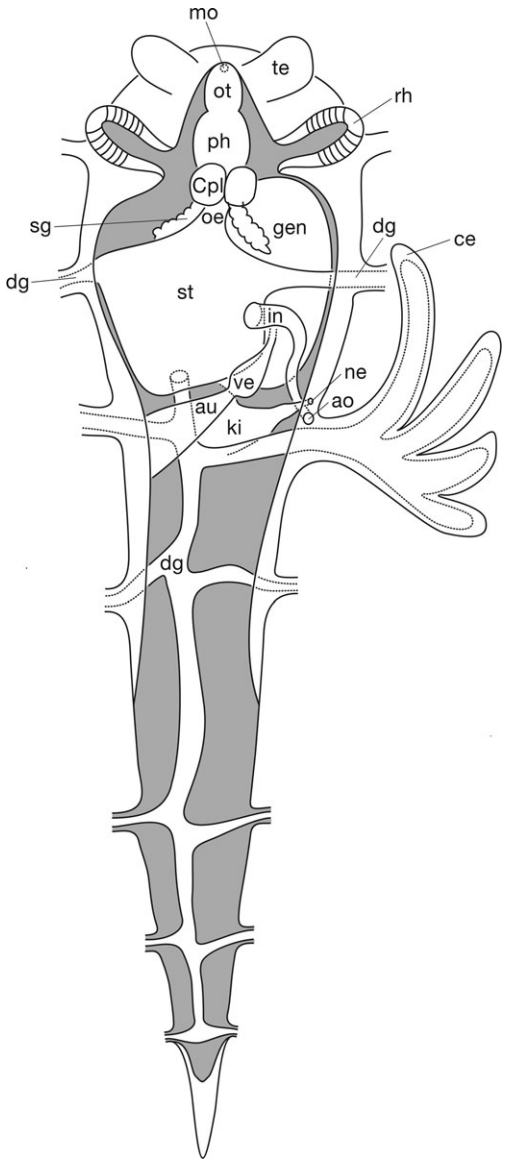

Figure 5. *Flabellina engeli lucianae* n. subsp., anatomical overview. Body wall opened dorsally; gonad, posterior portion of kidney, and some other organs omitted. Abbreviations: ao, anal opening; au, auricle; ce, ceras; Cpl, cerebro-pleural ganglion; dg, digestive gland; gen, distal reproductive organs; in, intestine; ki, kidney; mo, mouth; ne, nephroproct; oe, oesophagus; ot, oral tube; ph, pharynx; rh, rhinophore; sg, salivary gland; st, stomach; te, oral tentacle; ve, ventricle.

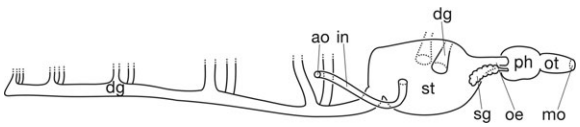

Figure 6. *Flabellina engeli lucianae* n. subsp. Schematic drawing of the digestive system, lateral (right) view; left salivary gland not shown. Abbreviations: ao, anal opening; dg, digestive gland; gen, distal reproductive organs; in, intestine; mo, mouth; ne, nephroproct; oe, oesophagus; ot, oral tube; ph, pharynx; salivary gland; st, stomach.

least include descriptions of cuticular structures within the digestive system such as radula, jaws, or stomach plates, and more or less detailed accounts on the morphology of soft parts, mainly the digestive and distal reproductive systems. Most modern workers also try to add anatomical information on integumental, central nervous, circulatory and excretory systems. Although always being destructive to a certain extent, careful dissection of large specimens is generally considered to reveal sufficient, reliable and largely reproducible results. However, retrieving anatomical information from small species such as the slender aeolid nudibranch *Flabellina engeli lucianae*, with a preserved body length of 8 mm, is problematic; the taxonomically relevant portions of major organ systems were concentrated within the anterior 2.5 mm portion of the body cavity. Either several specimens may be dissected and results combined; this has been tried, but without getting reliable information on tiny structures nor on their intraspecific variability. Or, in addition, histological methods need to be applied: (1) adding a new and useful character set, i.e. cellular and tissue structures (see Wägele, 1997; Wägele & Klussmann-Kolb, 2005); (2) confirming gross-anatomical judgments; e.g. allosperm receptacles can only be identified with certainty as being a receptaculum seminis (storing sperm) or a bursa copulatrix (disintegrating sperm) after histological analysis (see Wägele & Willan, 2000); and (3) allowing detailed and reproducible (micro) anatomical examination of tiny species such as mesopsammic acochlidians (e.g. Sommerfeldt & Schrödl, 2005; Neusser *et al*., 2006).

