REDESCRIPTION AND 3D MORPHOLOGY OF *WILLIAMIA GUSSONII* (GASTROPODA: SIPHONARIIDAE)

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ABSTRACT

Since it has never been described adequately, the taxonomic status of the type species of the siphonariid genus *Williamia*, *W. gussonii* (da Costa, 1829), is unclear. A redescription with the designation of a neotype is provided in the present study. Shell, radula and soft-part anatomy are investigated in detail by light microscopy, SEM and 3D-computer reconstruction and visualization of serial sections. The most significant features of the hard parts are the freely extending periostracum at the shell edge and the radula organization, with a tiny central tooth, bicuspid lateral teeth and scale-like trapezoid lateral-marginal teeth. These characters perfectly correspond to other *Williamia* species. In contrast, soft-part anatomy reveals features which differ strongly from those of other *Williamia* species. In particular the genital system lacks a bursa copulatrix but has other accessory structures, the pallial cavity that does not extend to the left side of the body, and there is only a single pulmonary vessel. Characters are discussed with regard to their usefulness for phylogenetic consideration, with a focus on siphonariid relationships. The hard-part characters appear to be more significant than the soft -part anatomy for the phylogeny of *Williamia*, and it is suggested that the genus is monophyletic.

INTRODUCTION

The pulmonate family Siphonariidae is limpet-shaped and mostly intertidal in habitat. It is one of approximately eight fairly heterogeneous families often grouped into 'archaeopulmonates' or primitive pulmonates which share the plesiomorphic feature of a veliger larval stage during development. The pallial cavity of siphonariids bears a ctenidium-like gill, which is exceptional for pulmonates. According to the main reviser of the family, Hubendick (1946, 1947), it comprises two genera only: *Siphonaria*, with the vast majority of species, and *Williamia*, with about six valid species.

The type species, *Williamia gussonii*, with a distribution ranging from the Mediterranean Sea to the adjacent Atlantic Ocean, is poorly known with regard to its anatomy, distribution and biology. The taxonomic status of this species is particularly obscure since the original description (Costa, 1829) is imprecise and the morphological information provided in subsequent studies (Mörch, 1877; Dall, 1879) is inadequate to characterize and identify the species. Therefore, a redescription with the designation of a neotype seems to be a prerequisite for further considerations of the systematics of *Williamia* and its relationship to *Siphonaria*. The availability of sophisticated new micromorphological methods, such as 3D reconstruction of serial sections, has allowed a detailed examination of the soft parts. The knowledge of soft-part anatomy of a member of the genus *Williamia* is of particular value as detailed anatomical accounts of siphonariids are scarce in the literature, and the genus *Williamia* is almost unknown in this respect. Information is available only on the western Pacific *W. peltoides* (='*W. vernalis*'), based on dissection of a single specimen (Hubendick, 1947).

MATERIAL AND METHODS

Material was examined from the Zoologische Staatssammlung München, Germany (ZSM), the Naturhistorisches Museum, Bern, Switzerland (NMB), the Naturhistorisches Museum Wien, Austria (NMW), the Naturhistoriska Riksmuseet, Stockholm, Sweden (NRS) and the Muséum National d'Histoire Naturelle, Paris, France (MNHNP).

Images of shells and whole specimens were prepared using a digital microscope camera (Spot Insight, Diagnostic Instruments Inc., or ProgRes C12plus, Jenoptik Laser) on a stereo microscope. Extended focus images were prepared by taking three to four photographs of one specimen at different focus levels. Subsequently, the areas of adequate focus were merged by hand with the help of standard image-editing software.

For radula preparation one shell containing dry soft parts was soaked in 30% ethanol for several days. The soft parts were then removed from the shell and immersed in 5% NaOCl for maceration. The radula was removed from the remaining tissue and rinsed repeatedly in distilled water prior to attaching it to the stub. It was sputter coated and observed on a LEO 1530VP scanning electron microscope (Carl Zeiss). The jaw of the same specimen was photographed during the maceration process on a stereo microscope.

For histological examination one specimen (ZSM Mol 20060118) was dehydrated in a graded series of ethanol/isopropanol and embedded in Paraplast. It was sectioned at a thickness of 5 μm and stained with AZAN (Adam & Czihak, 1964). Two specimens (ZSM Mol 20060119, ZSM Mol 20060120) were dehydrated in acetone and embedded in Araldite. Ribboned sections series (Ruthensteiner, Lodde & Schopf, 2006) with a thickness of 2 μm were prepared. These sections were stained with methylene blue-azure II (both 0.5% in 0.5% borax solution).

For 3D-computer reconstruction of the paraplast-embedded specimen, each section was photographed with a digital microscope camera (Kappa DX 30, KAPPA opto-electronics) on a Leica DMB-RBE microscope at a resolution of 1000 × 1300 pixels. Prior to 3D-treatment, the colour format of sections was changed to greyscale, images were enhanced and resolution was reduced to as low as 903 × 715 pixels with standard image-editing software. 3D-processing was carried out with the software Amira 2.3 and 3.11 (TGS Europe) on a PC as described by Ruthensteiner *et al.* (2006).

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SYSTEMATIC DESCRIPTION

Williamia Monterosato 1884

For synonymy see Marshall (1981). After an application by Rehder (1984) the ICZN made the decision to suppress the generic names *Allerya* and *Brondelia* and conserve the name *Williamia* (Tubbs, 1986: ICZN opinion No. 1410).

Williamia gussonii (da Costa, 1829)

Ancylus ? gussonii da Costa, 1829: 10 (Isola di Pantelleria, Sicily; no type material known; neotype here designated ZSM Mol 20060117, St. Paul's Bay, 45 m depth, specimens with soft parts in ethanol, fig. 1D).
Patella pellucida (emended to *Patella gussonii* p. 225)—Philippi, 1836: 111, pl. 7, fig. 7.
Piliscus (*Allerya*) *gussoni*—Mörch, 1877: 209.
Scutulum gussoni—Monterosato, 1877: 427.
Anisomyon gussoni—Dall, 1879: 286.
Williamia gussoni—Hubendick, 1946: 70, pl. 6, figs 34, 35, 37.
Ancylus drouetianus Bourguignat, 1853: 177 (ambiguous locality, no type material known). Bourguignat, 1854: 92, pl. 25, figs 10–17.
Brondelia drouetiana—Bourguignat, 1862: 21.

