

# Redescription of the Australian endemic nudibranch *Dendrodoris maugeana* Burn, 1962 (Gastropoda: Opisthobranchia: Doridoidea): new and reviewed features important for future phylogenetic analyses of porostomes

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## Abstract

The anatomy of the temperate Australian doridoidean nudibranch *Dendrodoris maugeana* Burn, 1962 was examined for the first time. This investigation was undertaken to address a recognised gap in our taxonomic knowledge and to increase our understanding of the structural diversity found within the radula-less taxon *Dendrodoris*. The structural detail of the ptyaline gland, oesophagus and pharynx in *D. maugeana* were the same as that found in other members of the genus. However, ptyaline gland shape, gonad position and form, prostate gland arrangement and vaginal-wall structure were different to any other member of the genus previously investigated. The ptyaline gland was found to have elongate, dorsal extensions, the gonad was totally separated from the digestive gland, the prostate gland was intimately folded around the bursa copulatrix, and the vagina contained ciliated pouches. In addition, the results contradict previous interpretations of the pharynx, oesophagus and salivary glands used in recent phylogenetic analyses of porostomes. This knowledge will contribute to elucidation of homologous structures and clarification of the relationships between radula-less (porostome) and radula-bearing doridoidean nudibranchs.

**Key words:** Australia, endemic, mollusc, radula-less, reproductive system, porostome, nudibranch, doridoidean, *Dendrodoris*

## Introduction

At least 3000 species of nudibranchs are estimated to exist world wide (Willan & Coleman 1984). Nudibranchs are characterized by loss of the protective molluscan shell, and are well known for their diversity, adaptation and specialisation (see Klussmann-Kolb & Brodie 1999; Wägele & Johnsen 2001; Coleman 2001; Wägele 2004). Doridoidean nudibranchs form a large subdivision of the Nudibranchia, and based on the presence of gill glands in *Dendrodoris nigra* Stimpson, 1855, Wägele *et al.* (1999) considered the genus *Dendrodoris* to be indisputably doridoidean. However, Wollscheid-Lengeling *et al.* (2001), who undertook a molecular phylogenetic analysis of the Nudibranchia, found the position of the genus *Dendrodoris* varied depending on the method and marker used. They acknowledged that while *Dendrodoris* possessed synapomorphies of the Doridoidea it also had many unique characters that distinguished it from other doridoideans and that additional species from the genus required closer examination. Later, Wägele *et al.* (2003), while analysing both 18S and 16S rDNA genes for a phylogenetic analysis of opisthobranchs, clearly showed assignment of the porostome genera *Dendrodoris*, *Doriopsisilla* and *Phyllidia* to the Doridoidea.

On the basis of a gill cavity and gill retractability, Doridoideans are further divided into the phanerobranchs and cryptobranchs (Wägele & Willan 2000). Rudman (1998) and the morphological study of Valdés & Gosliner (1999) suggested that porostomes constituted an internal branch of the cryptobranch dorids. This was confirmed in a more recent morphological phylogenetic and systematic revision

of the cryptobranch dorids in which Valdés (2002) found the radula-less porostomes to be classified within the cryptobranch dorids. Rudman (1998) and Valdés (2002), however, still disagreed over the monophyly of the porostomes.

Members of the porostome genus *Dendrodoris* Ehrenberg, 1831 are widespread, being found in the relatively shallow waters of the northern Pacific Ocean (Kay & Young 1969; McDonald 1983; Baba 1994), the south-west Pacific Ocean (Brodie *et al.* 1997), the Atlantic Ocean (Marcus & Gallagher 1976; Valdés *et al.* 1996), the west Indian Ocean (Edmunds 1971) and the deep-sea habitats of the tropical west Pacific (Valdés 2001). However, the taxon is not easily recognised as a cohesive group by its external features alone (Brodie 2004). In addition to this, their lack of hard parts (i.e. shell, radula and jaws) means that their distinguishing characteristics relate primarily to the anatomy of soft parts, e.g. possession of a ptyaline (oral) gland. It is therefore essential to know and understand the anatomical variability of these soft parts (organ systems) among species. To date, this information is lacking for many dendrodorids because their original descriptions are based on external characters alone, e.g. *D. rainfordi* Allan, 1932. This lack of basic information makes comparative analysis difficult and restricts the production of robust phylogenetic hypotheses at a broader level.

The absence of *D. maugeana* from most relevant faunal accounts (e.g. Basedow & Hedley 1905; Burn 1969; Wells & Bryce 1993; Edgar 2000; Rudman 2003) suggests that the species is either rare or that its favoured habitat has yet to be adequately sampled. However, it is mentioned in the faunal list of known Australian members of *Dendrodoris* (see Burn

1962a), the Australian dorid nudibranch list of Burn (1975) and the Australian nudibranch list of Willan & Coleman (1984). No prior investigation of the internal organs of this species has been conducted and the present study therefore provides an essential redescription, involving both anatomy and cellular structure in this temperate Australian endemic. In addition to species and generic level taxonomic information this study also includes information essential for broader phylogenetic analyses, as explored by Wägele (1998), and encouraged by Wägele & Willan (2000: 91) and Ponder & Lindberg (1997: 231).