Using histology for (micro) anatomical purposes requires serial sectioning of organs or entire specimens to be studied, and reconstructing their morphology through the series of histological slides. Major problems refer to (1) the knowledge required on how to interpret histological information, i.e. to identify certain organs on histological slides; and (2) the graphical reconstruction by hand; while qualitative information on single organs or systems can be obtained relatively rapidly (see Schrödl & Wägele, 2001), preparing exact 3-dimensional reconstructions of all major organ systems (e.g. Sommerfeldt & Schrödl, 2005) is a very time-consuming process that may be

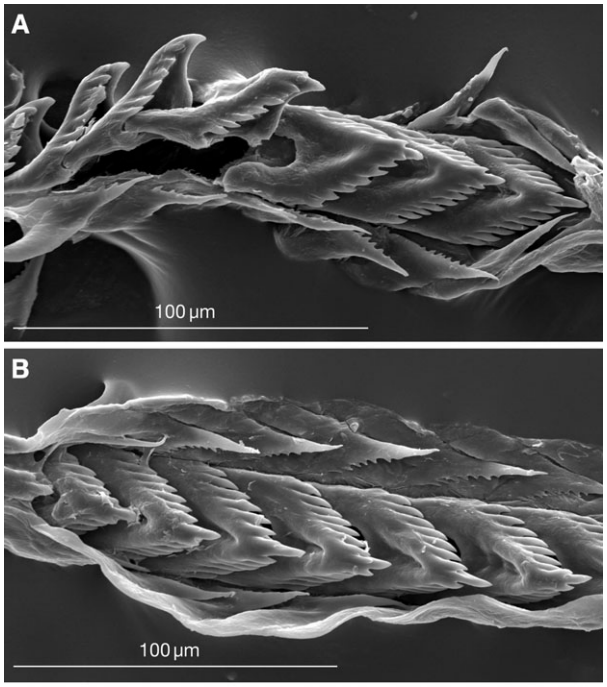

Figure 7. *Flabellina engeli lucianae* n. subsp. **A–B.** SEM-micrographs of radula; rachidian and lateral teeth. **A**. Rachidian teeth in lateral and dorsal view. **B**. Dorsal view. **C**. Light-microscopical photograph of left jaw with denticulated masticatory borders of both jaws (right jaw removed).

far from being trivial due to complexity of certain organs or poor quality of certain slides. Even if three dimensions (i.e. two angles of view plus thickness of sections) are considered during graphical reconstruction, results are presented as 2-dimensional drawings, with the third dimension only qualitatively indicated by overlap of organs. At least two illustrations from different angles of view must thus be given to permit an impression of

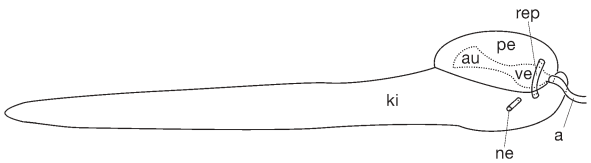

Figure 8. *Flabellina engeli lucianae* n. subsp. Schematic drawing of circulatory and excretory systems; lateral (right) view. Abbreviations: a, aorta; au, auricle; ki, kidney; ne, nephroproct; pe, pericardium; rep, renopericardioduct; ve, ventricle.

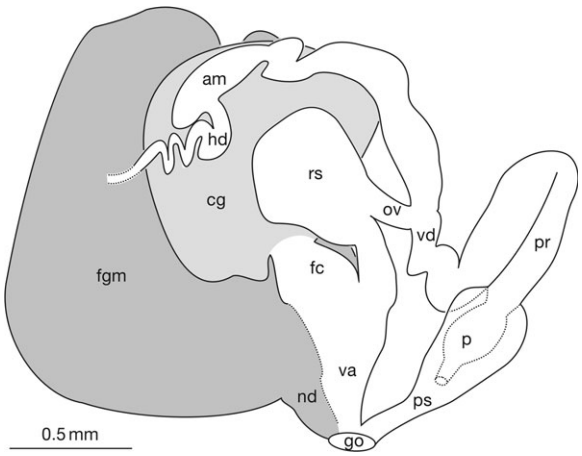

Figure 9. *Flabellina engeli lucianae* n. subsp. Schematic drawing of reproductive system (dorsal view); gonad not shown. Abbreviations: am, ampulla; cg, capsule gland; fc, fertilization chamber; fgm, female gland mass (except for capsule gland); go, genital opening; hd, hermaphroditic duct; nd, nidamental duct; ov, oviduct; p, penial papilla; pr, prostatic vas deferens; ps, penial bulb; rs, receptaculum seminis; va, vagina; vd, vas deferens.

shape and size (volume) of organs. Since usually every single organ system needs to be reconstructed and shown separately, relative positions of organs from different systems remain unknown and organ positions within the specimens are difficult to imagine.

