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MORPHOLOGY, ANATOMY AND HISTOLOGY OF FLABELLINA AFFINIS (GMELIN, 1791) (NUDIBRANCHIA, AEOLIDOIDEA, FLABELLINIDAE) AND ITS RELATION TO OTHER MEDITERRANEAN FLABELLINA SPECIES

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ABSTRACT

The Atlantic and Mediterranean flabellinid Flabellina affinis (Gmelin, 1791) (Opisthobranchia, Nudibranchia, Aeolidoidea) is examined morphologically, anatomically and histologically with special emphasis on characters which have been widely neglected in recent literature (e.g. the histological structure of the oral glands, typhlosole). The study provides detailed data about all organ systems which are compared to existing data of other authors. The species described as Flabellina affinis by Bergh (1875; 1886) is considered not to be conspecific with the Flabellina affinis examined in this and other studies. Furthermore, Flabellina affinis is compared to other Mediterranean species, especially F. ischitana (Hirano & Thompson, 1990). F. ischitana differs from F. affinis mainly by the structure of the genital system. The phylogenetic trees presented for the genus by Gosliner & Kuzirian (1990) and Gosliner & Willan (1991) are discussed.

INTRODUCTION

The taxonomy of the Flabellinidae has been subject to many inconsistencies in the past. The family was separated from the Coryphellidae by various authors (e.g. Pruvot-Fol 1954; Taylor & Sohl 1962; Odhner 1968; Vaught 1989). Marcus & Marcus (1967), however, have united both families as Flabellinidae. Today this is accepted by most authors, although the characters used for diagnosis vary depending on the position of the anus varies from pleuroproctic to acleioproctic, the radula is usually triseriate, but rarely monoseriate (in Calmell Nepalina cavolini, sensu Schmekel 1965) and one to three allosperm vesicles can occur. The arrangement of the cerata is also variable; they can be arranged separately or in rows and/or groups that are either placed on the natal ridge or on peduncles.

Gosliner & Griffiths (1981) considered Coryphella as a junior synonym to Flabellina which is accepted today by other authors, too. Flabellina comprises at least 70 species and has a worldwide distribution. There are numerous descriptions of single species containing anatomical details of the radula, jaws and genital organs (e.g. Baba 1987a; 1987b; Marcus & Marcus 1961; 1970). Henneguy (1925) provided histological studies of Flabellina pedata (Montagu, 1815) (described as Coryphella landsburgi Alder & Hancock, 1848). There are a number of further publications illuminating the histology of certain organ systems: Schmekel (1971), Schmekel & Wechser (1967; 1968) and Kalker & Schmekel (1976) provided some histological details of Flabellina affinis, yet no complete and coherent data about the histology of all organ systems of a single species is available. Aspects of the phylogeny within the family and the genus Flabellina have been discussed by Miller (1971), Gosliner (1980), Gosliner & Griffiths (1981), Gosliner & Kuzirian (1990) and Gosliner & Willan (1991).

In this paper all the organ systems of Flabellina affinis, the type species of the genus, will be described histologically and anatomically. Special emphasis is laid on new or controversial characters.

Detailed data about the arrangement of the genital organs and the structure of their tissues are provided.

MATERIAL AND METHODS

The material used in this study is listed in Table 1. Before fixation in 4% formaldehyde/seawater, the
Table 1. Number, location, length and application of each specimen.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of specimen</th>
<th>Location and date</th>
<th>Length</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Flabellina affinis</em> (Gmelin, 1791)</td>
<td>No. 1</td>
<td>Chalkidike/Greece 17.08.1980</td>
<td>14 mm</td>
<td>Macropreparation, histology of genital organs (cross section)</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>Chalkidike/Greece 17.09.1980</td>
<td>16 mm</td>
<td>Histology: cross sections</td>
</tr>
<tr>
<td></td>
<td>No. 3</td>
<td>Chalkidike/Greece</td>
<td>13 mm</td>
<td>Histology: longitudinal sections</td>
</tr>
<tr>
<td></td>
<td>No. 4</td>
<td>Isla Tarifa/Spain, 18 m, 30.07.1995</td>
<td>37 mm</td>
<td>Macropreparation</td>
</tr>
</tbody>
</table>

Results

External morphology (Fig. 1): Length of preserved, elongate specimens between 13 and 37 mm (Table 1). Maximum breadth 2 to 3 mm. No. 4 (Table 1) with irregular pink colouring and a darker patch of colour beneath the whitish cnidosac. Cerata arranged in six to nine groups on each side (Figs. 1A, E). Several cerata (2–15) originating from a common peduncle. Peduncles, uniform at their base, dividing into several branches. Cerata of variable length. First peduncles with four ramifications (Figs. 1C, E). Longest cerata were situated on the second and third peduncles. Rhinophores were strongly contracted due to fixation and annulate with 17–36 complete or incomplete annulations (Fig. 1D). Mouth hidden between the funnel-shaped outer lips (Fig. 1C). Labial tentacles shorter than cerata and approximately as long as rhinophores. Foot corners tentacular, bent posteriorly and with a deep groove on ventral side. Anus in front of second group of cerata, approximately on a level with the lower edge of the second peduncle. The notal ridge was absent. Genital openings at the base of first peduncle with an elongate anterior outer penial sheath and a posterior papilla with the female opening.