## Materials and Methods

The three specimens of *D. maugeana* from Tasmania were preserved in 1.25% glutaraldehyde and 0.5% paraformaldehyde in seawater. One of these specimens was dehydrated and then embedded whole in hydroxyethylmethacrylate resin (Kulzer Technovit 7100) before being serially sectioned (3 µm thick) using a powered microtome equipped with a tungsten-carbide knife. Sections were stained with toluidine blue to which 1% borax was added to assist stain retention. Structural features observed by this technique are termed microstructure or microanatomy throughout the remainder of this paper. Of the remaining two Tasmanian specimens, one was dissected to record anatomical detail and the other was lodged as a voucher specimen with the Museum of Tropical Queensland (MTQ), Townsville, Australia. The five additional specimens examined from Victoria, including the holotype, were preserved in 70% ethanol at the time of examination. Details of any previous preservative fluid used (e.g. formalin immediately after collection) is unknown, however their satisfactory state of preservation suggests this step was likely to have occurred. Of these five latter specimens, which are all lodged in the National Museum of Victoria (NMV), one was examined internally by dissection.

In both specimens dissected the vas deferens was removed from the rest of the reproductive system between the genital opening and the base of the prostate gland. The penis was located, severed and transferred to a glass slide. Once transferred, the penis was dehydrated in increasing concentrations of ethanol before being cleared in xylene. The presence of penial armature was confirmed during histological sectioning.

## Results

### Systematics

- Order Nudibranchia Blainville, 1814**  
**Suborder Anthobranchia Férussac, 1819**  
**Superfamily Doridoidea Rafinesque, 1814**  
**Cryptobranchia Odhner, 1934**  
**Porostomata Bergh, 1891**  
**Family Dendrodorididae ODonoghue, 1924**  
**Genus *Dendrodoris* Ehrenberg, 1831**

## *Dendrodoris maugeana* Burn, 1962

(Figures 1A–F, 2A–F)

### Synonymy

*Dendrodoris maugeana* Burn, 1962b: 104, text fig. 8; Willan & Coleman 1984: 53; Burn 1975: 516; Burn 1989: 769.

*Dendrodoris maugeana* Burn, 1961 [sic] Burn 1962a: 166; Macpherson & Gabriel 1962: 256

### Comments on Synonymy

*Dendrodoris maugeana* was described by Burn (1962b) and included in Willan & Coleman's 1984 checklist of Australian nudibranchs. The distribution of this species was compared to *D. albopurpurea* [sic] [= *albopurpura*] by Burn (1989).

### Type Material

**Australia, Victoria:** Holotype: Flinders, 1 specimen (10 mm preserved length), 25 May 1958, coll. R. Burn (NMV F20508), (intertidal).

### Comparative Material Examined

**Australia, Victoria:** Flinders, 2 specimens (5, 14 mm preserved length), 16 May 1959, coll. J. Macpherson. (NMV F20787). Walkerville, 2 specimens (23, 23 mm preserved lengths), Jan 1964, coll. J. Watson (NMV F24887).

**Tasmania:** City of Melbourne Bay, King Island, 1 specimen (40 mm long, live), 25 February 1996, coll. J. Marshall; Naracoopa, King Island, 1 specimen (30 mm long, live), 24 February 1996, coll. J. Marshall (intertidal); Coal Point, Adventure Bay, Bruny Island, 1 specimen (32 mm long, live), 31 December 1994, coll. J. Marshall (intertidal).

### Comment on Original Description

The original description of *Dendrodoris maugeana* by Burn (1962b) is relatively brief but contains detailed information on external features such as size (up to 36 mm), coloration (orange spotted with red), body shape (elongate) plus rhinophore and gill details (close to respective ends of body and eight branchiae). An associated line drawing shows a dorsal view of the living animal and provides a clear indication of the crenulated mantle margin.