As an alternative to traditional graphical methods, in the present study we used AMIRA-software for a computer based 3-dimensional reconstruction of all major organ systems from serial semithin sections. While equal knowledge is required on referring histological results to certain structures, other problems related to graphical reconstructions listed above are partly or fully resolved. The additional effort of taking digital photographs of histological sections and aligning them is more than compensated by the possibility to mark all structures on each image regardless of belonging to different organ systems, and by easy presentation of any single or combined systems from

Figure 10. Geographic distribution of *Flabellina engeli lucianae* n. subsp.

different angles of view (see Fig. 2D–G). If the 3-dimensional computer-model needs to be reduced to a paper illustration (as shown by a few examples herein), it still gives a better 3-dimensional impression than a drawing resulting from graphical reconstruction; as shown in Figure 4, (semi)schematic drawings can be derived from 3-dimensional models easily by hand. Most of all, on the condition that organ limits were correctly marked, the proportions and relative positions of reconstructed organs are always accurate.

Additional, analytical advantages of using AMIRA include the possibility (1) to follow certain structures (e.g. ducts) vertically through the different sections on the screen; (2) to control effects of trying different organ markings (i.e. alternative organ identification) on the completely reconstructed organ system; and (3) to analyse reconstructed single organs or entire systems in various angles of view.

In summary, there are no alternatives to applying histological techniques for anatomical studies on small opisthobranchs. Using software such as AMIRA, the reconstruction of serial sections is faster than by traditional graphical methods, analysis is improved by several tools, results are 3-dimensional, and proportions and positions are true. This is the powerful analysis and presentation tool that micromorphology has waited for.

Nervous system

The general arrangement of the nervous system of *F. engeli lucianae* matches that described as being usual for Aeolidioidea (e.g. Hoffmann, 1939; García & Cervera, 1985; Willan, 1998). However, there is little information on central nervous features of aeolid nudibranchs. Giant neurons are observed in the cortex of cerebropleural, pedal, buccal and gastro-oesophageal ganglia of *F. engeli lucianae*, while the cortex of the rhinophoral ganglia shows many small cells instead.

In addition to the cerebral commissure (a separate subcerebral commissure was not detected) and the cerebropleural-pedal and cerebro-buccal connectives, eleven nerves leave the right cerebropleural ganglion of *F. engeli lucianae*, 10 nerves leave the left. These are the connectives to the rhinophoral and buccal ganglia, the optic and static nerves, nervus labiotentacularis, nervus oralis, the short visceral loop, one pallial nerve, and two (left) or three (right) nerves that arise from the posterior portion of the cerebropleural ganglia and could not be identified. According to Huber (1993: Fig. 31F) four nerves leave the cerebral ganglia of nudibranchs: the rhinophoral and optic nerves, the nervus labiotentacularis and nervus oralis; the static nerve, however, was not mentioned. Based on Russell's (1929) description of *Aeolidia papillosa* (L., 1761) with cerebral and pleural ganglia separated by a superficial furrow, Hoffmann (1939) gave a detailed classification of CNS nerves. He mentioned an additional cerebral nerve (c4) that innervates the ventral portion of the mouth and oral tube; another potentially cerebral nerve (c5) leading to the lateral integumental muscles was found on the right side only. Three nerves were considered to be pleural; one of them may correspond to the tentative posterior pallial nerve of *F. engeli lucianae*, others cannot be clearly correlated. According to Hoffmann (1939), the unpaired visceral ganglion, which bears a single nerve, merges with the right cerebral ganglion. The existence of the visceral nerve may thus explain the different number of nerves (11 versus 10) leaving the right and left cerebropleural ganglia of *F. engeli lucianae*, respectively. The pedal ganglia of *F. engeli lucianae* each bear eight nerves, while Hoffmann (1939) only reports three nerves from *Aeolidia papillosa*.