Taxonomy: Williamia gussonii is widely regarded as having been described by da Costa (1829), but a closer examination of da Costa's paper shows, that the original description of *Ancylus gussonii* consists of a few imprecise remarks on shell morphology in Latin, and a reference that it looks similar to a fossil species drawn by Deshayes (1824) (Fig. 1F). However, the figure of Deshayes does not resemble *W. gussonii*; the shell is much flatter and the apex lies distinctly more anteriorly. Though it cannot be excluded that da Costa was dealing with *W. gussonii*, the information provided is clearly inadequate to identify or characterize the species. No type material was designated by da Costa. Philippi (1836) synonymized da Costa's species with a patellogastropod, but gave an illustration of the shell of a specimen from Sicily, which very likely represents *W. gussonii*. Mörch (1877) definitely was dealing with *W. gussonii*. He gave an accurate Latin description of the shell by, for example, dealing with details such as the radial stripes, but provided no illustration. He was the first to realize the siphonariid affinities of the species. Dall (1879) considered the systematic position by comparing morphological data. He included very accurate drawings of the jaw and radula, and confirmed the affinities with *Siphonaria*. Since the studies of both Mörch (1877) and Dall (1879) were clearly based on *W. gussonii* and provided morphological details, one of them might be taken as the original description of the species. However, both studies are inadequate too, because morphological information is poor and again no type material is known.

As *gussoni* is the type species of the genus *Williamia*, a neotype is of decisive importance for stabilizing systematics and nomenclature of this genus, and will provide a basis for clarifying questions such as the synonymy of *W. gussonii* and *W. krebsii* in future studies.

The shell dimensions of the neotype are: length, 4.4 (4.1 calcareous shell) mm; width, 3.8 (3.6 calcareous shell) mm. This specimen has been selected as the neotype because it is complete, with shell and soft parts. From its size, it also appears to be adult. It has a relatively spherical outline. The periostracal shell edge is present (Fig. 1H), and the protoconch is well-preserved. The radial rays are not visible, which might be due to the ethanol preservation as these rays generally are less obvious in wet-preserved material, or may also be due to the relatively deep locality from which the specimen was collected (see Discussion). Externally, the soft parts show the typical arrangement of the species. The tentacular lobes are extended anteriorly with the eyes showing through. The anal lobe is extended towards the right. The mantle edge encircles the foot in a regular curve without bulging out. The pedal sole is smooth and bent inwards.

The neotype was chosen from a locality in the Mediterranean (Malta) as close as was possible to where the species was first described (Isola di Pantelleria).

Williamia gussonii can be distinguished by shell morphology from *W. peltoides* (Carpenter, 1864), and from *W. radiata* (Pease, 1860) by its smaller size and more fragile shell. It differs from *W. subspiralis* (Carpenter, 1864) in its less high profile and less posterior and downturned apex. *Williamia magellanica* W.H. Dall, 1927 is more oval elongate and the apex lies distinctly further anteriorly at the posterior end of the anterior third. The differences from the very similar *W. krebsii* (Dall, 1877) are unclear; a comparative examination of material from different localities will be necessary in order to establish any possible synonymy.

Material examined: Malta: Bahar ic-Caghaq, 1 m depth, three specimens in ethanol, one sectioned and used for 3D-reconstruction (ZSM Mol 20060118), one with soft parts in ethanol (ZSM Mol 20060116), shell (ZSM Mol 20060130); St. Paul's Bay, 45 m depth, three specimens with soft parts in ethanol, one designated as neotype (ZSM Mol 20060117), two sectioned (ZSM Mol 20060119-20). France: Bouches du Rhone, Ensuès-la-Redonne, several shells (NMB 18935). Cyprus: W Girne, Alsancak, 2–3 m depth, several shells (NMB 18934). St. Helena: three shells (NMW 224 2/22). Italy: Sicily, Brucoli, Cozo dei Turchi, 37°17.1′N, 15°09.9′E, 20 m depth, several shells, some with dried soft parts (NRS, originally from MNHNP). Azores: Sao Miguel, Ponta Galera, several shells, some with dried soft parts (NRS, originally from MNHNP), one specimen used for radula preparation.

Shell: Limpet-shaped and symmetrical, elongate to oval with a length-width ratio ranging from about 1:0.75 to 1:0.88. Maximum dimensions: length 8 mm, width 6 mm, height 3 mm. It is thin and semitransparent, and the outer surface is shiny. The apex lies subcentrally near, or in many cases just beyond (Fig. 1A), the posterior end of the shell. If still attached, the larval shell (Fig. 1B, I) has 1.5–2 whorls and is bent to the left. Anterior and lateral slopes convex. Colour bright red-brown with about 16–19 weak radial rays sometimes hardly visible (neotype). The periostracum extends beyond the margin of the calcareous shell for about 150 μm (Fig. 1D, H) and is inwardly bent in dry specimens. Interior is paler than exterior.

External body morphology: The external appearance is limpet-shaped with an anterior head portion emerging from the posterior visceral/foot-portion. Except for the eyes, the body is completely unpigmented and pale-whitish. A dorsal, symmetrical mantle forms a fold that surrounds the whole body, enclosing a groove between it and the side of the foot. Anteriorly two inconspicuous tentacular lobes with pigmented eyes form the anterior sides of the head-portion (Fig. 1D). In fixed specimens the sides of the pedal sole are bent inwards (Fig. 2C). The pallial cavity opens (Fig. 2C) slightly anterior to the middle of the right side. The opening extends over about one-fourth of the animal's length. In the middle of the opening a conspicuous appendage, the anal lobe, protrudes from the mantle cavity floor approximately as far as the mantle fold (Figs 1D, 3A). This lobe bears the anal opening which lies dorsally in the middle, distinctly outside the pallial cavity. The pallial cavity (Fig. 3A) is large and extends anteriorly as far as the middle of the pharyngeal bulb, and posteriorly to the beginning of the intestine. The major part of the cavity lies on the right side of the animal. On the left side there is an indentation which is