Anatomy

Digestive System (Figs 2, 3): Narrow ducts of oral glands leading into a short oral tube. Oral glands consisting of numerous follicles arranged around primary and secondary ducts (see histology). Primary ducts leading dorsally to first pair of peduncles. In the peduncles, primary ducts branching into many narrow secondary ducts surrounded by further follicles smaller than ones of the primary ducts. Labial disc with smooth cuticle. Triseriate radula with 40 (*F. affinis* No. 4) to 42 (*F. affinis* No. 1) rows of teeth. Rhachidian teeth horse-shoe-shaped with one large central cusp and six to seven smaller lateral denticles (Figs 5A, C, D). Central cusp depressed and lying below the lateral denticles (Fig. 5D). Lateral teeth delicate, pointed at the upper end and with five to six small denticles at the inner edge (Figs. 3C, 5C, D). In *F. affinis* No. 4 jaws (Fig. 3D) 1.3 mm in length and 1.1 mm in breadth. Masticatory process with numerous denticles, arranged in irregular rows. Denticles of the outer row elongate, pointed and taller than remaining denticles (Fig. 5B). Salivary glands with very thin ducts, widening to a simple glandular tube with an irregular surface (Fig. 3A). Transition between oesophagus and stomach continuous. Three tubes of the digestive gland entering stomach. Intestine leaving stomach posteriorly without a clear delimitation. Dorsal typhlosole

Figure 1. *Flabellina affinis*. A. Dorsal view (No. 1). B. Ventral view of anterior third of the body (No. 3). C. Lateral view (No. 3). D. Rhinophores (No. 3), lateral and frontal view. E. Branches of peduncles (No. 3). Abbreviations: a, anus; c, cerata; cs, cnidosac; dgl, digestive gland; f, foot; fcg, foot corner groove; ga, genital apertures; lt, labial tentacles; m, mouth; p, peduncle; rh, rhinophores; tfc, tentacular foot corners; I-IV, branches of peduncles.
Figure 2. *Flabellina affinis* No. 4—arrangement of the organs. A. View after removal of notum, pericardium and heart. B. View after removal of kidney, stomach and canal of digestive gland. Abbreviations: am. ampulla; ao. aorta; cg. cerebro-pleural ganglion; cgl. capsule gland; dgl. digestive gland; e. oesophagus; gf. gonadal follicle; i. intestine; k. kidney; lt. labial tentacle; mgl. mucous gland; ogl. oral gland; p. pharynx; pr. prostate; sgl. salivary gland; rg. rhinophoral ganglion; tpl. terminal part 1.
Figure 3. Flabellina affinis—digestive organs. A. View of the digestive tract without glandular parts of the digestive gland (No. 4). B. Histological structure of the glandular epithelium of the digestive gland (No. 3). C. Detail of the radula (No. 1). D. One half of jaw (No. 4). Abbreviations: A, A-cell; B, B-cell; D, D-cell; dgl, digestive gland; dt, dorsal typhlosole; e, oesophagus; i, intestine; j, jaw; l, lateral tooth; mp, masticatory process; ogl, oral gland; p, pharynx; st, stomach; str, white stripe.
Figure 4. *Flabellina affinis*—genital and nervous system (No. 4). A. Anterior genital complex *in situ*. B. Scheme of genital complex. C. Dorsal view of the nervous system and some nerves (No. 4). D. Half-schematic drawing of the cerebro-pleural and pedal ganglia and their nerves (according to No. 1). Abbreviations: am, ampulla; cg, cerebro-pleural ganglion; cgl, capsule gland; dmgl, distal mucous gland; did, distal oviduct; e, oesophagus; ey, eye; fc, fertilisation chamber; gd, gonoduct; mgl, mucous gland; N1–10, Nerves according to Russell (1929); p, pharynx; pam, postampullary duct; pg, pedal ganglion; pes, penial sheath; pmgl, proximal mucous gland; pr, prostate; ps, penis; rg, rhinphoral ganglion; r1, r2, receptacula 1 and 2; sta, statocyst; tp1, tp2, terminal parts 1 and 2; vd, vas deferens.
ANATOMY AND HISTOLOGY OF FLABELLINA AFFINIS

starting at the posterior end of the stomach and proceeding to proximal part of intestine (Fig. 3A). Epithelium of lateral tubes as well as of the posterior central canal thin and translucent. Paired white stripe (only present in F. affinis No. 4), probably consisting of strong epithelial foldings, on whole length of central canal and also reaching into the peduncles (Fig. 3A). Glandular part of digestive gland (Fig. 3B) restricted to cerata.

Nervous system (Figs 4C,D): The following anatomical descriptions of the nervous system refer to F. affinis No. 4 unless there is a reference to another specimen. Cerebro-pleural ganglia (approximately 515 μm in length) lying upon the oesophagus. Pedal ganglia beneath cerebro-pleural ganglia. Eyes sessile. Rhinophoral ganglia stalked. Several nerves identifiable (names according to Russell 1929): N1 = Rhinophoral Nerve; N3 = Sensory Nerve (labial tentacles); N4 = Motoric Buccal Nerve; N8 = Pleural Nerve (Russell 1929) or Posterior Pallial Nerve (Hoffmann 1939); N9 = Anterior Pedal Nerve; N10a = Middle Pedal Nerve; N10b = Posterior Pedal Nerve. Buccal ganglia under cerebro-pleural ganglia and in front of pedal ganglia. Optical ganglia (55 μm in diameter) present. Statocyst (86 μm in diameter) nearly spherical with numerous statocones of variable diameter. Gastro-oesophageal ganglia only observed in No. 2 and No. 5 on right side.