While such tiny nervous features still need to be studied and confirmed by special staining techniques, the pair of thick rhinophoral nerves leaving each rhinophoral ganglion of *F. engeli lucianae* is easily detectable by standard staining (Richardson *et al*., 1960), and may have some taxonomic significance. Usually, either a single rhinophoral nerve runs to the base of the rhinophore and then ramifies more or less irregularly, or several nerves may already leave the rhinophoral ganglion as in e.g. *Spurilla neapolitana* Delle Chiaje, 1823 by Garcá & Cervera (1985). Paired rhinophoral nerves as found in *F. engeli lucianae* were also reported from *F. bicolor* (as *Samla annuligera* Bergh, 1900) and *Facelina rubrovittata* (as *Hervia berghii* Vayssière, 1888) by Hoffmann (1939), as well as for *Flabellina affinis* by Schulze & Wägele (1998: Fig. 4C).

Digestive system

The general arrangement of digestive organs of *F. engeli lucianae* is as usual for aeolids, i.e. a layer of oral glands without distinct efferent ducts is present, the pharynx is cuticularized and contains a pair of jaws and the radula, and a pair of tubular salivary glands that enter the posterior pharynx. The stomach bears three digestive gland ducts, two of them laterally, one posteriorly. According to Wägele & Willan (2000), the intestine leaves the stomach anteriorly in nudibranchs, except for *Bathydoris* Bergh, 1884 and *Tritoniella* Eliot, 1907. The intestine of *F. engeli lucianae* arises right laterally (slightly dorsally) from the central portion of the stomach, but proportions may be influenced by fixation. Wägele & Willan (2000) mention a typhlosole for *Flabellina*. The intestine of *F. engeli lucianae* is short; there are longitudinal folds in its proximal portion but no clearly detectable typhlosole.

Reproductive system

The arrangement of distal reproductive organs of *F. engeli lucianae* is androdiaulic (definition after Ghiselin, 1965; Wägele & Willan, 2000; Schrödl, 2003); it is characterized by a separate vas deferens, a vagina that is attached to and partly fused with the nidamental duct, and a proximal receptaculum seminis inserted by both oviduct and vagina. Some other *Flabellina* species such as *F. babai* Schmekel, 1972 and *F. affinis* (Gmelin, 1791) have also been described as diaulic (Schmekel & Portmann, 1982; Schultze & Wägele, 1998). Others, such as *F. marcusorum* Gosliner & Kuzirian, 1990 have been repeatedly described as triaulic, e.g. by Gosliner & Kuzirian (1990) and Gosliner & Willan (1991). However, gonoducts are never fully separated in any known *Flabellina* species, thus they are all (andro)diaulic (see Wägele & Willan, 2000).

The genital systems of the Flabellinidae may bear one to several allosperm receptacles, with not always clarified function and homology; their number, position and arrangement vary considerably (Wägele & Willan, 2000). For example, two receptacles may be present distally (e.g. *F. affinis*), or one distally and one proximally [e.g. *F. nobilis* (Verrill, 1880), *F. ischitana* Thompson, 1990, *F. capensis* (Thiele, 1925)], or one proximally (e.g. *F. babai* Schmekel, 1972; *F. baetica* García, 1984), or two proximally [e.g. *F. pedata* (Montagu, 1815), *F. bertschi* Gosliner & Kuzirian, 1990] (Wägele & Willan, 2000). *Flabellina marcusorum* bears three receptacles (see Gosliner & Kuzirian, 1990), two proximally and one distally situated. *Flabellina engeli lucianae* bears only one proximal receptaculum seminis; the histological examination shows a muscular layer and sperm that are directed with heads pointing to the vesicle wall. According to Wägele & Willan (2000), this number and arrangement is the plesiomorphic condition for cladobranchs.

Taxonomy

The slender body of our specimens, combined with the triseriate radula with denticulate, triangular rachidian and denticulate lateral teeth, clearly indicates a generic placement within *Flabellina*. The genus *Flabellina* Voigt, 1834 (including *Coryphella* Gray, 1850 and *Coryphellina* O'Donoghue, 1929) contains numerous, morphologically diverse species (see e.g. Gosliner & Kuzirian (1990); Gosliner & Willan, 1991) with still uncertain relationship to other aeolidioideans (Wägele & Willan, 2000).