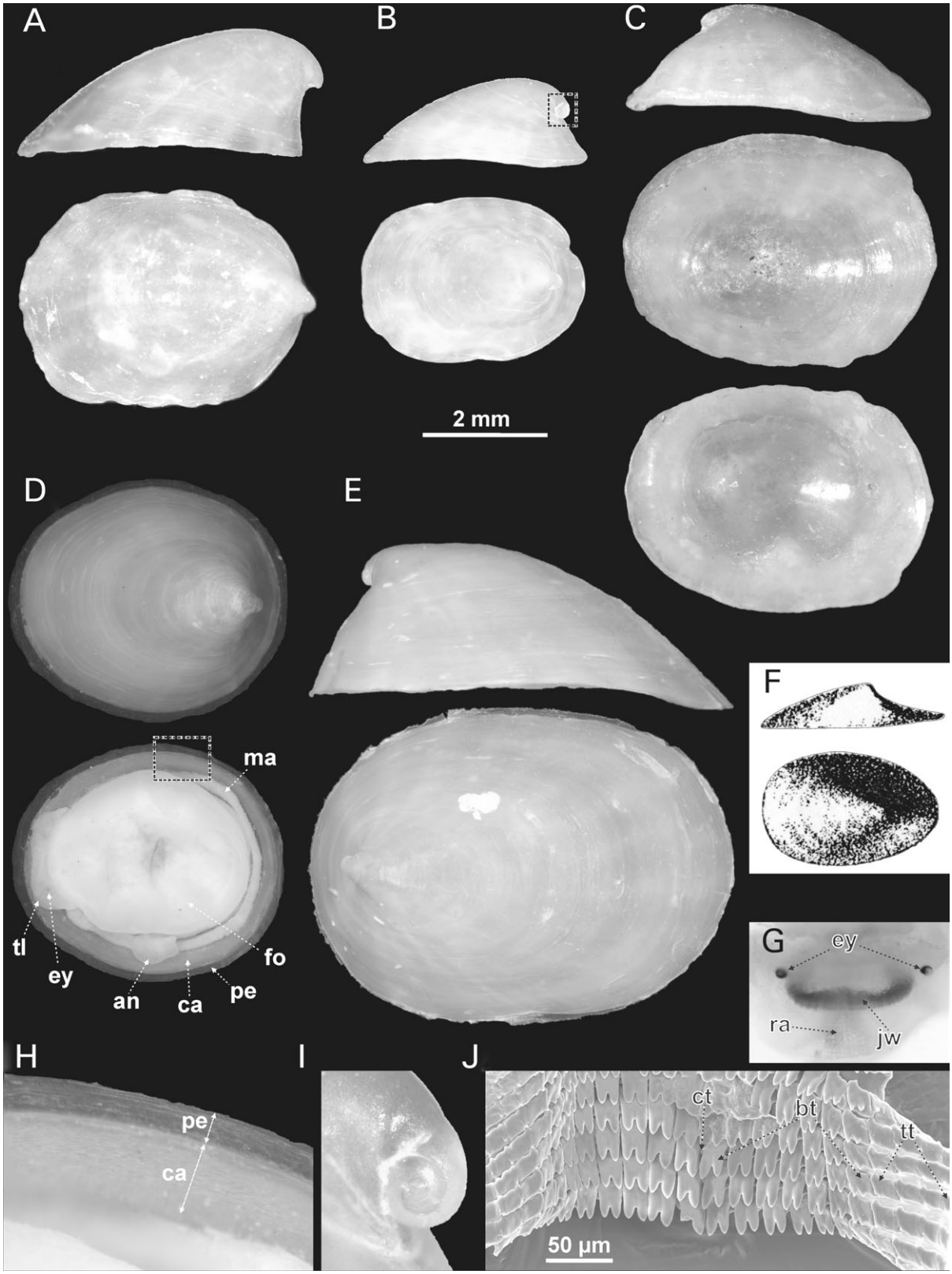

Figure 1. *Williamia gussonii*. **A, B.** Shell, left and dorsal views. Cyprus (NMH 18934). Stippled rectangle enlarged in **I**. **C.** Shell, right, dorsal and ventral views. St Helena. (NMW 224). **D.** Neotype of *Ancylus gussonii* da Costa, 1829. Total specimen in ethanol, dorsal and ventral views. Malta (ZSM Mol 20060117). Stippled rectangle enlarged in **H**. **E.** Shell of specimen used for radula preparation, right and dorsal views. Azores (NHRM). **F.** '*Ancylus depressus*'. Original drawing, slightly enhanced, from Deshayes (1824: fig. 13). **G.** Head of specimen used for radula preparation during the maceration process, dorsal view. **H.** Shell edge of neotype. **I.** Larval shell. **J.** Scanning electron micrograph of radula. Abbreviations: an, anal lobe; bt, bicuspid lateral teeth; ca, calcareous shell; ct, central tooth; ey, eye; fo, foot; jw, jaw; ma, mantle edge; pe, periostracum edge of shell; ra, radula; tl, tentacular lobe; tt, trapezoid lateral teeth. Scale bar below **B** applies to **A–E**.