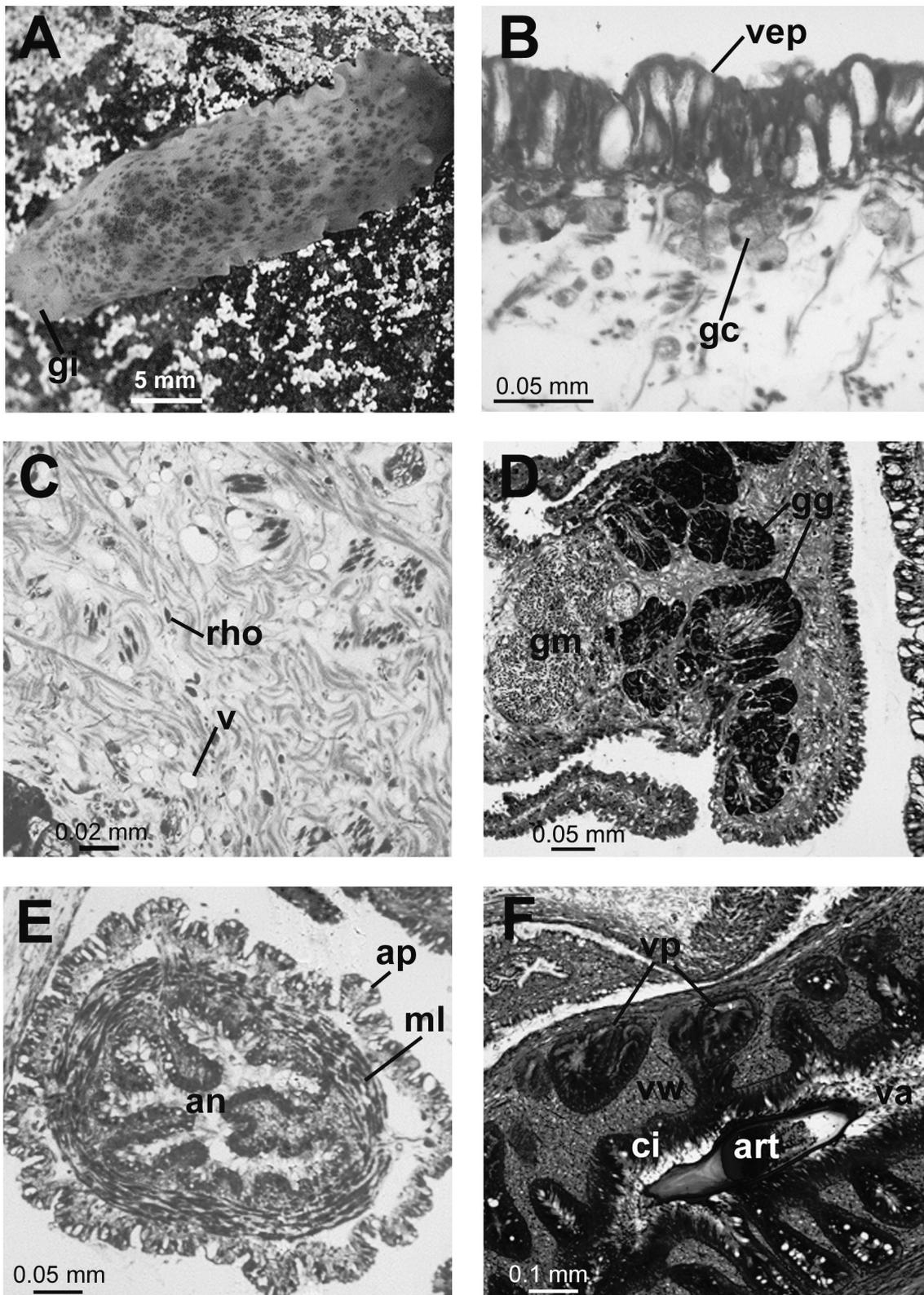
### Redescription

The following redescription is based on preserved material, photographs of live material, the original unpublished sketches by Burn of living animals and the original description by Burn (1962b). Three specimens were investigated internally, two by dissection and the third histologically.

The elongate body has a low profile and the notum overlaps the pale foot by a narrow margin. The crawling length of living adults extends to 40 mm and the foot extends either a very small amount or not at all beyond the posterior mantle edge. The smooth dorsum is orange with reddish brown spots (Figure 1A) that are still visible in preserved material. These spots are larger and closer together centrally than at the notal margins. The notal edge is crenulate and thin

with the underside of the mantle showing the dorsal reddish brown spotting. The foot is uniform pale orange. The rhinophores are orange to dark brownish red with white or yellowish apices. The clavus, with 11–12 sloping lamellae,

is longer than the stalk. The rhinophoral pockets are smooth, slightly raised and located very close to the anterior edge of the body.



**FIGURE 1.** Photographs of *Dendrodoris maugeana* showing: A, living animal (32 mm, live length) from Bruny Island, Tasmania. Photograph by J. Marshall. B, submarginal gland cells beneath the epithelium of the ventral notal edge. C, connective tissue of the notum showing non-staining vacuole-like spaces and rhogocyte-like structures. D, gill glands at the gill base. E, the anal papilla muscle. F, the internal walls of the vagina showing a close up of the long cilia on the epithelium of the vaginal pockets. an = anus, ap = anal papilla, art = artifact, ci = cilia, gc = gland cell, gg = gill glands, gm = gill muscles, ml = muscle layer, rho = rhogocyte-like structures, v = vacuole, va = vagina, , vep = ventral epithelium, vp = vaginal pockets, vw = vaginal wall.