Two *Flabellina* species were known from Brazil, *F. verta* (Marcus, 1970) from the subtropical Cananeia, southern Brazil (Marcus, 1970), and *F. marcusorum* from tropical northern Brazil (see Gosliner & Willan, 1991). The first species has smooth rhinophores and cerata arranged on elongate ridges, while specimens of *F. engeli lucianae* have perfoliate rhinophores and cerata arranged on elevated, narrow peduncles. *Flabellina marcusorum* has papillated rhinophores, and cerata form preanal rows and postanal arches. Further significant differences between *F. engeli lucianae* and *F. marcusorum* are the slightly elevated versus depressed central cusp of rachidian radular teeth, and the single proximal versus two proximal and one distal allosperm receptacles. The circumtropical *F. bicolor* (Kelaart, 1858) shows perfoliated rhinophores, cerata with orange bands and arranged on peduncles, elevated central cusps of rachidian teeth, and a CNS that is quite similar to that of *F. engeli lucianae*: cerebropleural ganglia are completely fused, rhinophoral ganglia are attached to the cerebral ganglia, two rhinophoral nerves leave each rhinophoral ganglion, the pedal commissure is short; gastro-oesophageal ganglia were only detected in *F. engeli lucianae* n. subsp. However, both species are clearly distinct with regard to coloration, the number of cerata per peduncle and their spatial arrangement, and due to *F. bicolor* having an additional, distal allosperm receptacle.

Four Atlantic *Flabellina* species with perfoliated rhinophores and cerata arranged on peduncles were recently reviewed by Calado *et al*. (2005), i.e. *F. babai*, *F. ilidioi* Calado, Ortea & Caballer, 2005, *F. llerai* Ortea, 1985, and *F. engeli* Marcus & Marcus, 1968; in addition to colour differences, these authors emphasized consistent differences regarding structure and shape of cuticular buccal organs such as jaws and radula. *Flabellina engeli lucianae* resembles the northeastern Atlantic and Mediterranean *F. babai* regarding well-developed jaw denticles, denticles on the inner side of the lateral teeth, and the shape of the rachidian teeth; in addition, both species have a single proximal receptaculum seminis. *Flabellina engeli lucianae* differs from *F. babai* in its whitish body and cerata with orange tips, less cerata per peduncle, and having more radular rows (24–35 versus 19–20). *Flabellina engeli lucianae* clearly differs from both *F. ilidioi* and *F. llerai* from Cape Verde Islands in coloration, i.e. in lacking white to orange dorsal markings, lateral spots, and orange ceratal rings, by having rachidian radular teeth with depressed main cusps, and by differences regarding the denticulation of jaw and lateral teeth.

The Caribbean *Flabellina engeli*, from here on referred to as *F. engeli engeli*, at first sight is very similar to Brazilian specimens studied herein. All these slender aeolids are characterized by a translucent to salmon or pinkish body with a more or less regular or broken line of opaque white to yellow pigment along the notal border and across the head; cerata have orange or brown markings and white tips. Table 2 compares external and internal features of Caribbean specimens and indicates *F. engeli engeli* is quite variable regarding coloration, number, arrangement and shape of cerata, and jaw and radula structures. External descriptions of the original material and that redescribed by Edmunds & Just (1983) are brief and superficial; this might explain some of the colour differences to the specimens illustrated in living condition by Rudman (2001–2006) and Calado *et al*. (2005). The Cuban specimen described by Ortea & Espinosa (1998) is, however, unique in having orange notal spots. Orange (or brown) markings on the cerata of *F. engeli engeli* appear to be well-defined rings in specimens examined by Marcus & Marcus (1968), Edmunds & Just (1983), and those shown by Rudman (2001). The two specimens illustrated by Valdés *et al*. (2006) have cerata with orange rings which are, however, faded considerably on posterior cerata of the Venezuelan specimen. An additional specimen listed as '*Flabellina* sp. 2' was regarded as either an undescribed species or a colour form of *F. engeli engeli* by Valdes *et al*. (2006); other than stated by these authors, we do not see any differences regarding tail lengths of the specimens illustrated. Colour differences between '*Flabellina* sp. 2' and *F. engeli engeli* refer to a more slender, yellow notal line which is not composed of large blotches, to a yellow lateral line which is not broken, and to the lack of orange ceratal rings. However, a hue of orange ('brown') is visible on the photograph. In contrast, a specimen from Costa Rica illustrated by Calado *et al*. (2005) and all specimens of *F. engeli lucianae* show broad and marginally diffuse orange bands. The rhinophores of all Caribbean specimens have a white tip, except for specimens illustrated by Valdés *et al*. (2006) which have rhinophores with translucent tips, and upper or all lamellae are white. Similarly, rhinophores of Brazilian specimens are translucent with only some opaque white scattered subapically.