Genital system (Figs 2B, 4A,B): Gonadal follicles with a diameter of 750 μm. Gonoduct widening in proximity of anus and forming ampulla (Figs. 4A,B). Ampulla voluminous (2.5 mm in length and 1.6 mm in diameter in the 37 mm specimen). Postampullary gonoduct dividing into vas deferens and proximal oviduct. Vas deferens widening to long, sinuous prostate, distally ending in a short ductus ejaculatorius connected with the seminal sheath. Penis unarmed. Proximal oviduct widening to a lobulate fertilisation chamber, distally narrowing again and joining the distal oviduct. Two receptacula inserting at distal oviduct: receptaculum 1 not stalked, receptaculum 2 smaller and stalked.

Capsule gland yellowish and consisting of several narrow loops. Mucus gland large and mainly situated on left side.

Kidney (Fig. 2A): Voluminous organ lying upon dorsal surface of central canal of the digestive gland as multi-lobed, ramified organ with a whitish surface. Nephroproct directly anterior to anus. Branches of kidney reaching into peduncles and situated between gonadal follicles and tubes of digestive gland.

Histology

Digestive System: Ducts of oral glands beginning as narrow and winding tubes within oral tube, subsequently widening to the glandular parts (Figs 6D,E). Ciliated epithelium of primary ducts with glandular cells containing purple granules (toluidine blue) and a large nucleus with a distinct nucleolus. Multicellular follicles (approx. 45 μm in diameter) around primary ducts with similar granules as in the glandular epithelium cells. Secondary ducts with a pavement epithelium. Some of the epithelial cells containing deep blue granules. Smaller follicles (25–30 μm in diameter) consisting of one single cell or few cells arranged around secondary ducts.

Glandular part of salivary glands (Fig. 6C) consisting of two different, alternating cell types. One of them with a large, central nucleus, uniformly blue cytoplasm and sometimes several lightly stained vacuoles. Second type with weakly stained cytoplasm and filled with small purple granules. Nucleus not visible. Anterior oesophagus with a flat epithelium and lined with cuticle. Cuticle absent from posterior part, epithelium folded and ciliated, epithelial cells cuboidal to columnar. Epithelium of stomach not folded, thin and ciliated. Dorsal typhlosole visible as a deep groove (Fig. 6B), filled with compact connective tissue staining lightly purple with toluidine blue. Second typhlosole beginning at transition between stomach and intestine, lying caudo-ventrally.

Epithelium of central canal of digestive gland similar to epithelium of stomach. Transition between canal and glandular part of digestive gland at base of cerata abrupt. Epithelium of digestive gland consisting of several layers, staining dark blue and folded into longitudinal grooves. Flabellina affinis No. 4 with three different functional cell types (Fig. 3B) (according to Schmekel & Wechsler 1967, 1968a): A-cells (undifferentiated cells); D-cells (excretion cells); and B-cells (digestive cells). Cells of digestive gland beneath the cnidosac sometimes containing nematocysts.

Cnidosac connected to the digestive gland by a narrow duct. Cnidophagous cells large, arranged in one layer around a central lumen and alternating with narrow interstitial cells.
ANATOMY AND HISTOLOGY OF FLABELLINA AFFinis

**Figure 5. Flabellina affinis**—radula and jaw. A. Rhachis tooth; scale bar = 10 μm. B. Masticatory process; scale bar = 10 μm. C. Dorsal view of radula; scale bar = 10 μm. D. Lateral view of radula; scale bar = 20 μm. Abbreviations: cc, central cusp; l, lateral tooth; rha, rhachis tooth.

with a basal nucleus. Cnidophagous cells filled with numerous nematocysts (6.5–7.5 in length x 4.5 μm in diameter) of apparently only one type. Nematocysts concentrated in apical part of cell. Some cnidocytes opening to exterior with a small pore at apical ends.

**Nervous System:** All ganglia surrounded by a rather thick sheath (Fig. 6A) of connective tissue. Nervous cells in periphery of ganglia. Cells of different types (according to their size) are present in the cerebro-pleural ganglia as described by Schmekel & Wechsler (1986b).

**Genital System:** Gonadal follicles containing eggs in cortical zone and spermatogonia and autosperm in medullary zone (Fig. 7D). Eggs with a diameter of up to 85 μm and large nucleus (up to 35.5 μm).

Epithelial cells of ampulla flat and containing dark blue stained vacuoles of variable size and with relatively large nuclei. No cilia visible by means of light microscopy. Ampulla densely packed with autosperm in No. 2 and No. 3; No. 1 full of eggs. Sperm only present in periphery of ampulla in No. 1. Postampullary hermaphroditic duct with ciliated epithelium. Proximal vas deferens containing numerous sperm; its epithelium with a similar structure to hermaphrodite duct. Epithelium of prostate consisting of a simple layer, with cylindrical glandular cells alternating with ciliated supporting cells. Cells filled with one type of vacuole containing blue-stained secretions. Ductus ejaculatorius surrounded by a thick, muscular layer.