The small compact gills are uniform pale orange in colour and found in a very posterior position on the dorsum. There are up to eight branchia but this varies depending on the size of the animal. The two anterior-most branchia tend to be slightly smaller. The anal papilla is of medium size and located centrally within the gill circle. No oral tentacles were observed on any of the preserved specimens examined, including the holotype. However the original description, based on live material, mentions the presence of small; leaf-like ... orals (Burn 1962b: 104).

#### *General Histology*

In the single specimen sectioned (30 mm, from King Island, Tasmania), a thin layer of simple gland cells, with pale purple-staining contents, were present subdermally on the ventral side of the notal edge (Figure 1B). Very few ciliated cells are present on the notal epithelium, but the epithelium of the foot consists of cells with long cilia. Distinctive multi-vacuolated cells are visible in the rhinophoral epithelium but were not observed in the notal epithelium.

No spicules were seen within the connective tissue of the notum. However, a number of small spicules were present in the central core of the rhinophores and even smaller sparse spicules in the gill rachi and foot. Within the notal connective tissue, many non-staining rounded spaces suggest storage vacuoles and many small spindle-shaped structures (possibly rhogocytes) are dispersed throughout (Figure 1C). Very distinct compact gill glands, without obvious deferent ducts, were located at the base of the gill branchiae (Figure 1D).

#### *Digestive System*

The mouth is ventral, within a notch in the antero-ventral surface of the foot (Figure 2A).

The radula and jaws are absent. A generalised schematic diagram of the arrangement of organs within the anterior digestive system is shown in Figure 2B. The oral tube duct is narrow and uniform in width. A separate ptyaline (= oral) gland is connected by a single duct to the lumen of the distal oral tube. In both of the specimens dissected the relatively large ptyaline gland was distinctively shaped (Figure 2C). Two elongate extensions of the gland arise dorsally, one on either side of the pharynx. *In situ*, the knob-like apex of each extension lies either side of the central cerebro-pleural ganglia. The pharynx is long and tubular with an external surface that is smooth and shiny. A pair of very small, rounded salivary glands is present, one on either side, at the junction of the pharynx and oesophagus (Figure 1B). No deferent ducts were seen to be associated with the salivary glands. The broader oesophagus follows on from the pharynx, and then enters the stomach, which is deeply embedded within the large digestive gland. Externally, the oesophagus is less uniform in shape and not as smooth as the pharynx. In both of the specimens dissected, the posterior of the digestive gland was not distinctly bilobed, as it is in many other members of the genus. Although embedded within this gland, the stomach is not clearly distinct from it

and cannot be separated from it by dissection. A simple, narrow intestine emerges dorsally from the stomach, and exits the digestive gland dorsally. At this point, where the intestine bends before running posteriorly to the anus, a distinct pyloric bulb was seen in the specimen from Tasmania (40 mm) but not in the specimen from Victoria (F24887). The intestine is tubular, thin-walled and dorso-ventrally flattened. It runs posteriorly over the digestive gland, and narrows before terminating in an anal papilla that is located to the centre of the gill branchia.

#### *Histology of the Digestive System*

A loose layer of purple staining gland cells surrounds the proximal oral tube. The tissue of the ptyaline gland consists of very distinctive cells that are closely packed and irregular in shape. The outermost cells are full of small non-staining vacuoles. Centrally, within the glands core, there is a large number of darker staining glandular cells. Located around the main muscular ducts leading out of the gland are many variably sized gland cells that stain very deeply.

In cross-section, the pharynx is very distinctive and consists of several layers. A layer of muscle surrounds a thick layer of very elongate cells that form an open spongy network-like structure of cells with large non-staining vacuoles. The lumen of the pharynx is characteristically Y-shaped and its epithelium possesses a thin cuticular covering. In cross section, the pharynx and the oesophagus differ significantly in structure. Although also surrounded by an outer layer of muscle, internally the oesophagus is extremely glandular. The gland cells are large, irregular in shape, and they stain deep pinkish purple. The glandular development is very extensive and there is no cuticle or epithelial lining present in the narrow oesophageal lumen. The extensive glandular develop does not however continue all the way to the stomach, and a relatively short section of the proximal oesophagus is non-glandular and narrow, with a typhlosole-like thickened groove present for a very short distance along the dorsal surface of the lumen.

The salivary glands were difficult to locate in the histological sections probably because of their very small size. The digestive gland was composed of numerous lobes. The areas between the lobes are interspersed with kidney tissue. No consistent layering of the two tissues is present. The stomach is differentiated within the digestive gland by a folded epithelium that, close to its junction with the oesophagus, is covered in tufts of long cilia. The epithelium of the intestinal lumen is very folded at its origin from the stomach but a typhlosole (thickened groove) is not discernible. No glandular cells were seen in association with the pyloric bulb area.

