

Australian *Aphelodoris* (Mollusca: Nudibranchia): two new species, sperm ultrastructure and a redescription of *Aphelodoris greeni* Burn

Nerida G. Wilson

Centre for Marine Studies, University of Queensland, St Lucia Qld 4072 Australia
nwilson@marine.uq.edu.au

Abstract – Two new species of *Aphelodoris* Bergh are described from the north-western coast of Australia. *Aphelodoris gigas* sp. nov. has been found only in the Dampier and Kimberley regions in Western Australia, while *A. karpa* sp. nov. is known to occur in northern Western Australia and also in the Northern Territory. These species both possess a two-part prostate; this is the first time this has been reported in the genus *Aphelodoris*, and thus lends support for inclusion in the family Halgerdidae. Redescription of *A. greeni* Burn from Tasmania indicates that a two part prostate also occurs in at least one southern Australian species. The range extension reported here for *A. greeni* indicates that it is sympatric with *A. juliae* Burn in southern Tasmania, and not allopatric as was previously thought. The sperm morphology of *A. gigas* sp. nov., *A. greeni* Burn and *A. varia* (Abraham) is described, and shows acrosomal internal structure (short, angular striations through part of the pedestal) that is to date unrecorded among the Doridina. The spermatological characteristics of these *Aphelodoris* species appear most similar to the dorid genera *Sclerodoris* and *Asteronotus*.

INTRODUCTION

Bergh (1879) introduced the genus *Aphelodoris* to accommodate his species *A. antillensis* from the West Indies. Since that time, nine other valid species have been described, all from the Southern Hemisphere. The addition of two new species described here brings the total to eleven. There remain several other undescribed species in Western Australia (Wells and Bryce, 1993; Coleman, 2001; unpublished observations) and at least one in South Africa (Gosliner, 1987).

Initially Bergh (1879) described *Aphelodoris* as a genus within the Chromodorididae, united by details of gill pinnation and the morphology of the oral tentacles. Odhner (1926) introduced the subfamily Halgerdinae (within the Dorididae) for the genera *Halgerda*, *Aphelodoris*, *Asteronotus* and *Sclerodoris*. Later, Thiele (1931) renamed Odhner's family as Asteronotinae but also included *Aporodoris* (now synonymized elsewhere with *Taringa*). Yet in spite of having priority with the family name Halgerdidae, Odhner in Franc (1968) continued to use Asteronotidae. In more recent years, Halgerdidae has been more commonly used to accommodate these four genera (Boss, 1982; Vaught, 1989; Fahey and Gosliner, 1999). A recent phylogenetic analysis (Valdés and Gosliner, 2001) indicated that *Sclerodoris* was better placed with other genera bearing caryophyllidia, but confirmed a close relationship between *Halgerda* and *Asteronotus*. No representative of *Aphelodoris* was included in the analysis and its systematic placement within the Dorididae requires further study.

This paper describes two new *Aphelodoris* species and redescribes *A. greeni* Burn. As the morphology of sperm ultrastructural features is known to assist in elucidating supraspecific relationships amongst nudibranch taxa (Healy and Willan, 1991; Wilson and Healy, 2002), the sperm morphology of *Aphelodoris gigas* sp. nov., *A. greeni* and *A. varia* (Abraham) is described and compared to other dorid nudibranch genera.

MATERIALS AND METHODS

All material studied is deposited in the collections of the Western Australian Museum (WAM), Perth, and the Australian Museum (AM), Sydney. Much material was collected during the Woodside Dampier Marine Biological Workshop 2000, organized by the Western Australian Museum. The relevant states and territories of Australia are abbreviated as follows: VIC, Victoria; NSW, New South Wales; QLD, Queensland; NT, Northern Territory; WA, Western Australia; SA, South Australia; TAS, Tasmania. Specimens were anesthetized with magnesium chloride (8%) mixed with seawater before being fixed in 10% neutral-buffered formalin. The specimens examined ultrastructurally for sperm morphology (WAM S12652; AM C203862, AM C203863) were cut along the notum to expose the visceral mass, and fixed in 3% glutaraldehyde at ~4 °C for at least 12 hours. The reproductive system was later dissected and the ampulla removed. Tissue from the ampulla was post-fixed in 1% osmium tetroxide, dehydrated in ethanols and embedded in Spurr's low-viscosity medium (see Wilson and Healy, 2002). Semi-thin sections (1 µm) were cut using a glass knife, while ultrathin sections (60–90 nm) were cut using a DiATOME ultra 45° diamond knife both on an LKB 2088 Ultratome V. Ultrathin sections were stained with uranyl acetate and lead citrate (Daddow, 1986) on uncoated, 150 µm mesh copper grids. Grids were examined using a Hitachi transmission electron microscope operated at 75kV. Radulae were examined on a JEOL JSM-6300F scanning electron microscope at 8kV.