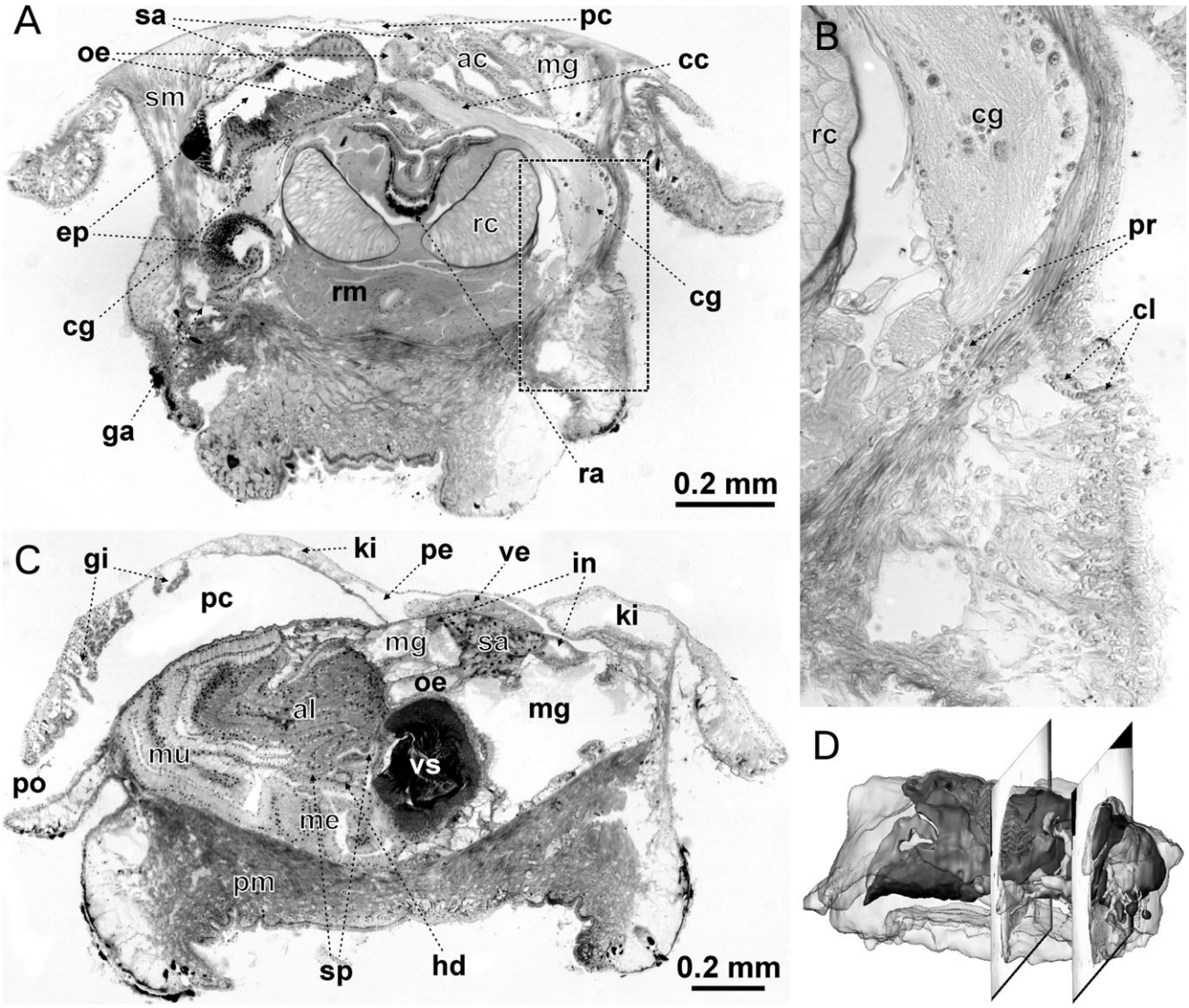

Figure 2. *Williamia gussonii*, Malta (ZSM Mol 20060118). **A.** Histological transverse section in the anterior area. Stippled rectangle enlarged in **B**. **C.** Histological transverse section in the middle area. **D.** 3D reconstruction of the total specimen obliquely from the right. 'Ortho slices' indicate the position of sections in **A**, **B** and **C**. Abbreviations: ac, accessory genital gland; al, albumen gland; cc, cerebral commissure; cg, cerebral ganglion; cl, cerebral gland; ep, epiphallus; ga, genital atrium; gi, gill; hd, hermaphrodite duct; ki, kidney; in, intestine; me, membrane gland; mg, midgut gland; mu, mucus gland; oe, oesophagus; pc, pallial cavity; pe, pericardium; pm, pedal muscle; po, pallial cavity opening; pr, procerebrum; ra, radula; rc, radula cartilage; rm, radula musculature; sa, salivary glands; sm, shell muscle; sp, spermoviduct; ve, ventricle; vs, vesicula seminalis.

caused by the heart (Fig. 3A). Inside the pallial cavity lies a gill (Figs 2C, 3A); this consists of a row of dorsally inserting lamellae. It extends from the pallial cavity opening slightly posteriorly to the left side of the pallial cavity and consists of about 17 lamellae, which increase in size from the left to the right. On the roof and on the bottom of the pallial cavity run ciliary stripes lying opposite each other (Fig. 3A). They extend from the posterior part of the pallial cavity opening towards the posterior of the end of the pallial cavity. The dorsal one runs directly posterior of the gill. The foot is surrounded by a number of irregularly arranged multicellular glands (Fig. 3E). These epidermal glands have a conspicuous, compact, often spherical, inner body which lies distinctly underneath the epidermis. They discard their contents via a thin duct to the body surface. In total there are about 30 of these glands.

Digestive system: There is a muscular pharynx with a radula (Figs 1G, J, 3A) and two radula cartilages (Fig. 2A). The radula of the specimen from Sao Miguel, Azores (Fig. 1G, J) has a tiny unicuspid central tooth, six pairs of subequally bicuspid lateral teeth, six pairs of scale-like trapezoid lateral-marginal teeth with a trace of a tiny median cusp and one pair of scale-like teeth with two short cusps. The latter pair combines features of the lateral and the latero-marginal teeth. Accordingly, the radula formula could be given as: $13 + 1 + 13$. The radula of the sectioned specimens from Malta (Fig. 2A) shows the same general pattern. At the bottom of the pharynx above the mouth opening lies the crescent-shaped jaw (Fig. 1G). It is fairly large and reaches laterally nearly as far as the eyes. It is built up of densely packed small rods. The oesophagus emerges antero-dorsally from the pharyngeal bulbus (Fig. 3C) and runs posteriorly underneath the salivary glands. The paired salivary glands (Figs 2A, C, 3C) are voluminous. Their main body extends backwards deep into the visceral cavity. Their anteriorly running ducts are delicate and open above the radula into the pharyngeal cavity. Anteriorly, after