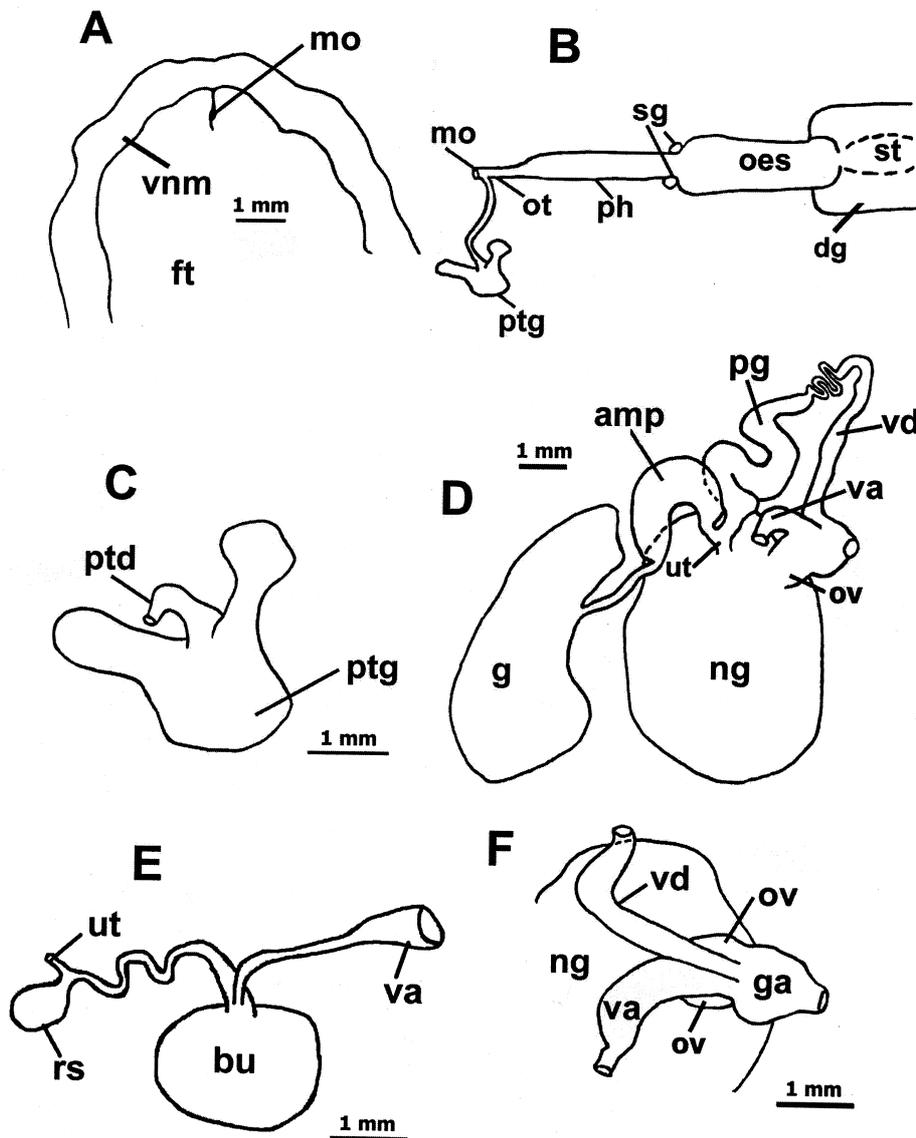
The anal papilla is folded apically and a ring of circular muscle sits within the wall of the anal papilla (Figure 1E).

#### *Reproductive System*

The gonad of *D. maugeana* is totally separated from the digestive gland mass. A drawing of the reproductive system (minus the bursa copulatrix and receptaculum seminis for clarity) is shown in Figure 2D. *In situ*, the compact, solid,

gonad lies immediately in front of the digestive gland and adjacent to the nidamental gland mass. The large, compact nidamental (= female) gland mass has several intimately connected regions that cannot easily be teased apart. The gonad is also substantial in size but flattened on both the anterior and posterior faces. The hermaphroditic duct is relatively long and wide and it leaves the gonad in the centre of its anterior face. This duct leads into a smooth shining tubular ampulla. The lobate cream prostate gland is irregularly elongate but *in situ* folds back on itself, around the bursa copulatrix, giving it a flattened disc-like appearance. In both specimens dissected, the prostatic covering is difficult to remove from the rounded thin-walled

bursa. The prostate narrows considerably before entering the vas deferens. The proximal vas deferens is very narrow, twisted and convoluted but expands in width distally before terminating at the penis. The penis is armed with numerous spines that are very elongate and sharply pointed. The thin-walled rounded bursa and the smaller oval receptaculum seminis individually lead off the vaginal duct (Figure 2E) before it enters the nidamental gland. The distal vagina is non-muscular and relatively wide (Figure 2F). The vagina, vas deferens and oviduct all merge before exiting at a common genital orifice. The genital aperture is located well forward, almost below the right rhinophore, on the body wall beneath the notum.