Epithelium of penial sheath densely ciliated. Retracted penis with a slightly curved tip (Fig. 7A). Absence of cuticular structures confirmed by histology.

Fertilisation chamber in No. 2 and No. 3 filled with allosperm. Heads of those often directed towards the epithelium (i.e. outwards). Fertilisation chamber in No. 1 enlarged and containing numerous eggs and few allosperm; the latter situated marginally with their heads still directed towards the epithelium (Fig. 7B).

Receptacula 1 and 2 (Fig. 7C) packed with allosperm. Receptaculum 1 surrounded by a thick muscle layer and originating in the vestibulum without a distinct demarcation. Epithelium of receptaculum 1 cylindrical and strongly folded. Heads of the sperm directed towards, or even embedded in, epithelial cells. Receptaculum 2 with a thinner muscle layer; epithelium flatter and less folded. Nuclei not as regular at base as in receptaculum 1. Heads of sperm also directed towards the epithelium.

Female glandular mass with five histologically different areas. Glandular epithelium similar to prostatic epithelium with alternating glandular and supporting cells.

1. Capsule gland (Fig. 8A): Glandular cells containing a nucleus (11 μm in diameter) with a large nucleolus. Cells packed with secretion granules, staining dark blue to purple basally and light blue apically.

2. Proximal mucous gland (Figs 4B, 8B): Glandular cells hardly stained, without granules; nuclei pycnotic. Remains of secretions, especially at base, forming irregular fibers.

3. Distal mucous gland (Fig. 8C): Glandular cells completely filled with red to purple staining secretions; nuclei small. Dark purple cells with separate secretion granules; contents rather homogenous in red cells. Distal mucous gland in direct contact with proximal mucous gland and on its other end with terminal part 2, but also with distal oviduct (Fig. 4B).

4. Terminal part 1 (Figs 4B, 8D): Forming a loop originating from the mucous gland but also being in contact with terminal part 2. Epithelial cells highly columnar (145 μm). Nuclei lying basally to medially. Cytoplasm without granules and not stainable with toluidine blue.

5. Terminal part 2 (Fig. 8D): In contact with terminal part 1 and distal oviduct. Epithelium flatter than in glandular parts described above. Cytoplasm of glandular cells appearing homogenous apically and containing fibrous blue secretions basally.

**Flabellina affinis** No. 1 with eggs in all parts of the glandular mass (Fig. 8). Eggs arranged in rows within capsule gland. Thin layer of secretion lying upon cilia of glandular epithelium. Few sperm also visible. Line of eggs in proximal mucous gland surrounded by a thin purple layer.

In contrast to the other specimens, red and purple regions of distal mucous gland clearly separated without transition in *Flabellina affinis* No. 1. Accumulations of purple secre-
ANATOMY AND HISTOLOGY OF FLABELLINA AFFINIS

Figure 6. Flabellina affinis. A. Cross section of a cerebro-pleural ganglion, asterisk: sheath of connective tissue; scale bar = 50 μm. B. Cross section of the typhlosole, asterisk: lumen of proximal intestine; scale bar = μm. C. Longitudinal section of a part of the salivary gland; scale bar = 50 μm. D. Position of the oral gland in the first peduncle, arrow: primary duct; scale bar = 100 μm. E. Detail of the oral gland; scale bar = 50 μm.

Abbreviations: pd, primary duct; sd, secondary duct.

Discussion

Comparison with existing data

Because of its abundance in the Mediterranean, Flabellina affinis has been examined and described by numerous authors, especially Bergh (1875; 1886), Pruvot-Fol (1951; 1954), Schmekel (1970; 1971) and Schmekel & Portmann (1982). In addition to a morphological and anatomical description, Schmekel (1971) provided detailed data about the histology of the genital system. The data of other authors and the results of this study will be compared and examined in so far as they characterise the species.

Heart and pericardium: Epithelia of atrium and pericardium delicate. Atrium with some connective tissue in its ventral part. Ventricle consisting of strong, muscular fibres. No pericardial glands present.

Kidney: Epithelium of kidney consisting of highly vacuolated cells. Syrinx funnel-shaped, its inner foldings bearing long cilia directed towards the lumen of the kidney.

Anterior pedal gland: Mainly developed on anterior part of foot, consisting of densely packed multicellular follicles lying subepidermally. Glandular cells with large nuclei and deep purple secretions.

Epidermis: Consisting of highly vacuolated cells. Vacuoles not staining with toluidine blue. Nuclei (5-6 μm in diameter) without a discernible nucleolus. Dispersed epidermal glandular cells with black-purple staining secretions.

Glandular stripe: Dispersed glandular cells (approx. 24 μm in diameter) with a large nucleus (approx. 10 μm in diameter) present on right side starting behind genital openings and extending into ceratal peduncles; rare in posterior part. Cells containing lightly stained vacuoles.

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Morphological descriptions: The data on the arrangement of ceratal clusters vary somewhat. Bergh (1886) found eight to nine, Pruvot-Fol (1954) found six to seven, Schmekel & Portmann (1982) found seven (25-mm specimen) and Garcia Gómez (1986) found six to eight. The number of ceratal clusters apparently does not always correlate with the length of the specimen. Thus, in this study nine groups of cerata have been found in the 37 mm specimen as well as in the 14 mm specimen.