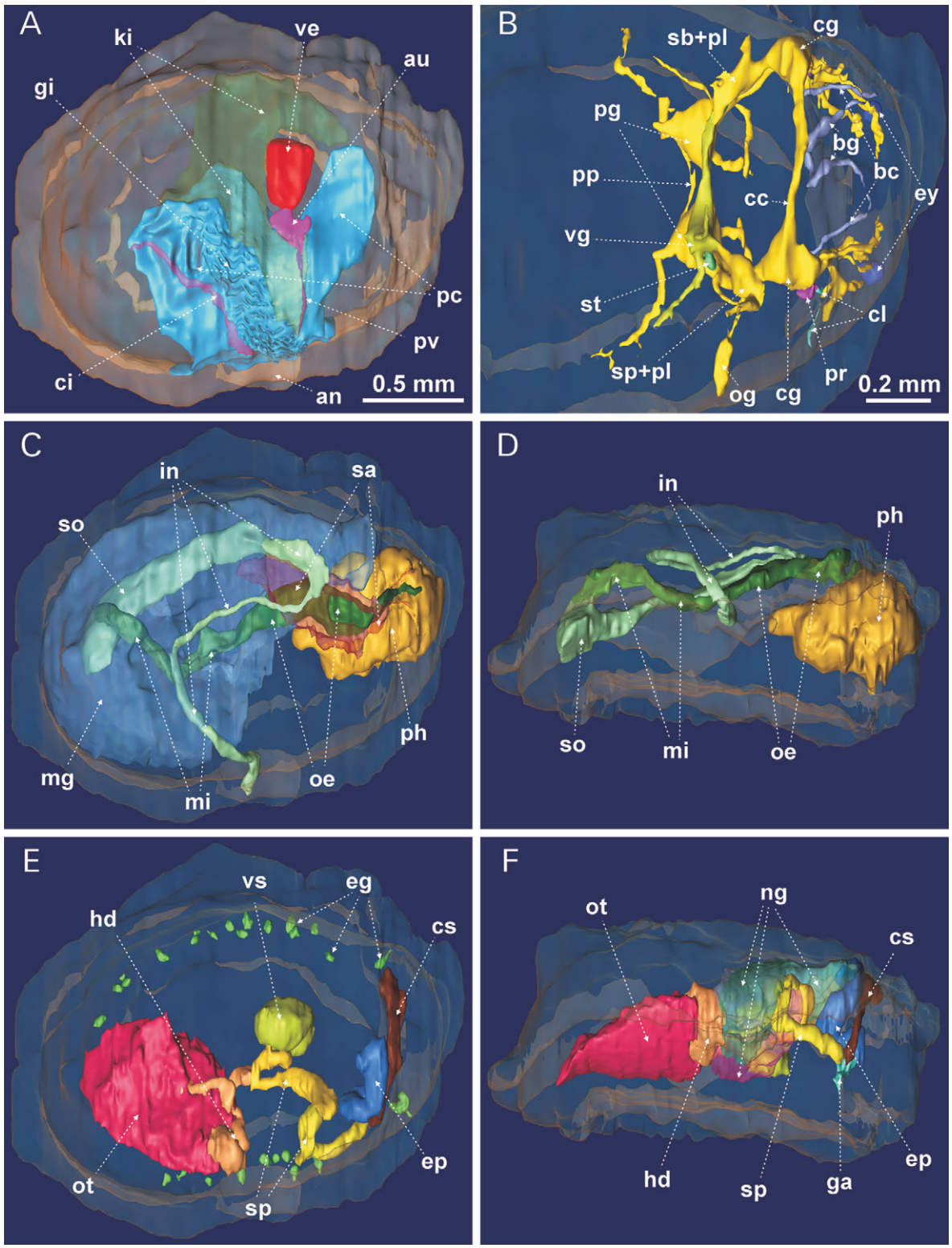

Figure 3. *Williamia gussonii*. Same specimen as Figure 2. Soft-part anatomy by 3D reconstruction and visualization. Body surface brownish transparent (less transparent in **A** than in **B–F**). Anterior to the right. **A.** Renopericardial system and pallial cavity, dorsal view. **B.** Nervous system, dorsal view. **C.** Digestive system, dorsal view. Midgut and salivary glands transparent. **D.** Digestive system from the right. Midgut and salivary glands not shown. **E.** Genital system and epidermal glands, dorsal view. Nidamental glands not shown. **F.** Genital system from the right. Nidamental glands transparent. Abbreviations: an, anal lobe; au, auricle; bc, buccal commissure; bg, buccal ganglion; cc, cerebral commissure; cg, cerebral ganglion; ci, (dorsal) ciliary stripe of the pallial cavity; cl, cerebral gland; cs, copulatory sac; eg, epidermal glands; ep, epiphallus; ey, eye; ga, genital atrium; gi, gill; hd, hermaphrodite duct; in, intestine; ki, kidney; mg, midgut gland; mi, midgut; ng, nidamental glands; oe, oesophagus; og, osphradial ganglion; ot, ovotestis; pc, pallial cavity; pg, pedal ganglion; ph, pharyngeal bulb; pp, parapedal commissure; pr, procerebrum; pv, pulmonary vein; sa, salivary glands; sb+pl, subintestinal ganglion + pleural ganglion; so, stomach; sp, spermoviduct; sp+pl, supraintestinal ganglion + pleural ganglion; st, statocysts; ve, ventricle; vg, visceral ganglion; vs, vesicula seminalis. Scale bar in **A** applies also to **C–F**.

emerging from the pharynx, the oesophagus (Fig. 3C, D) is extremely narrow. Posteriorly at the height of the posterior end of the pharynx it shows a conspicuous widening. The midgut (Fig. 3C, D) is narrow and bears a single very long opening towards the midgut gland. The most posterior portion of the gut forms a pouch-like stomach (Fig. 3C, D). The intestine (Fig. 3C, D) emerges anteriorly from the stomach and gradually narrows to a thin tube. It forms a single loop that runs anteriorly until the middle of the posterior portion of the pharynx and backwards to the right underneath the bottom of the pallial cavity towards the anal opening. There is a single, voluminous midgut gland (Figs 2C, 3C).

Nervous system: The nervous system shows a high degree of concentration with few ganglia connected by short connectives and commissures (Fig. 3B). A pair of separate cerebral (Figs 2A, B, 3B) and pedal ganglia is linked by connectives to the pedal ganglia (Fig. 3B). There is a single cerebral commissure (Figs 2A, 3B) and pedal and parapedal (Fig. 3B) commissures. One pair of ganglia (Fig. 3B) lies posterior of the cerebral ganglia and is connected to the cerebral as well as to the pedal ganglia via connectives. Because of this innervation the latter ganglia must be at least partly interpreted as pleural ganglia but probably also contain the subintestinal ganglion on the left and supraintestinal ganglion on the right side (Fig. 3B). From both of these ganglia a connective leads posteriorly to the only separate ganglion of the visceral loop, the visceral ganglion (Fig. 3B). The latter lies dorsally, tightly attached to the right pedal ganglion (Fig. 3B). In summary, the central nervous system consists of seven distinct ganglia: one pair of cerebral ganglia, one pair of pedal ganglia, pleural-subintestinal ganglion on the left, pleural-supraintestinal ganglion on the right and visceral ganglion. The whole visceral loop is very short with the pedal ganglia extending further posteriorly than the visceral ganglion. Another ganglion nearly as prominent as the central nervous system ganglia is the osphradial ganglion (Fig. 3B). It lies in front of the anterior end of the pallial cavity opening and is innervated by the right pleural-supraintestinal ganglion. It has two components which are innervated separately by two branches of the osphradial nerve. The base of the osphradial nerve, which leaves the pleural-supraintestinal ganglion anteriorly, has a ganglionic swelling. Paired buccal (Fig. 3B) ganglia sit on top of the muscular pharynx with a commissure running underneath the oesophagus where the latter leaves the pharynx. Long connectives innervating the buccal ganglia can be traced emerging medio-ventrally from the cerebral ganglia. These connectives then enter the outer muscular layer of the pharynx thus forming a long loop by running anteriorly and post wards inside this muscle layer. The cerebral ganglia latero-anteriorly bear a partly separate portion, the procerebrum (Figs 2B, 3B). From the procerebrum a string of tissue can be traced running towards the lateral body surface: a delicate tube-like organ, the cerebral gland (Figs 2B, 3B). The major nerves emerging from the central nervous system are (Fig. 3B): two nerves anteriorly from the cerebral ganglia with the ventral ones more conspicuous than the dorsal ones; the osphradial nerve (see above) from the pleural-supraintestinal ganglion; one nerve dorsally and one posteriodorsally from the pleural-subintestinal ganglion; one nerve anteriorly from the right pedal ganglion probably innervating copulatory components; one medio-anterior and one medio-posterior nerve from each pedal ganglion; one nerve dorsally and one nerve to the right from the visceral ganglion.

Anteriorly on the head, underneath the epidermis, lies a pair of eyes (Fig. 3B) with a well-developed lens and pigment layer. One pair of statocysts is embedded dorsally in the pedal ganglia (Fig. 3B), containing a number of statoconia. The osphradium is represented by the epithelium above the osphradial ganglion. It is highly differentiated with high prismatic cells which are densely ciliated.

Renopericardial system: The heart occupies a transverse position at the end of the anterior third of the animal, with the auricle extending to the right and the ventricle to the left (Fig. 3A). Anterior to the kidney, on the roof of the pallial cavity, a single pulmonary vein runs transversely towards the heart (Fig. 3A). The voluminous kidney (Figs 2C, 3A) surrounds the pericardium (Fig. 2C) posteriorly, with one branch extending on the left side more anteriorly than the pericardium. The branch that lies in the roof of the pallial cavity extends to the right until the area of the pallial cavity opening. Here it lies in the space between the pericardium, pulmonary vein and gill (Fig. 3A). Near the right termination of this branch lies the simple renal pore that opens dorsally into the pallial cavity.