**FIGURE 2.** Composite drawings of *Dendrodoris maugeana* showing: A, a ventral view and details of the mouth area. B, a generalised schematic diagram of the arrangement of organs within anterior digestive system. C, unusual shape of the ptyaline gland. D, reproductive system (with the bursa copulatrix and receptaculum seminis removed). E, arrangement of the bursa copulatrix and receptaculum seminis. F, relatively wide distal vagina. amp = ampulla, bu = bursa copulatrix, dg = digestive gland, ft = foot, g = gonad, ga = genital atrium, mo = mouth, ng = nidamental gland, oes = oesophagus, ot = oral tube, ov = oviduct, pg = prostate gland, ph = pharynx, ptd = ptyaline duct, ptg = ptyaline gland, rs = receptaculum seminis, sg = salivary glands, st = stomach, ut = uterine duct, va = vagina, vd = vas deferens, vnm = ventral notal margin.

### *Histology of the Reproductive System*

In the gonad of the specimen sectioned (30 mm live length), large well-developed male acini are present, each surrounded by a thin layer of connective tissue. Each acini contained many sperm at different stages of development, with the more mature tailed sperm located centrally. Smaller immature or spent acini were also present and it was difficult to determine if these were male or female. No recognisable oocytes or well-developed ova were observed. The prostate gland is large, and consists of two consecutive parts each with a different cell type; the distal section has much larger paler staining cells; the lumen of both parts is distinct and contains mature sperm.

As suggested by its external appearance, the walls of the unusually wide distal vagina are very glandular and contain numerous, unusual, thick-walled pockets (Figure 1F). The epithelial cells of these pockets are fringed by extremely long cilia creating a very dense thick border along the lumen of the duct and the pockets. The pockets are round in cross-section and the thick area of vaginal wall between them consists of spongy looking cells with large dark staining-nuclei and non-staining vacuoles. The cilia are so long that they completely fill the cavity of the pockets; however, even larger non-staining vacuoles, than found in the cells of the vaginal wall, are seen surrounded by cilia within the lumen of the pockets themselves. No sign of any annexed vestibular glands was seen in the histological sections made.

### *Nervous System*

The nervous system is well developed with a ring of ganglia surrounding the distal pharynx. This circumpharyngeal nerve ring is equivalent to the circumesophageal ring of ganglia, typical of many gastropod nervous systems. The smooth surfaced pharynx can move freely backwards and forwards through this circumpharyngeal nerve ring. A separate pair of small cream-coloured ganglia is also located well behind this principal ring, very close to the salivary glands. Without close inspection, it is possible that these buccal ganglia could be confused with salivary glands.

All ganglia of the circumpharyngeal nerve ring have smooth contours and are surrounded by a thin, loose layer of connective tissue. Two small, dark concentrations of photosensory pigments are located dorso-laterally and anteriorly on this ring. The nerve cells are located primarily around the margins of the ganglia. The relatively thick-walled statocyst is located close to the ganglia and contains several, small, oval statoconia.

### *Histology of the Circulatory System*

The blood gland is non-pigmented, irregular in shape and flattened dorso-ventrally. It is composed of a compact mass of very small, loose, uniform cells. There is no apparent internal structure, and no epithelium or connective tissue surrounds the gland.

### *Histology of the Excretory System*

In cross-section, the non-staining kidney cell tissue extends over, around, and throughout the digestive gland. A relatively large, rounded renal syrx is present and located on the right side, in the posterior-most section of the body cavity. The internal structure of the renal syrx consists of large folds. The appearance of these folds varies from the base to the apical region. Tufts of long cilia are present within the apical folds giving the area a very structured geometric appearance. However, basally there are no cilia, the folds become less defined and the cells contain large non-staining vacuoles.

### *Occurrence & Habitat*

The type locality is Mushroom Reef, Flinders, Victoria, Australia (Burn, pers. comm.). Willan & Coleman (1984) state the range of this species as being Victoria, Tasmania and Western Australia in their list of Australian nudibranchs. Occurrence may well be ephemeral because the species was reported as common in one year but was then unreported in the same location for the following three years (Burn 1962b). According to Burn (1962b) *D. maugiana* is usually taken intertidally under stones. It is possible that *D. maugiana* may be present in New Zealand (R. Willan, pers. comm.) but to date this is unconfirmed.

### *Diagnostic Features*

The diagnostic external features of *D. maugiana* are: (a) its elongate body shape and (b) smooth texture, in combination with (c) its spotted, orange colour pattern, (d) the extreme posterior position of the gill, (e) the very anterior position of the genital aperture, (f) the very anterior position of the rhinophores and, unlike other *Dendrodoris* with an elongate, smooth body profile, (g) the anal papilla located centrally within the gill plume.

Internally, *D. maugiana* is the only member of the genus known to possess (h) a gonad, which is totally separate from the digestive gland. Besides this gonadal arrangement, the most distinctive internal feature of this species is (i) the numerous unique glandular pockets, lined with long cilia, in the walls of the distal vagina. In addition, (j) the reproductive organs are situated more anteriorly *in situ* than found in other members of the genus.