External differences between *F. engeli lucianae* and *F. engeli engeli* refer to the rhinophores which are elongate with only 10 complete, broad lamellae ($<$15 including incomplete ones) in Brazilian specimens (Fig. 1D), while rhinophores are stouter but have 25 complete, thin lamellae in the type material of *F. engeli engeli*; all other Caribbean specimens known in sufficient detail so far also show many more and much thinner lamellae than *F. engeli lucianae*. The number of cerata per cluster appears to be higher in Brazilian specimens with comparable body sizes; the total number of cerata is 30–69 in Caribbean specimens with information available, while up to $\sim$100 in Brazilian specimens. While Marcus & Marcus, 1968 described *F. engeli engeli* as having cerata on 'footstalks', Edmunds & Just (1983) mentioned cerata arranged on transverse ridges. All *F. engeli lucianae* examined have their cerata on well-defined narrow peduncles. The cerata of *F. engeli lucianae* show a very special kind of basal ramification and arrangement (Fig. 1B) of which details are, however, still unknown for Caribbean *F. engeli engeli*. Cerata of *F. engeli lucianae* appear to be longer (up to 4 mm) and more slender than those of *F. engeli engeli* specimens (up to $\sim$ 2 mm) at least regarding those illustrated in living condition by Edmunds & Just (1983), Rudman (2001–2006) and Valdés *et al*. (2006).

While *F. engeli* was originally described to possess central radula teeth with a very long and projecting median cusp, Edmunds & Just (1983) showed the length of central cusps to be variable in their material. Central teeth of *F. engeli lucianae* have moderately projecting central cusps with 6–8 saw-like cusps on each side (Fig. 7A,B), while *F. engeli engeli* shows 7–11 cusps. The central cusp is depressed and overgrown by lateral cusps in Caribbean *F. engeli engeli*, while central cusps of *F. engeli lucianae* specimens are more elevated and remain devoid of cusps on their dorsal side. Calado *et al*. (2005) described the lateral teeth of *F. engeli engeli* as having a ventral striation rather than true cusps, while *F. engeli lucianae* shows well-developed, irregularly sized and shaped cusps. Both *F. engeli engeli* and *F. engeli lucianae* have an androdiaulic genital system with a receptaculum seminis in a proximal position (Marcus & Marcus, 1968; this study), thus the two subspecies cannot be distinguished by their reproductive systems with present knowledge.

Table 2. Comparison of southern Brazilian and Caribbean specimens of *Flabellina engeli*.

	Flabellina engeli lucianae n. subsp.	*F.engeli engeli* Marcus & Marcus, 1968						
Data source	Present study	Marcus & Marcus, 1968	Edmunds & Just (1983)	Ortea & Espinosa (1998)	Rudman (2001–2006)	Calado *et al.* (2005)	Valdés *et al.* (2006)	Valdés *et al.* (2006), as '*Flabellina* sp. 2'
Collecting data	Atlantic coast of southern Brazil (Búzios, Trindade, Ilha Arvoredo, Santa Catarina); shallow subtidal	Piscadera Baai, Curacao, Caribbean Sea; 'on *Halimeda*', depth unknown	Bellairs Research Institute, Holetown, Barbados, Caribbean Sea; among *Galaxaura*; at 1 m depth	Pool of Hotel Comodoro, La Habana, Cubaunder stone, at 2 m depth	Tobago, South Florida and Grand Cayman; at 1.5–13 m depth	Manzanillo, Caribbean Sea of Costa Rica	Punta Tigrillo, Venezuela; Pidgeon Island, St Lucia	Venezuela
Maximum body size (living/ preserved)	20/8 mm	20/12 mm	20 mm/?	10 mm/?	10–15 mm/?	9 mm/?	'up to 25 mm'/?	'up to 19 mm'/?
No. of specimens	>8	3	8	1	3 (at least)	1	2 (at least)	1 (at least)
Ground colour	Translucent bluish whitish; some specimens with pinkish gonads shining through integument	Translucent pinkish white with scarce brown pigment granules	Translucent orange with a fluorescent bluish tinge	Translucent violet	Translucent pinkish or violet; specimen from Tobago with dark pink viscera (gonad?) shining through body wall	Translucent violet	Translucent violet	Translucent violet ('gray')
Colour of head	Translucent with opaque white spots in between and posterior to each tentacle base	Opaque white spots in between and posterior to each tentacle base	Yellow spots in between oral tentacles and on head sides in some but not all specimens. Orange spot in front of each rhinophore	Reddish mandibles shining through tissue; circular reddish zone between rhinophores and pericardium	Translucent, with orange mandibles shining through tissue; opaque white spots in between and posterior to each tentacle base	Opaque white spots in between and posterior to each tentacle base	Translucent, with orange mandibles shining through tissue; opaque white to yellow spots in between and posterior to each tentacle base	Translucent, possibly with orange mandibles shining through tissue; yellow spots in between and posterior to each tentacle base
Colour of oral tentacles	Translucent at base and tips, white in between	?	White or cream distally		Translucent at base, apical two-thirds white	Translucent at base, apical two-thirds white	Translucent at base, apical two-thirds yellow to white	Translucent at base, apical two-thirds bluish whitish
Colour of rhinophores	Translucent at base and tip, some opaque white pigment on lamellae in upper third	White tip	White tip	Upper two-thirds pinkish salmon coloured	Translucent at base, upper two-thirds either white or with a pinkish translucent band, tip white	Translucent at base, upper two-thirds pinkish violet, tip white	Translucent at base and tip, upper two-thirds pinkish violet, white subapical band	Translucent at base and tip, upper two-thirds club salmon with white on lamellae