Genital system: From a posteriorly located ovotestis (=-hermaphrodite gland, 'gonad') the hermaphrodite duct, with an expanded region (the ampulla), leads to the middle portion comprising the fertilization area and the voluminous nidamental glands (=female genital glands). The spermoviduct runs anteriorly to the right towards the genital atrium which opens at the body surface. The genital system, with a single efferent duct and a single genital opening, is therefore monaulic and monotremous.

The genital atrium (Figs 2A, 3F) with the genital pore lies anteriorly on the right side of the animal. Beside the spermoviduct, two other organs open into the genital aperture. The epiphallus (Figs 2A, 3E, F) opens into the anterior of the genital atrium. It is a voluminous pouch-like organ with a thick glandular wall, extending from the genital atrium dorsally and medially above the middle of the pharynx. The gland that forms the main mass of the organ consists of high prismatic cells containing numerous spherical dark-staining granules. Its base is formed by glandular cells with uniform contents. The entire inner surface towards the lumen bears cilia. Just posterior to the epiphallus, an organ of unknown function and homology, here termed the copulatory sac (Fig. 3E, F), opens into the genital atrium. The copulatory sac is a long thin sac-like structure that widens slightly toward its distal end. Its wall is a simple epithelium with a ciliary surface on the inside. In the three individuals investigated the organ was detectable in the two larger ones, which also showed full (female) sexual maturity. In the reconstructed specimen it displayed a uniform content; in another it was totally collapsed with no lumen. The copulatory sac lies in front of the epiphallus and extends transversally to the left nearly until the left edge of the pharynx.

The spermoviduct (Figs 2C, 3E, F) emerges posteriorly from the common genital atrium. Its anterior portion runs inside the musculature of the body wall. It has no glandular epithelium. The opening towards the nidamental glands lies just posterior of the point where the spermoviduct enters the body cavity. Distal to the nidamental glands the duct widens and then narrows again and joins the hermaphrodite duct. The anteriormost end of the latter runs for a short distance inside the spermoviduct. At that point both ducts enter a large spherical bulb together. This structure, the vesicula seminalis (Figs 2C, 3E), is thin-walled and densely filled with sperm. This sperm might be autosperm as it is regularly arranged, with the tails towards the outer wall and the heads inwardly directed.

There are four nidamental glands or glandular portions (Fig. 3F). The membrane gland (Fig. 2C) lies ventrally. The lumen of this gland has several connections with the spermoviduct and the other glands. It consists of high prismatic cells with bright homogenous contents. The whole inner surface is densely ciliated. The mucus gland (Fig. 2C) is the most voluminous gland. It lies on the right side and, like the membrane

gland, consists of high prismatic cells with uniform contents. The cell content is brighter than that of the membrane gland and there is also less ciliation. The lumen of this gland has a connection to the three other genital glands. The albumen gland (Fig. 2C) lies partly covered by the mucus gland in the middle of the animal. Its cells are less high and darker staining than those of the other glands. The lumen of this gland is collapsed, but there is one opening detectable where the mucus gland opens into the spermoviduct. The fourth gland, here termed accessory genital gland (Fig. 2A), lies anteriorly. Its high prismatic cells with bright lumen are similar to those of the mucus gland, but the cells of the accessory gland are distinctly smaller. The lumen of this multilobed accessory gland shows connections to the mucus gland and the albumen gland posteriorly.

Between vesicula seminalis and ovotestis, the thin walled hermaphrodite duct (Fig. 3E, F) forms a loop to the right of the body side. This loop has a conspicuous widening, the ampulla, that is full of regularly arranged sperm and is obviously another autosperm storage organ.

The ovotestis (Fig. 3E, F) forms a compact mass that is located at the floor of the posterior portion of the visceral cavity. The main mass of the organ lies on the right side of the animal. Oogenetic and spermiogenetic areas can be found adjacent to each other.

Distribution: Williamia gussonii is known from several localities throughout the Mediterranean Sea. It can also be found in the adjacent eastern Atlantic Ocean on islands including St Helena and the Azores. It has been found subtidally at a depth range of 1 to 45 m.

DISCUSSION

Hard-part morphology

The shell of *Williamia gussonii* shows a number of features in common with other species of *Williamia*. All species are bilaterally symmetrical except for details like the apex or muscle scar on the inside. The surface is smooth except for a fine circular striation. This character differs distinctly from *Siphonaria*, where there is generally some kind of radial rib pattern present. The symmetry of the shell is also typical for *Williamia*, which lacks the siphonal groove usually found in *Siphonaria* that takes the form of a bulge on the right side above the opening of the pallial cavity. This siphonal groove of *Siphonaria* is accompanied by varying degrees of asymmetry. If known, larval shells of *Williamia* species are twisted to the left, as in *W. gussonii* (this study), or backwards as in *W. radiata* (Harbeck, 1996). Radial coloured rays similar to those in *W. gussonii* are also known from other *Williamia* species, e.g. *W. radiata*: Marshall, 1981; *W. peltoides*: McLean, 1998; *W. magellanica*: Dall, 1927. According to Marshall (1981) the intensity of this radial colour pattern appears to decrease with increasing water depth in *W. radiata*. This might be the same in *W. gussonii* and explain the lack of these rays in the neotype, which was collected at 45 m depth. Another distinctive feature is the protruding periostracum edge of the shell, which appears to be generally present in *Williamia*. Although not mentioned by the authors, it can be seen in photographs of *W. radiata* (Marshall, 1981: fig. 2B), *W. peltoides* and *W. subspiralis* (McLean, 1998: figs 3, 5), and might represent a diagnostic generic feature.

The radula of *Williamia gussonii* has already been accurately drawn by Dall (1879), although he gave a lower number (four) for the scale-like trapezoid lateral-marginal teeth. The most marginal of these, however, are very delicate and can easily be overlooked if investigated without an SEM. The radulae of *W. peltoides* (Hubendick, 1947) and *W. radiata* (Marshall, 1981) are also known, and are nearly identical to that of *W. gussonii*. All these radulae are highly characteristic, with a formula of $12 - 13 + 1 + 12 - 13$. Radular morphology in *Siphonaria* (Jenkins, 1981, 1983, 1984; Harbeck, 1996; Hodgson, 1999) differs strongly from that of *Williamia*. In *Siphonaria* bicuspid lateral teeth lie next to a prominent median tooth. The median denticle of these lateral teeth is much higher than the lateral one. The marginal lateral teeth are tricuspid; scale-like teeth as in *Williamia* are completely missing. The arrangement of the radula, therefore, might be diagnostic for *Williamia*.