There is a similar variability in data related to the number of rhinophoral annulae, which vary between 14 (Geiger 1993) and 36 (Schmekel & Portmann 1982). Schmekel & Portmann (1982) mentioned aberrant specimens with almost smooth rhinophores. A problem when determining the number is that some of the annulae only surround half the rhinophore, or even less. Bergh (1886) overcame this problem by including interrupted annulae in his count. Furthermore, there may be rings of contraction as artifacts of fixation which are incorrectly counted as annulae. Hence, the number of rhinophoral annulae should only be used, if at all, as a character in the diagnosis of living specimens. More important than the actual number is the presence or absence of annulae in other species.

Hirano & Thompson (1990) claim a pleuroproctic site of the anus for the genus Flabellina, contrary to Schmekel & Portmann (1982) who postulated an acleioproctic position for most Flabellina species, but avoided the term when describing F. affinis. The terms ‘pleuroproctic’, and ‘acleioproctic’ were introduced by Odhner (1939). He described ‘pleuroproctic’ as a position on the side of the body especially in forms which still have a notal brim. In the
'aclesioprotic' position he hypothesised that the anus had shifted dorsally to the interhepatic space. This means that Odhner restricted use of the term 'interhepatic space' to a dorsal area, on a level with the tubes of the digestive gland. According to our results the anus is shifted somewhat dorsally compared to other Flabellina species that still have a natal ridge, and the position could be determined as aclesioprotic, in the interhepatic space.

**Digestive system:** The number of radula rows reported for Flabellina affinis range between 30 (Vayssière 1888) to 36 (Schmekel & Portmann 1982). The radulae examined in this study contained 40 to 42 rows, respectively. The latter is the largest number ever found, but it does not deviate essentially from former findings. Concerning the number of denticles (six to seven) of the radicidial tooth the data are consistent with those of other authors that report the number to vary between five and seven (Schmekel & Portmann 1982; Gosliner & Griffiths 1981). Scanning electron micrographs (unpublished data of Wägele) show a radula of F. affinis with seven to ten denticles at the radicidial tooth. Gosliner & Willan (1991) considered the degree of elevation of the central cusp as a character which is important for phylogenetic analyses; in many derived Flabellina species (as F. affinis) it is depressed between the lateral denticles.

The statements about the number of denticles on the inner edge of the lateral tooth vary between five and six (Hirano & Thompson 1990; Schmekel & Portmann 1982; Gosliner & Griffiths 1981). Most authors agree that the outer edge of the lateral tooth is smooth. Only Bergh’s (1875) findings are contradictory. He described the inner, and often the outer, edge as delicately and quite irregularly serrated. Furthermore, he described one or several rows of teeth on the jaw’s masticatory process, whereas Schmekel (1972) found five rows and Schmekel & Portmann (1982) up to five. The specimens examined in this study also bear approximately five rows, but since the rows of denticles on the masticatory process are very irregularly arranged their number can never be precisely determined.

Few comparative data are available giving the position, size and size relations of the digestive organs. Bergh (1875) reported the pharynx of a specimen he examined to have a length of 1.5 mm. This is consistent with the length of the pharynx of the 37 mm specimen in this study. It should be realised, however, that the specimen examined by Bergh was only 8 mm in length; a pharynx of 1.5 mm is extremely large. Furthermore, Bergh (1875) described the salivary glands as a paired organ, originating in front of the middle of the pharynx and hanging down to the ventral edge of the jaws. In our specimens they are located dorsally and are relatively inconspicuous. He (1889) later presented drawings of structures that could be oral glands, but they were described as part of the kidney. Data about the oral glands of Flabellina affinis can be found in the list of phylogenetically relevant characters presented by Gosliner & Kuzirian (1990) (dorsal and ramified) and Gosliner & Willan (1991) (only presence is mentioned). Pruvot-Fol (1954: 414) provided a drawing of the glands for which she used the term 'glandes pylinales'. However, she did not determine the insertion of the ducts. Histological and ultrastructural data about the digestive system of Flabellina affinis are restricted to the digestive gland (Schmekel & Wechsler 1968) and the cnidosacs (Kälker & Schmekel 1976). We did not find all four types of cells of the digestive gland that have been described by Schmekel & Wechsler (1967). In both the specimens we examined histologically the C- or secretion cells seem to be absent or they cannot be identified by means of light microscopy. Kälker & Schmekel (1976) reported two types of nematocysts in the cnidosac of Flabellina affinis; atrich haplonemes and microbasic euryteles. Only one type was found in this study, which can probably be assigned to the former category.

**Genital system:** The variability of statements about a penial armature is remarkable. Whereas Bergh (1875; 1886) subsequently cited by Hirano & Thompson (1990) mentioned the presence of a penial spine, all other authors (Table 2) described the penis as unarmed. The absence of a spine is confirmed in this study.
Figure 8. *Flabellina affinis* – female glands. A. Capsule gland with eggs surround by a mucous layer; scale bar = 20 μm. B. Proximal mucous gland with eggs; scale bar = 20 μm. C. Distal mucous gland with eggs, arrow; egg not yet surrounded by secretions; scale bar = 20 μm. D. Terminal parts 1 and 2; scale bar = 50 μm. Abbreviations: eg, egg. tp1, tp2. terminal parts 1 and 2.