Colour of cerata	Translucent at base and tip, white in between with rather broad, marginally diffuse orange band at half way up	Light brown, each with a darker brown ring	Pale yellow with a diffuse band of orange in the middle (text); while illustrated as a narrow and relatively well defined orange ring	Apically pinkish or reddish (translucent?), central area orange or yellow, base pinkish (translucent?)	Translucent at base and tip, white in between with narrow orange ring in specimens from Tobago and Florida. Cayman specimen with a broader, diffuse orange band	Whitish with broad diffuse orange band	Translucent at base, rest whitish with narrow orange ring	Translucent at base, rest whitish with subapical faded orange ('pale brown') marking, white tip
Colour of notum	No median spots	?	?	Large median orange spots	Translucent; Cayman specimen with some white spots	?	Translucent; at least Venezuelan specimen with some white markings	Translucent
Colour of notal border	More or less broken, irregular opaque white line	Opaque white streaks and (crown shaped) spots between footstalks of cerata	Cream or white markings dorsolaterally joining the bases of cerata clusters	Large sulphur-yellowish spots around ceratal bases	More or less broken, irregular opaque white line around the ceratal bases	White spots	Broad irregular opaque white line around the ceratal bases	Yellow line
Colour of body sides	Row of usually 7 white blotches		Yellow spots	Large sulphur-yellowish spots	Row of white blotches	?	Row of white spots	Continuous yellow line which is white posteriorly
Colour of foot sides	Line of bluish iridescent spots	?	?	?	Row of bluish iridescent or white spots	?	?	?
Colour of propodial tentacles	Opaque white with translucent base	?	?	?	Translucent; specimen from Grand Cayman with some opaque white pigment	Translucent	Translucent with white tip	Translucent with white tip (?)
Colour of tail	White median line	White median stripe	White median line	Large sulphur-yellow 'spot' (but line in Fig. 1A)	White median line	?	White median line	White median line
Rhinophores	Perfoliate, long and slender club with $<$15 broad lamellae, of them, 10 or less complete ones; elongate tip	Perfoliate, stout club with '25 complete thin leaves', stout knob	Perfoliate ('lamellate' see text); short stem with a stout club, bearing 'up to 20' (complete?) lamellae	Perfoliate, with 30 very densely arranged transverse lamellae	Perfoliate, stout club with many densely arranged lamellae	Perfoliate, stout club with many densely arranged lamellae	Perfoliate, stout club with many densely arranged lamellae	Perfoliate, stout club with many densely arranged lamellae

(Continued)

Table 2. Continued.