SYSTEMATICS

Family Halgerdidae Odhner, 1926

Genus *Aphelodoris* Bergh, 1879

Type species

Aphelodoris antillensis Bergh 1879, by monotypy.

Diagnosis (from Burn, 1966)

“Glossodoridiform” or high, slender, elongate body shape with usually narrow notal brim, smooth notum, high conical rhinophoral and branchial sheaths, laterally grooved oral tentacles, five-branched branchia, smooth labium, hook-shaped radular teeth without denticles, unarmed penial sheath, large prostatic part in male duct, and spermatheca (here termed bursa copulatrix) and spermatocyst (here termed receptaculum seminis) arranged serially (Odhner, 1924) or semiserially.

Aphelodoris gigas sp. nov.

Figures 1A, 2A, 3A–D, F, 4, 8.

Aphelodoris sp. Debelius, 1998, p258.

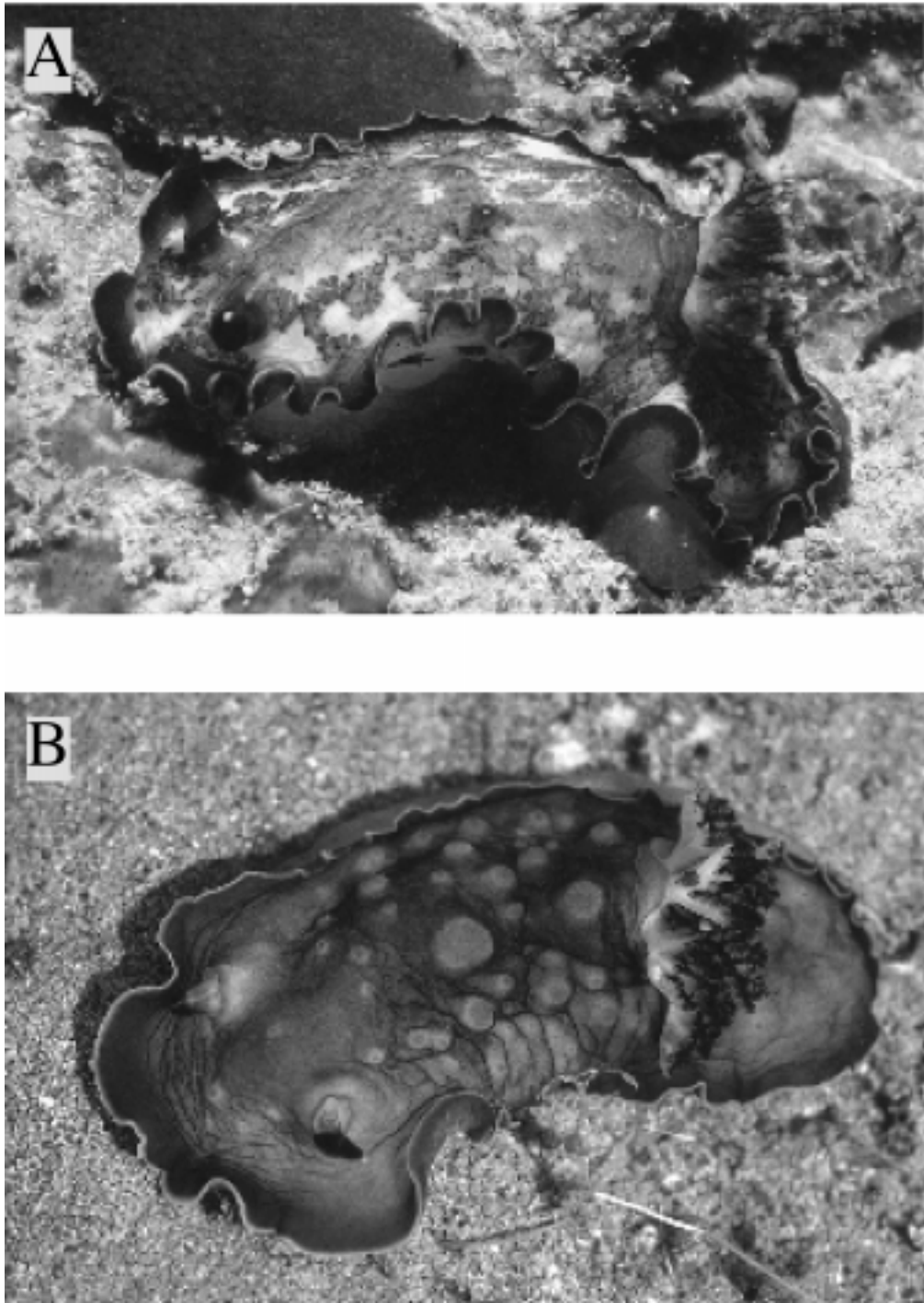


Figure 1 A. *Aphelodoris gigas* sp. nov. (WAM S24940). B. *Aphelodoris karpa* sp. nov. (paratype, AM C204865).

Aphelodoris sp. Wells and Bryce, 1993, p97, pl. 112.

Material examined

Holotype

One specimen, north-west Enderby Island, Dampier Archipelago, Western Australia, 20°35.119'S, 116°28.908'E, 8 m, 9/8/00, live length 123 mm, preserved length 60 mm, coll. S. Fahey, WAM S12641.

Paratypes

Two specimens, north-west Enderby Island, Dampier Archipelago, Western Australia, 20°35.119'S, 116°28.908'E, 8 m, 9/8/00, live lengths 113 and 125 mm, preserved lengths 51 and 55 mm, coll. S. Fahey, WAM S12715; one specimen, north-west Enderby Island, Dampier Archipelago, Western Australia, 20°35.119'S, 116°28.908'E, 8 m, 9/8/00, live length 110 mm, preserved length 50 mm, coll. S. Fahey, AM C203864.