Table 2. Summary of data about the genital system.

<table>
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<th>Authors</th>
<th>Number of receptacula</th>
<th>Position and shape</th>
<th>Bursa copulatrix</th>
<th>Fertilisation chamber</th>
<th>Penis</th>
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</thead>
<tbody>
<tr>
<td>Bergh (1875)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bears a straight, colourless thorn of 0.1 mm length with a small pore at its tip and longitudinal stripes</td>
</tr>
<tr>
<td>Bergh (1886)</td>
<td>2</td>
<td>one stalked</td>
<td></td>
<td></td>
<td>Penis with the shape of a truncated cone (length appr. 0.2 mm); thorn almost as long as the penis (Ø 0.3–0.035 mm)</td>
</tr>
<tr>
<td>Goslinger &amp; Griffiths (1981)</td>
<td>1</td>
<td>distal, rarely proximal</td>
<td>present</td>
<td>distal</td>
<td>long, conical, unarmed</td>
</tr>
<tr>
<td>Goslinger &amp; Kuzirian (1990)</td>
<td>1</td>
<td>proximal semiserial</td>
<td>present</td>
<td>stalked</td>
<td>unarmad</td>
</tr>
<tr>
<td>Goslinger &amp; Wilan (1991)</td>
<td>1</td>
<td>single, serial</td>
<td>present, stalked</td>
<td></td>
<td>armed with a chitinous thorn</td>
</tr>
<tr>
<td>Hirano &amp; Thompson (1990)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>unarmad (refers to the whole genus)</td>
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The elongate structure visible in front of the papilla with the female opening has been described as a penis (Hirano & Thompson 1990), but it is only an outer penial sheath. The varying data about the number and position of the allosperm receptacles can be reconciled as a confusion of terminology. All authors find two vesicles. One of them has a distal position and is stalked. This is called the ‘bursa copulatrix’ by Gosliner & Wilan (1991) and ‘receptaculum seminis’ by all other authors. In this study it is called the receptaculum 2. As proven histologically, the heads of the sperm in the vesicle are directed towards the epithelium. Hence the organ does not seem to be a bursa copulatrix (Schmekel 1971). The second vesicle, which all authors agree it is a receptaculum, is not stalked, and its position is in most cases also described as distal. Gosliner & Griffiths (1981), Schmekel (1970) and Schmekel & Portmann (1982) mentioned that the position may exceptionally also be proxi-
The fertilisation chamber of egg-laying specimens. It is apparent from our sections that the granules are delivered into the duct as intact units. The vacuoles of the prostatic cells do not seem to merge with each other before ejection. This is consistent with the findings of Schmekel (1971). Her descriptions were summaries based upon examinations of nudibranchs of different taxa (Flabellina affinis among others). Most of the details, but not all, are consistent with our observations.

Schmekel (1971) found a ciliated strip in the ampulla of Doridoidae and Dendronotoidea along which the eggs are transported through the organ without interfering with the auto-sperm. This observation cannot be confirmed for Flabellina; in F. affinis No. 1 the ampulla was so densely packed with eggs that there was very little space for sperm. According to Schmekel (1971), there are two different types of secretions in the prostrate gland which in some taxa are stored in separate vacuoles (Peltodoris), or even in separate cells (Chromodoris). She assumed that in Flabellina both types of secretions are mixed within each vacuole. This is consistent with the findings of this study (only one type of vacuole has been found). The vacuoles of the prostatic cells do not seem to merge with each other before ejection. It is apparent from our sections that the granules are delivered into the duct as intact units.

Schmekel (1971) did not find sperm in the fertilisation chamber of egg-laying specimens. But our Flabellina affinis No. 1 definitely contained sperm as well as eggs in this chamber. Furthermore, since the heads of the sperm, as described above, are directed towards the epithelium, they can be presumably stored and nourished there for some time. Thus the fertilisation chamber functions partly as a receptaculum in F. affinis. Possibly it represents a rudiment of a proximal receptaculum or an evolutionary stage in the development of a third receptaculum.

Schmekel divided the female gland mass into a capsule and a mucus gland. She assumed that the mucus gland consisted of a mucus producing part and an adhesive producing part; the latter secreting material to glue the line of eggs to the surface. Schmekel stated that the cells of the mucus gland are merokrin in nature and that in certain zones they remain intact with a pycnotic nucleus following the delivery of the secretion granules. Her description implies that zones of intact glandular and supporting cells alternate with zones of spent cells. This conclusion cannot be confirmed from our specimens. There is just one zone of spent glandular cells in the proximal part of the mucus gland. Wägele (1989) has found a zone in Antarctic nudibranchs which stained differently from the rest of the mucus gland. This area sometimes has a flatter epithelium and may be composed of intact and spent cells. Wägele (1989) has introduced the term ‘proximal mucus gland’ for this zone. In our study, the area between capsule gland and distal mucus gland is composed of cells which have delivered all their contents. Therefore, it is difficult to decide whether the area described here is the same as the proximal mucus gland or not.