The present observations on the jaw of *Williamia gussonii* also confirm the findings of Dall (1879). The jaw of *W. peltoides* is also known (Hubendick, 1947), and is very similar to that of *W. gussonii*. A jaw seems to be generally present in *Siphonaria* too (Hubendick, 1947), but it appears to be relatively smaller and less curved.

Soft-part morphology

The general shape of the gut has previously been investigated in several *Siphonaria* (Hubendick, 1947) and two *Williamia* species. The comparison of *Williamia peltoides* (Hubendick, 1947) and *W. radiata* (Schopf, 2003) with *W. gussonii* shows large differences and there is no common pattern in *Williamia* that generally differs from that of *Siphonaria*. In the latter genus a vast variety of patterns is present too. This suggests that this character complex is highly variable and thus unsuitable for consideration even at shallow phylogenetic levels.

The organization of the renopericardial system relative to the pallial cavity and gill has been used by Hubendick (1947) for systematics within *Siphonaria*. He categorized three types, which, however, do not completely correspond to his sub-groupings of the genus. Consequently, this character complex appears to be of limited reliability for systematic use, and thus it is not surprising that *W. gussonii* is considerably different from all three types found in *Siphonaria*. The kidney extends distinctly further to the left than the pericardium, but has no portion lying at the bottom of the pallial cavity. The heart lies almost medially instead of on the left as in *Siphonaria*. The ventricle does not lie posteriorly as in *Siphonaria*, but on the left of the atrium, and there is only a single vessel leading to the heart. However, other members of *Williamia* differ strikingly from *W. gussonii* in the organization of this organ complex too. Therefore, the arrangement seen in *W. gussonii* is not typical of the genus. Both *W. peltoides* (Hubendick, 1947) and *W. radiata* (Schopf, 2003) differ from *W. gussonii* in having two vessels leading to the heart, a feature that they share with *Siphonaria*. Additional differences are that in *W. peltoides* the kidney does not extend to the right until the pallial cavity opening, and that in *W. radiata* the heart lies on the left body side and also to the left of the kidney with the atrium located posterior of the ventricle. The organization of the pallial cavity of *W. gussonii* is unique among known siphonariids in that (apparently because of position of the kidney) it does not reach to the left side of the animal. All these renopericardial-pallial cavity characters provide a rather diffuse overall picture and obviously are unsuitable for systematics within the family Siphonariidae. In terms of the renopericardial and pallial cavity complex *W. radiata*, for example, is much more similar to members of the genus *Siphonaria* than to other *Williamia* species.

The overall organization of the central nervous system of siphonariids has been investigated in only a few older studies. The nervous system of *Siphonaria obliquata* (Cottrell, 1911) and *S. pectinata* (Dieuzeide, 1934) are very much alike. The only differences are in the visceral loop which has a separate pleural ganglion in *S. pectinata*, and which appears to be fused with the supraintestinal ganglion in *S. obliquata*. In this respect *W. gussonii* resembles *S. obliquata*, having no separate pleural ganglion on the

right side. The degree of concentration of the siphonariid nervous system has been interpreted by Haszprunar & Huber (1990) who consider most of the ganglia to be a result of fusion of five ganglia of the euthyneuran ancestor, which had a pair of additional, 'parietal' ganglia (lying posterior of the pleural ganglia) in addition to subintestinal, supraintestinal and visceral ganglia in the visceral loop. According to this 'pentaganglionata' concept (Haszprunar, 1988; Haszprunar & Huber, 1990), the 'left' ganglion in siphonariids consists of pleural and left parietal ganglia, the 'posterior' ganglion consists of subintestinal and visceral ganglia and the 'right' ganglion consists of pleural, supraintestinal and right parietal ganglia. The 'pentaganglionata' concept has been dismissed by Dayrat & Tillier (2000), who doubt that there is evidence for 'parietal' ganglia in the euthyneuran ancestor. Indeed the siphonarian visceral loop can be interpreted more easily the Dayrat-Tillier way, because there is no evidence for 'parietal' ganglia, although sites of individual ganglia in fused ganglia are difficult to identify. The only clearly identifiable sites are those of both the pleural and the supraintestinal ganglia. These can be identified by the descending nerves running to the cerebral/pedal and osphradial ganglia in *W. gussonii*. Without assuming 'parietal' ganglia to be present in the visceral loop, only the intestinal ganglia are (partly) fused with other ganglia in siphonariids. Accordingly, the subintestinal ganglion is fused with the left pleural ganglion in all species known, and the supraintestinal ganglion is fused with the right pleural ganglion in *W. gussonii* and *S. obliquata*. The interpretation of the siphonariid nervous system given by Haszprunar & Huber (1990: Fig. 3b) is obviously misleading regarding the position of the osphradial nerve, which at least in *W. gussonii*, descends closely posterior to the pleural ganglion. This fact in particular does not support the 'pentaganglionata' hypothesis. It seems unlikely that there is a remnant of a 'parietal' ganglion between the right pleural ganglion and the supraintestinal ganglion in *W. gussonii*.

The procerebrum-cerebral gland complex in the cerebral ganglion of *W. gussonii* is of particular significance. The procerebrum is the anterodorsal portion of the cerebral ganglion, which usually gives rise to the tentacular nerves. According to Van Mol (1967) it is generally present in pulmonates and Dayrat & Tillier (2002) regard it as a synapomorphic character of this taxon. Laterally, on the procerebrum, sits the cerebral gland which differs in size and organization among the pulmonates. It is presumably a neurosecretory organ that ontogenetically represents the remnant of the invagination trough of the cerebral ganglion (Ruthensteiner, 1999). It has a tube-like extension to the body surface at the base of the tentacle in several adult ellobiid pulmonates (Van Mol, 1967; Ruthensteiner, 1999). The lack of such a tube-like extension in other pulmonates may be explained by the loss of this structure during ontogeny. *W. gussonii* is the first species other than ellobiids known to have this tube-like portion of the cerebral gland. This structure, however, could be more widely distributed among siphonariids since it is very delicate and hard to detect. In addition, hardly any detailed histological studies have been carried out on the nervous system of members of this family. The organization of this part of the cerebral ganglion could, nevertheless, have an impact on deeper phylogenetic considerations of euthyneurans or pulmonates. The plesiomorphic/apomorphic condition of the cerebral gland in pulmonates is unclear at present. The identical organization in ellobiids and siphonariids gives additional evidence for the placement of the latter within the pulmonates. This is of interest since the position of *Siphonaria* as the sister taxon of all other pulmonates in a recent morphological phylogenetic analysis of euthyneurans (Dayrat & Tillier, 2002) indicates that the placement of siphonariids within the pulmonates has not yet been fully established.