Other material

One specimen, dissected, west Nelson Rocks, Dampier Archipelago, Western Australia, 20°26.506'S, 116°40.261'E, 19 m, 29/7/00, preserved length 61 mm, coll. P. Hutchings, WAM S12625; one specimen, north-west Legendre Island, Dampier Archipelago, Western Australia, 20°21.206'S, 116°50.439'E, 27 m, 30/7/00, preserved length 40 mm, coll. M. Berggren, WAM S12626; one specimen, dissected, north-west Legendre Island, Dampier Archipelago, Western Australia, 20°21.206'S, 116°50.439'E, 12 m on sponge, 30/7/00, preserved length 53 mm, coll. N. Wilson, WAM S12652; one specimen, dissected, north side Long Reef, Kimberley, Western Australia, 13°58'S, 125°38'E, 17/7/88, preserved length 43 mm, coll. C. Bryce and F. Wells, WAM S24940; one specimen, photo examined only, Legendre Island, Dampier Archipelago, Western Australia, WAM S12043.

Distribution

Aphelodoris gigas is presently known only from the Dampier Archipelago and the Kimberley region in north-western Western Australia.

Etymology

This species is named for its large size.

Natural History

Aphelodoris gigas has been recorded feeding on the sponge *Penares* sp 1 (Figure 2A) in the Dampier Archipelago. It is typically found in high current areas, in both silty and clean water situations and is usually actively crawling during the day. The species, like some other *Aphelodoris*, can swim strongly as an escape response. This action is achieved by repetitive dorso-ventral flexion.