The differently stained parts of the distal mucus gland may either consist of cells with different secretions or of different developmental stages of cells. Schmekel (1971) described secretory vacuoles that grow in size during maturation while space for the cytoplasm is decreasing. She also claimed that the membranes of the vacuoles remain intact, contrary to the cells of the capsule gland.

The terminal part 1 is clearly separated from the purple stained mucus gland as a separate loop. It apparently does not contain acidic mucopolysaccharides, as the cells are just slightly stained with toluidine blue. Possibly the terminal part 1 is equivalent to the adhesive producing part described by Schmekel (1971).

The function of the terminal part 2 is unknown. It may represent another part of the adhesive producing zone. Schmekel (1971)
mentioned the presence of undifferentiated cells in the foldings of the mucus gland. We could not find these cells.

After passing the fertilisation chamber, the fertilised eggs are apparently transported to the distal oviduct and from there to the capsule and mucous gland. Before entering the terminal part 1, the line of eggs probably passes the distal oviduct again. The eggs are finally transported through terminal parts 1 and 2 and then laid.

**Nervous system:** Our findings agree with those of Bergh (1875; 1886) insofar as the cerebropleural ganglia are kidney-shaped, the pedal ganglia are smaller and the buccal and rhinophoral ganglia are very similar. Bergh (1875) also claimed that he detected visceral ganglia, only a little smaller than the pedal ganglia. Yet, in all arminoid, dendronotoid and aeolidoid nudibranchs, the visceral ganglion is supposed to have merged with the right cerebropleural ganglion (Schmekel & Portmann 1982). We could not locate the visceral ganglion in our material either anatomically or histologically.

Bergh (1886) also mentioned the presence of gastro-oesophageal ganglia with a diameter about one-tenth that of the buccal ganglia. This suggests that the ganglion found at the right side of *Flabellina affinis* actually is a gastro-oesophageal ganglion, because the size relation is consistent with Bergh’s description. The fact that no corresponding ganglion on the left has been found, is surprising. Usually the gastro-oesophageal ganglia are paired as shown for example in *Flabellina pedata* (examined by Trinchese, 1881 as *Coryphella landsburgi*). As the gastro-oesophageal ganglia are very small it may be assumed that the left one was present in some of our sections that were lost. Furthermore, Bergh described a ganglion at the base of the penis which could not be found in this study.

**Epidermis:** The epidermis with its highly vacuolated cells represents a typical epithelium of special vacuoles as described by Schmekel & Portmann (1982).

**Glandular stripe:** A glandular stripe has been described in the Charcotiidae (Wägele et al. 1995a) and *Notaeolidia gigas* (Wägele et al. 1995b). Since then the glandular stripe has been detected in many other representatives of the Nudibranchia (Wägele in press 1997). We still have too little information about the distribution of this gland to speculate about possible relationships based on this character.

**Remarks on the description of Flabellina affinis by Bergh (1875; 1886):** The discrepancies in the descriptions of *F. affinis* by Bergh can be summarised as follows:

The outer edge of the lateral teeth is described as serrated by Bergh and as smooth by us and other authors. Bergh described one or several rows of denticles on the masticatory process whereas all other authors found about five rows. While in our specimens the salivary glands were relatively inconspicuous and dorsal, Bergh described them as large structures that also extended ventrally. He did not mention the presence of oral glands. But it is likely that he misinterpreted the oral glands as salivary glands. Contrary to Bergh, a penial spine was not described by most other authors. In relation to the length of the examined specimen, its pharynx is extremely large according to Bergh’s description. A visceral ganglion is absent in our specimens and apparently present in his.

Because of the differences listed above compared to our results and those of other authors it is assumed that the species described by Bergh is not conspecific with the otherwise described *Flabellina affinis*.

**Comparison with other Flabellina species**

Five species of *Flabellina* are known from the Mediterranean and the Straits of Gibraltar. *F. affinis* (Gmelin, 1791), *F. ischitana* Hirano & Thompson, 1990, *F. babai* Schmekel, 1972, *F. baetica* García-Gómez, 1984 and *F. insolita* García-Gómez & Cervera, 1989; are compared here. *Flabellina ischitana* and *F. affinis* are very similar in many details. Both species are characterised morphologically by an arrangement of the cerata on well developed peduncles and by annulate rhinophores, which are approximately the same length as the oral tentacles. Schmekel & Portmann (1982) mentioned that they have also found specimens of *F. affinis* with almost smooth rhinophores. Smooth rhinophores can apparently also occur in *F. ischitana* (Cervera, pers. comm.). Thus, the structure of the rhinophores seems to vary intraspecifically within both species.

Both species bear peduncles which divide into several branches. According to Hirano & Thompson (1990) the first pair of peduncles has three branches in *Flabellina ischitana* and...
four branches in *F. affinis*. In our opinion the branches I and II in *F. affinis* are not sufficiently clearly separated (Fig. 1E) to use the character for the diagnosis of the species. Geiger (1993) pointed out that Pruvot-Fol (1954) presented a drawing of a specimen of *F. affinis* which has four ramifications on the right and three on the left demonstrating that this is also a variable character. Apart from a general purple body colour which occurs in both species, the colour is slightly different:

In *Flabellina affinis* the rhinophores, oral tentacles and the upper third of the cerata are usually darker than the rest of the body. This could still be observed in the specimen from Tarifa. In *F. ischitana*, on the other hand, the colour is described as ‘uniformly violet’ by Hirano & Thompson (1990). Furthermore they stated that an opaque white ring surrounds the cnidosac of *F. ischitana*, but this has also been described in some specimens of *F. affinis* (Schmekel & Portmann 1982). Possibly Schmekel & Portmann actually examined specimens of *F. ischitana* without realising.