	Flabellina engeli lucianae n. subsp.	*F.engeli engeli* Marcus & Marcus, 1968						
Front edge of foot	Propodial tentacles with upper lip notched	Propodial tentacles with upper lip notched	Propodial tentacles with upper lip notched	Propodial tentacles	Propodial tentacles	?	Propodial tentacles	Propodial tentacles
Cerata	Different sizes, long and slender, elongate taperind tip; maximum length 4 mm (living)	Different sizes, living shape and dimensions unknown	Different sizes. Long and pointed (text); up to 3 mm moderately elongate, tip rounded (illustration)	Different sizes; moderately elongate, rounded tips	Different sizes; moderately elongate, rounded tips	Different sizes. Long and slender	Different sizes; moderately elongate, rounded tips	Different sizes; elongate with slender tips
Cerata arrangement	Up to 7 pairs of ceratal clusters, two of them prepericardial; each cluster on narrow, rounded and elevated peduncle, with up to 11 cerata, arranged in groups with up to 4 cerata joined basally	6 pairs of ceratal clusters, one (text) or two (Fig. 2) of them prepericardial; each cluster on 'footstalk' with up to 10 smaller and larger cerata forming groups 'like the branches of a tree'	Up to 8 pairs of ceratal clusters, two of them prepericardial; each cluster on transversely set ridges, with up to at least 6 cerata	5 pairs; first cluster preperiacardial (on two peduncles), up to at least 5 cerata per cluster	6–8 pairs, two of them prepericardial; up to 6 cerata per cluster	?	7 pairs, two of them prepericardial; up to more than 10 cerata per cluster	8 pairs, two of them prepericardial; up to more than 10 cerata per cluster
Number of cerata	~ 100	69	Up to 61	~ 30	~ 30–60	?	?	?
Genital opening	Below 2nd ceratal cluster	Below 1st ceratal cluster (text, Fig. 1)	?	?	?	?	?	?
Anus position	Anterior to 1st postcardiac ceratal cluster	Anterior to 1st postcardiac cluster	?	?	?	?	?	?
Jaw denticles	up to 4 rows of cusps; marginal cusps well-developed elongate, others low	One series of pointed cusps near the hinge and several rows on the free end	?	Absent	?	1–2 rows; some marginal denticles elongate, others low	?	?
Radula formula	19–20 × 1.1.1	*c.* 20 × 1.1.1	3 specimens with 19 × 1.1.1	19 × 1.1.1	?	?	?	?
Central cusp of rachidian tooth	Moderately long. Not depressed, lacking dorsal denticles	Very long and projecting. Depressed, with dorsal denticles	Slightly to strongly projecting. Depressed, with dorsal denticles	Projecting. Depressed with dorsal denticles	?	Projecting. Depressed, with dorsal denticles	?	?

Rachidian denticles (each side)	6–8	7–11	7–10	8–9	?	8	?	?
Denticles of lateral teeth	6–7 well-developed denticles	5–10 denticles	6 denticles	5–6 irregular and fine denticles	?	5–6 denticles (ventral striation)	?	?
Allosperm receptacles	1 proximal receptaculum seminis	1 proximal receptaculum seminis	?	1 proximal receptacle	?	?	?	?
Vas deferens	Prostatic, short	Prostatic, short	?	Short	?	?	?	?
Penial papilla	Bulbous, unarmed	Tongue-shaped, unarmed	?	?	?	?	?	?

In summary, there are many features including the complex and unique coloration pattern shared by Brazilian specimens examined herein and *F. engeli engeli* from the Caribbean (see Table 2). Therefore, an origin from a common ancestor is very likely. Table 2 shows that several features such as body coloration and length of the central cusp of rachidian teeth are variable within Caribbean specimens and include the range of variation observed in Brazilian specimens. Some other features, such as shape and structure of rhinophores, and the depressed versus more elevated position of central rachidian cusps which are devoid of or dorsally covered by denticles, however, differ consistently and suggest reproductive isolation and independent anagenetic changes. Further potentially relevant distinguishing features such as the special ceratal branching and arrangement on narrow peduncles in Brazilian specimens (versus, e.g. insertion on ridges as stated by Edmunds & Just, 1983) cannot be clarified without comprehensive re-examination of Caribbean material. Unfortunately, the type material of *F. engeli* is not in the collection of the São Paulo Museum anymore and, thus, not available for re-examination; most probably all three types have been lost.

With present, limited knowledge, *F. engeli engeli* and *F. engeli lucianae* have markedly disjunct distributional ranges: the first was found at various localities throughout the truly tropical Caribbean Sea, while *F. engeli lucianae* seems restricted to the subtropical southern Brazil with a known range from Búzios, Rio de Janeiro State, to Ilha do Arvoredo, Santa Catarina State. Until more information on the morphology and distribution of Caribbean *F. engeli* is available we regard the southern Brazilian *F. engeli lucianae* as a new subspecies that has common ancestors with but is isolated from the Caribbean *F. engeli engeli*.

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Padula & Santos (2006) briefly described several Brazilian specimens of '*Flabellina engeli*' from Cabo Frio, Rio de Janeiro state, and Ilhabela, São Paulo state. This material largely agrees with ours regarding external and radular features; thus we refer these specimens to *F. engeli lucianae* as well. There are 24 lamellae per rhinophore, but this number also includes several incomplete lamellae (Padula, pers. comm.).

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