### **Discussion**

Unlike any other anatomically-investigated member of the genus *Dendrodoris*, including *D. elongata* Baba 1936, which it may be considered to superficially resemble, the gonad of *D. maugiana* is totally separate from the digestive gland mass. This is a significant discovery, since in all other members of the genus investigated internally to date, the gonad is intimately associated with the digestive gland, often to the point of separation being impossible by dissection. Within the genus, the widespread acceptance of this relationship, is clearly shown by the gonad being drawn in association with the digestive system in the illustrations of major publications relating to the genus (see Valdés *et al.*

1996, Figures 3A, 5A, 7A, 9A, 13A, 14A and particularly 10 and 11A; Valdés & Gosliner 1999, Figure 4L) and never within the reproductive system drawings (e.g. Edmunds 1971; Valdés *et al.* 1996; Valdés & Gosliner 1999; Brodie 2004). This latter practice is common across illustrations of nudibranch reproductive systems (e.g. Marcus & Marcus 1967; Millen & Gosliner, 1985; Gosliner & Brehrens 1988; Wägele 1995; Miller 1996; Schrödl 2000; Schrödl & Wägele 2001; Rudman 2004) presumably because the occurrence of a separate, solid, gonad that is immediately in front of the digestive gland, as found here in *D. maugeana*, is somewhat unusual within the order Nudibranchia (see Wägele & Willan 2000: 139). Additionally, in comparison to the totally separated condition found in *D. maugeana* during the current study, the intermingled position of gonad, kidney and digestive gland reported by Wägele *et al.* (1999) for *D. nigra* and by Brodie (2001) for *D. fumata* (Rüppell & Leuckart, 1828) reflects a very contrasting extreme. Moreover, an intermediate layered arrangement has been documented in several other *Dendrodoris* species (Brodie 2002) and the porostome genus *Doriopsilla* (Brodie 2001).

The line drawings of Valdés *et al.* (1996) show two gonad conditions in Atlantic members of the genus *Dendrodoris*. These are where the gonad (= hermaphroditic gland of Valdés *et al.*) is considered as either differentiated or undifferentiated from the digestive gland. These states are further defined as separate and interdigitated respectively by Valdés & Gosliner (1999) in their phylogenetic analysis of radula-less dorids. The condition described here for *D. maugeana* however, is quite different to both of these states; as confirmed by my own dissection of the Atlantic species, *D. limbata* (Cuvier, 1804) and *D. grandiflora* (Rapp, 1827).

The arrangement of a gonad totally separate from the digestive gland (as found here in *D. maugeana*) is not considered to be a feature found in the majority of doridoidean nudibranchs. However, it is found more broadly in many opisthobranch molluscs including: the nudibranch sister taxa Pleurobrancoidea (Wägele & Willan 2000); *Bathydoris*, a nudibranch considered to be a sister taxon to the doridoideans (Wägele & Willan 2000); and *Doridoxa*, an enigmatic nudibranch considered to be part of the Dexiarchia and sister taxon to the Anthobranchia [= Bathydoridoidea + Doridoidea] by Schrödl *et al.* (2001). The arrangement is certainly unique among species of *Dendrodoris* and its discovery was quite unexpected in the present study.

The distal vagina of *D. maugeana* is uniquely glandular and the thick, spongy vaginal walls are dominated by unusual looking gland cells and a network of large, ciliated pockets. I am not aware of any previous documentation of such structures in nudibranchs. In light of the unique findings of symbiotic bacteria within similar, highly ciliated, deep-staining glandular areas of the distal reproductive system in *D. nigra* (see Klussmann-Kolb & Brodie 1999) these vaginal tissues require further microstructural (TEM) investigation and comparison to a broader range of doridoideans using similar techniques.

As stated by Valdés *et al.* (1996) most species of *Dendrodoris* are found to have a tubular prostate gland (e.g.,

*D. elongata* Baba, 1936 (see Baba 1994). However, a flattened prostate that *in situ* surrounds the bursa (resulting in a disc-like arrangement that is quite often found in *Doriopsilla*, see Valdés & Ortea 1997) was unexpectedly found in *D. maugeana*. This means that prostate shape or arrangement cannot be considered as a constant generic feature for *Dendrodoris*.

In a review of the microscopic anatomy of opisthobranchs, Gosliner (1994) acknowledged that oral glands appeared to provide significant phylogenetic information for several opisthobranch clades but that further information was required to ascertain the variability and homology of these structures. Despite its unusual shape, the tissue of the ptyaline gland in *D. maugeana* has the same very distinctive spongy appearance found in the ptyaline gland of other members of the genus that have been examined histologically, e.g., *D. nigra* (see Wägele *et al.* 1999: 86 and Figure 2B) and *D. guttata* (Odhner, 1917) (see Brodie 2001). According to Wägele *et al.* (1999: 90) this type of oral gland tissue is unique i.e., to date unknown in any other genera of doridoidean nudibranchs.