They described a pigment-free white area in front of, and beneath, the rhinophores, oral tentacles and the upper third of the cerata which has four ramifications. The denticles of the outer row are hooked and shield the other rows. In *Flabellina affinis* it is only one distally lying receptacle with a very smooth inner edge of the lateral teeth of the radula. In *F. ischitana*, by the smooth inner edge of the lateral teeth from each other. Nonetheless, *Flabellina ischitana* is maintained as separate based on two additional characters:

1. Hirano & Thompson (1990) presented a drawing of the masticatory process. It is short and pointed, and it bears numerous, irregular rows of denticles. In *Flabellina affinis* it is wedge-shaped and bears only few rows of denticles. The denticles of the outer row are hooked and shield the other rows.

2. The genital system of *Flabellina ischitana* differs in some aspects from that of *F. affinis*. In *F. affinis* there are two uniform sperm vesicles which are both situated distally, whereas in *F. ischitana* one of the vesicles is situated proximally and seems to be bilobed. In *F. affinis*, the prostate is more curled than in *F. ischitana*, although it is possible that in the latter case Hirano & Thompson (1990) have torn apart the loops in an effort to clarify the drawing. And finally, *F. affinis* has a conspicuous fertilisation chamber which has not been described for *F. ischitana*.

Hirano & Thompson (1990) mentioned that the genital arrangement which they present shows a resemblance to the aberrant arrangement of *Flabellina affinis*, observed by Schmekel (1970). Cervera (pers. comm.) is of the opinion that this aberrant arrangement actually belongs to a specimen of *F. ischitana*.

However, the proximal receptaculum in Schmekel’s drawing is extremely large and uniform compared to Hirano’s and Thompson’s drawings. This may be due, though, to a different development stage of the specimen or a different degree of expansion.

In summary, *F. ischitana* can be distinguished from *F. affinis* by its more uniform colour, the shape of the masticatory processes and the presence of a proximal receptaculum. Future research needs to evaluate the validity of these characters. The other three Mediterranean flabellinids which were originally described in the genus *Flabellina*, can easily be differentiated from *F. affinis*. *F. babai*, *F. baetica* as well as *F. insolita* are different in colour; *F. babai* is milky white with orange zones on the rhinophores (Schmekel 1972), *F. baetica* is uniformly white (García Gómez 1984) and *F. insolita* hyaline white. Contrary to *F. affinis*, *F. babai* has two clearly separate rows of preanal cerata, which are arranged upon small, non-ramified peduncles. Apart from the colour, *F. baetica* differs from *F. affinis* by its papillate rhinophores.

More differences between the species can be detected in the genital system. *Flabellina babai* has a very characteristic genital arrangement: There is only one receptaculum which is serial according to the drawing by Schmekel (1972) but semiserial according to Gosliner & Kuzirian (1990) and Gosliner & Willan (1991). The penis is described as shaped like a duck’s bill (Schmekel 1972) and is surrounded by a sheath bearing glandular papilae. *F. baetica* also has only one distally situated vesicle which García Gómez (1984) considered as a bursa copulatrix. Furthermore, this species differs from *F. affinis* by the smooth inner edge of the lateral teeth of the radula. *F. insolita* also has only one distally lying receptacle with a very long stalk.
Phylogenetic relationships of Flabellina affinis

Two cladograms including Flabellina affinis have been published (Gosliner & Kuzirian 1990; Gosliner & Willan 1991). The latter comprises more of the derived species and more characters, resulting in a slightly different topology. In both analyses, the presence of peduncles, highly ramified oral glands and annulate rhinophores are considered to be synapomorphies for a group of Flabellina species which includes the Mediterranean species F. ischitana and F. babai, but not F. baetica (Gosliner & Willan 1991). The latter is assigned to another monophyletic cluster within the genus Flabellina due to the presence of an 'anterior liver arch', papillate rhinophores and bilobed receptaculum.

According to the most parsimonious tree, annulate rhinophores as a derived character state must have evolved more than once within the genus Flabellina as it also occurs in the species F. pricei, F. trilineata, F. fusca (Gosliner & Willan 1991). Smooth rhinophores are assumed to be plesiomorphic within Flabellina although Notaeolidia which was used as an outgroup has wrinkled to annulate rhinophores. Thus, the structure of the rhinophores seems to be relatively variable and of restricted use for phylogenetic analysis.

Gosliner & Willan (1991: 128) distinguish the peduncles as 'low' or 'elevated'. This character is too subjective and may vary intraspecifically or even individually. According to a drawing presented by the authors we would judge the first peduncles of F. exoptata, for example, as rather elevated, but the following pairs as low.

Although our results do not definitely elucidate the phylogenetic relationships of F. affinis, we hope that further studies on other flabellinids will yield more comparative information that will permit its position to be determined and the species groups of Flabellina to be better understood.

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