External morphology Figure 1A, 2A

The body is very soft and fleshy, broadly ovate, with a wide mantle overlap. The extended crawling lengths of living specimens ranged from 110–125 mm. The notum is brown, varying in

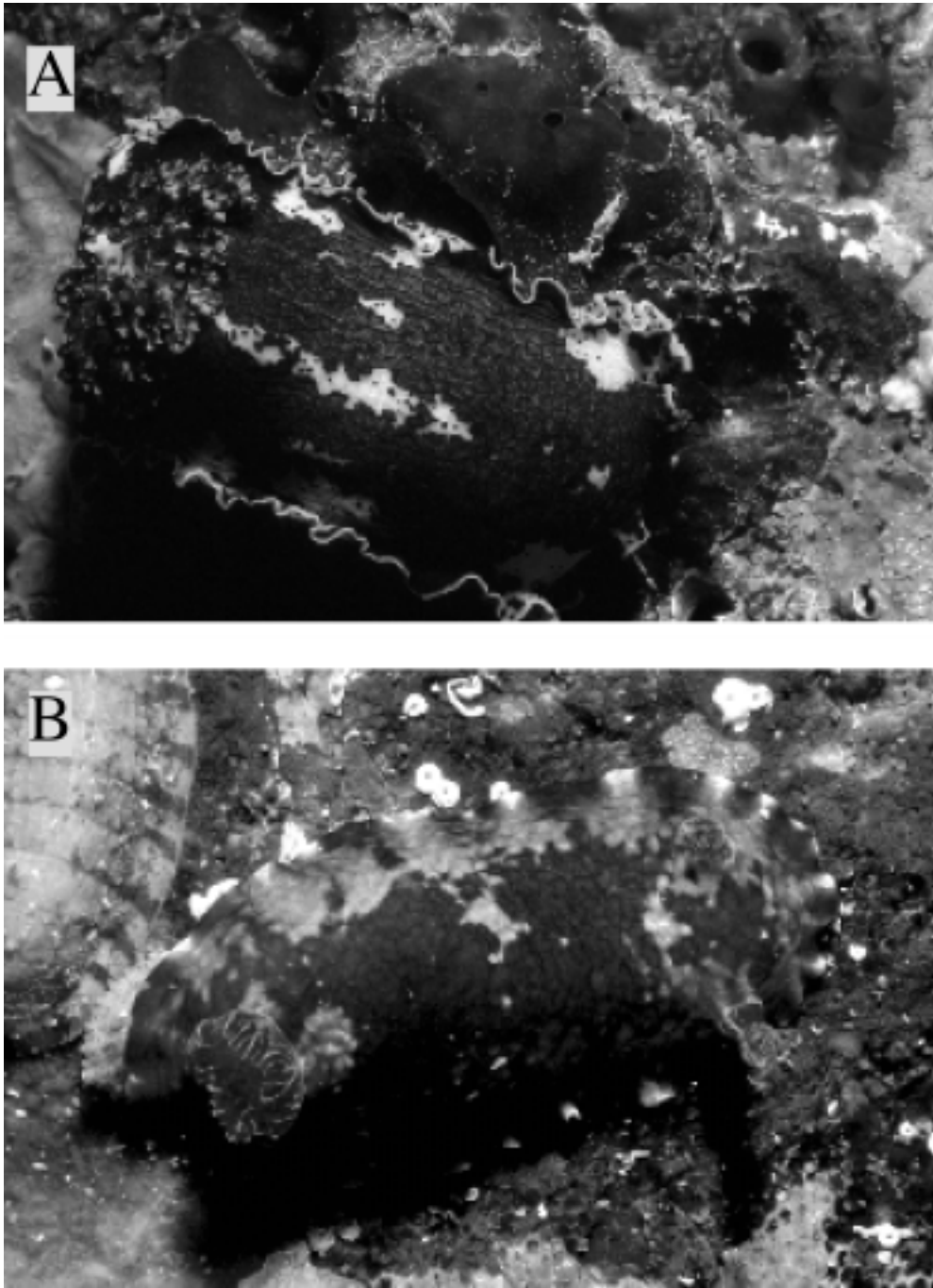


Figure 2 A. *Aphelodoris gigas* (WAM S12652) feeding on *Penares* sp., dark gray sponge in background. B. *Aphelodoris greeni* Burn, 1966.