The presence of a very glandular oesophagus, already well documented and shown by Wägele *et al.* (1999, Figure 3A, B) in *D. nigra* and several other *Dendrodoris* species by Brodie (2001, see Figure 3C), was confirmed in *D. maugeana*. According to Wägele *et al.* (1999) similar glandular cells can be found in the oesophagus of *Jorunna tomentosa* (Cuvier, 1804) and *Hypselodoris tricolor* (Cantraine, 1835), however they are not found in anywhere near the same quantity and thickness. The results of the current study therefore confirm that the pronounced secretory function of the oesophagus, first documented in *D. nigra* by Wägele *et al.* (1999), appears to extend across the genus.

As has been well documented in other species of *Dendrodoris* (see Wägele *et al.* 1999, Figures 2C, 3A and Brodie 2001, Figure 3A) the microstructure of the pharynx in *Dendrodoris* is very distinctive and obviously has a very different function to the very glandular oesophagus. This marked difference between the pharynx and oesophagus is now confirmed in *D. maugeana* and the reader is referred to the previous publications for more structural detail of these organs (see Wägele *et al.* 1999 Figure 3A; Brodie 2001 Figures 3A, C). This difference however is significant because these areas of the digestive system are misinterpreted in several recent studies (e.g. Valdés *et al.* 1996; Valdés & Gosliner 1999; Valdés 2002), with the pharynx being considered as part of the oesophagus. This misinterpretation of the beginning of the oesophagus has in turn resulted in *Dendrodoris* salivary glands being coded erroneously as absent within these important analyses.

Gosliner (1994) acknowledged that salivary glands appear to exhibit the same basic structure throughout the Opisthobranchia but that the proportion of different cell types varied between species. More recently (Wägele *et al.* 1999) reported that salivary glands in nudibranchs have a rather uniform structure, with glandular cells that stain in two different ways, one with and one without evidence of the

production of acidic mucopolysaccharides. However, the results of the present study were unable to confirm that the salivary glands of *D. maugeana* consist of only one type, as previously shown and discussed for *D. nigra* by Wägele *et al.* (1999, see Figure 2D).

My interpretation of the anterior part of digestive system and the possession and placement of salivary glands in *Dendrodoris* is supported by several previous workers, particularly Young (1969), who undertook a very detailed study of the functional morphology of feeding in several dorid groups including *Dendrodoris*. Although the salivary glands are very reduced, their location at the proximal end of the very elongate pharynx (which consists of radula-bearing cushions and a cuticle, see Wägele *et al.* 1999) is convincing. Bergh (1880), Salvini-Plawen (1988) and Wägele *et al.* (1999) also support this interpretation of the relative positions of the pharynx, oesophagus and salivary glands.

Unlike several other members of the genus *Dendrodoris* (e.g., *D. guttata* and *D. albobrunnea* Allan, 1933) there is no sign of a posterior gap in the arrangement and location of the gill branchia. I consider this important because the position of the anus is a characteristic feature in several other porostome taxa including the genus *Doriopsilla* (see Valdés & Ortea 1997) and the family Phyllidiidae (see Brunckhorst 1993).

To my knowledge possession of a distinct intestinal sphincter muscle close to the anus has not been recorded previously for doridoideans but is known to be variable among *Dendrodoris* species in both its presence and development (Brodie 2002).

Discovery of a distinctly vacuolated rhinophoral epithelium (as well shown in Wägele 1998 Figures 3, 4) within *D. maugeana* is significant as these specialized vacuolated cells have not been previously found in *Dendrodoris* (Wollscheid-Lengeling *et al.* 2001: 254) and are considered to be phylogenetically important (Wägele 1998). Wägele (1998) also considered rhogocytes, dark-staining spindle-shaped cells that function in hemocyanin production, (see Haszprunar 1996; Albrecht *et al.* 2001) to have significant phylogenetic potential, and therefore recording their possible presence in *D. maugeana* may be useful for future phylogenetic analyses. However, confirmation of rhogocyte presence requires cellular detail beyond the scope of the methods used in the current study (see Wägele 1998).

## Conclusions & Future Studies

*Dendrodoris maugeana* is a distinct species that is undoubtedly correctly placed within the genus *Dendrodoris* because of its possession of ptyaline glands. The microstructure of these glands is unique and to date has only been found within the genus *Dendrodoris*. *D. maugeana* does however possess at least two significant features (gonad position and the locational relationship between the prostate gland and bursa copulatrix) that make it important for inclusion in any future phylogenetic analyses of porostome, cryptobranch or doridoidean nudibranchs. In the future it

would also be valuable to compare the anatomy of *D. maugeana* with the similarly coloured but apparently larger *D. citrina* (Cheeseman 1881) reported from New Zealand by Morton & Miller (1973) and Willan & Morton (1984).

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