In general, the hermaphroditic reproductive system of siphonariids is relatively simple compared to other euthyneurans. The genital system of *W. gussonii* possesses a number of features unusual for siphonariids. Anteriorly there is the copulatory sac, whose function and homology are unclear, as it is not detectable in all individuals and it may be established very late during development. It might, however, have a function in female reproduction as it was detected only in specimens with fully mature female systems. A homology with the flagellum that is present in most *Siphonaria* species and, like the copulatory sac, also descends from the genital atrium, seems very unlikely. The flagellum usually contains a spermatophore and has an elongated shape, while the copulatory sac is rounded and is filled with irregular contents. Another peculiarity is the absence of a bursa copulatrix. With a single exception, Hubendick (1946, 1947) found this structure in all *Siphonaria* species. It is also present in the two other *Williamia* species investigated (Hubendick, 1947; Ruthensteiner *et al*., 2006). The organization of the vesicula seminalis of *W. gussonii* is very unusual for siphonariids too. In the illustrations given by Hubendick (1947) no similar structures can be seen. It is much more voluminous than any comparable structure in the fertilization region of the genital system of any other siphonariid investigated thus far. This is also true of the other two *Williamia* species investigated: *W. radiata* displays a 'normal' organization with a small, blind-ended fertilization pouch and a coiled spermatheca at the comparable region (Ruthensteiner *et al*., 2006) and thus differs considerably from *W. gussonii*. The organs in those *Williamia* species investigated differ to such a degree that even the homology of individual components remains unclear. Nevertheless, the vesicula seminalis of *W. gussonii* might be the homologue of the fertilization pouch of *W. radiata*. I, however, prefer to retain the differences in terminology and apply the rather neutral term 'vesicula seminalis' for the structure in *W. gussonii* as long as its relationship is not definitely resolved. The differences in the epiphallus-copulatory organ organization of *W. gussonii* and *W. radiata* are also considerable. While there is a muscular, penis-like copulatory organ in *W. radiata* (Ruthensteiner *et al*. 2006), there is no such structure in *W. gussonii*. Until now, the only siphonariid in which the nidamental glands have been examined in detail by histology is *W. radiata* (Ruthensteiner *et al*., 2006). Again, there is a difference between this species and *W. gussonii*: with the additional accessory gland, the latter species has four instead of three glandular portions.

Systematic conclusions

Overall comparison of all the morphological characters of *Williamia gussonii* with those of other members of the genus *Williamia*, as well as the comparison within the family Siphonariidae reveals a remarkably heterogeneous scenario. There are two character complexes that point in opposite directions in terms of systematic affinities.

Soft-part anatomy suggests that there is no close relationship of *W. gussonii* to *W. radiata* and *W. peltoides* within the family Siphonariidae. The renopericardial, together with the pallial cavity organization, also shows major differences. In this respect the similarity between *W. radiata* and *Siphonaria* species is significantly greater than that of *W. radiata* with the other *Williamia* species investigated, which again shows considerable differences between each other. Characters of further organ systems, such as the gut loopings, also show no significant similarities among the *Williamia* species. Finally, the genital system displays striking differences, and with regard to this organ system *W. gussonii* is distinguished more distinctly from the other *Williamia* species than they are from *Siphonaria* species.

Hard-part morphology provides a completely different story. The shells of all *Williamia* species show very characteristic common features that are not found in *Siphonaria*. The same is

true for the radula. As far as is known, the radulae of all *Williamia* species are highly characteristic, very similar between the species, and totally different from those of *Siphonaria*. Therefore, the hard parts strongly suggest that the species currently attributed to the genus *Williamia* indeed represent a natural systematic unit.

Accordingly, the question arises which one of the two character complexes, the soft parts or the hard parts, are more significant for systematics of these taxa. The comparison of soft-part anatomy within *Siphonaria* shows that most organ systems have a wide range of organizational types (Hubendick, 1947). The differences are at least as large as those of *Williamia*. For instance, there are genital systems in *Siphonaria* both with or without a bursa copulatrix, flagellum or copulatory organ. The gut loopings also vary considerably and there are major dissimilarities in renopericardial-pallial organization. This suggests that caution should be applied when using soft-part anatomy for systematics within the Siphonariidae.

The most significant organ indicating monophyly of *Williamia* is the radula. In particular, because of its highly specialized scale-like lateral marginal teeth, it appears very unlikely that its particular organization evolved more than once. This radula organization, therefore, probably is synapomorphic for the taxon. Furthermore, the shell with, for example, its freely extending periostracum shows features that could hardly be regarded as analogous between the *Williamia* species.

Accordingly, at the present state of knowledge, it can be concluded that the genus *Williamia* is a natural systematic unit, most probably representing a clade within the Siphonariidae, which can be defined by its hard-part morphology. Soft-part anatomy, in contrast, is of limited use for categorizing the existing siphonariid subgroups.

Tentatively, a number of diagnostic characters can be given for the genus *Williamia*, but which nevertheless need to be confirmed by the detailed examination of more species. The shell is small to medium-sized, limpet-shaped, symmetrical except for the apex and elongate to oval. The outer surface is fairly smooth and often shiny. The larval shell is twisted to the left or backwards. Often there are bright or dark radial rays. The periostracum extends beyond the margin of the calcareous shell. The radula has a tiny unicuspid central tooth, several pairs of subequally bicuspid lateral teeth, several pairs of scale-like trapezoid lateral-marginal teeth with a trace of a tiny median cusp, and one pair of scale-like teeth with two short cusps, combining features of the lateral and the lateral marginal teeth.

Nearly all the characters given by Hubendick (1947) for *Williamia* must be dismissed. Most of them are based on the soft-part anatomy of *W. peltoides*, which turns out to differ strongly from the other species investigated (see above). In addition, the shell of *W. radiata* is not semitransparent (Marshall, 1981). The character of a more-weakly developed anterior portion of the shell muscle in *Siphonaria* than in *Williamia* seems doubtful too, as *S. pectinata*, for example, shows a well-developed muscle scar anteriorly (unpublished observation).

Distribution and habitat

Williamia peltoides and *W. radiata*, which are more similar to each other anatomically than either is to *W. gussonii*, are distributed in the Pacific Ocean, while *W. gussonii* has a Mediterranean-Atlantic Ocean distribution. This might suggest separate evolutionary branches due to geographic separation. However, more data, particularly on other Atlantic species, like *W. krebsii* are required in order to obtain a clearer view.

The habitat of *W. gussonii* is remarkable as it has only been found subtidally. Because of the depth range of localities one can assume that members of this species never come into contact with atmospheric oxygen and the pallial cavity never functions as a lung. This behaviour was overlooked in a review of the biology of the Siphonariidae (Hodgson, 1999), where it was assumed that all species live intertidally. *W. subspiralis* also appears to be fully subtidal (McLean, 1998), whereas *W. radiata* is known from both subtidal and intertidal localities (Marshall, 1981). The genus *Williamia*, therefore, is unique among the Pulmonata, as a fully subtidal pattern of life is unknown for any other taxon.

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