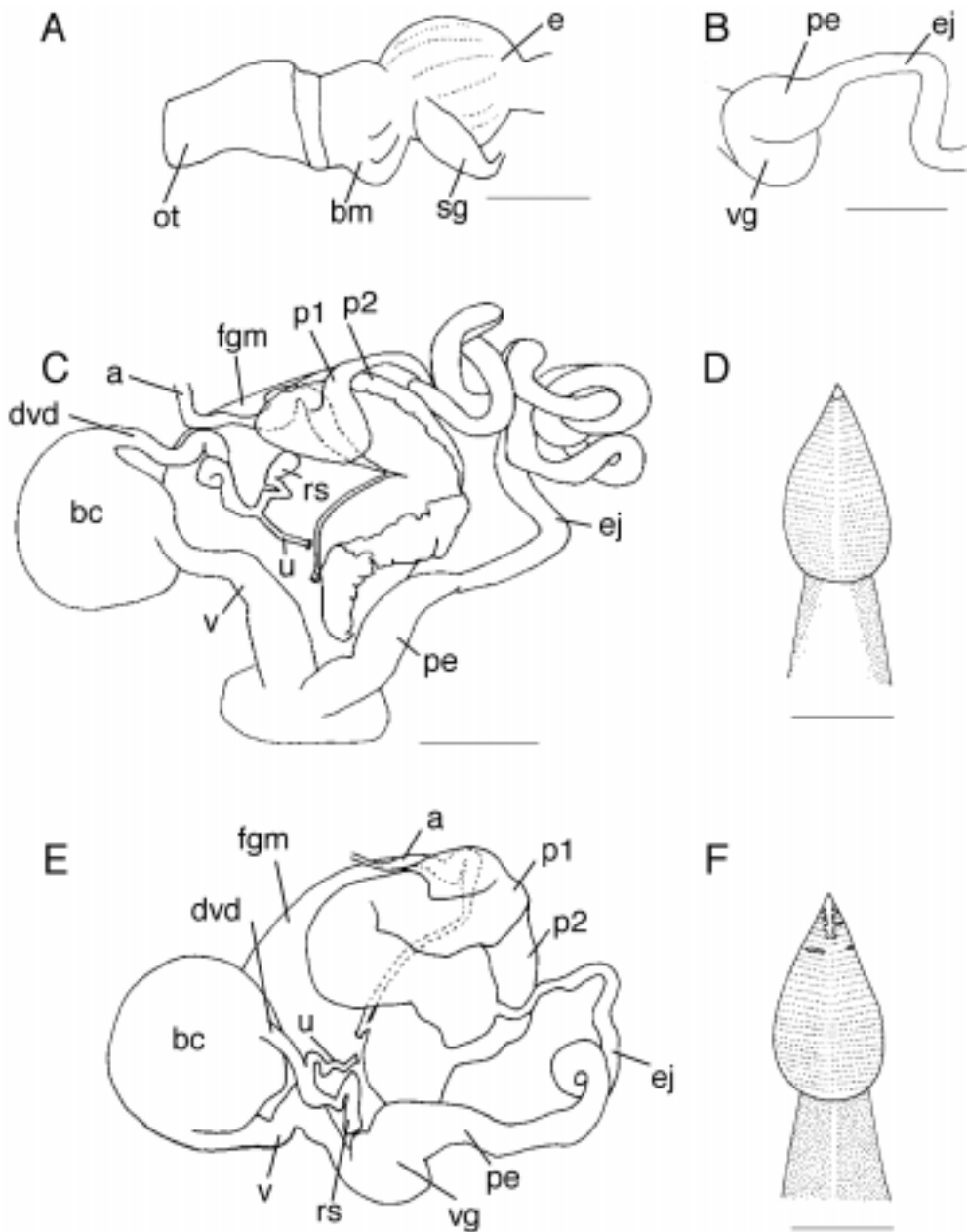


Figure 3 A. Buccal mass of *Aphelodoris gigas*, retractor muscles removed. B. Penial sheath of *Aphelodoris gigas* with vestibular gland intact. C. Reproductive system of *Aphelodoris gigas* with vestibular gland removed. D. Rhinophore of *Aphelodoris gigas*, anterior view. E. Reproductive system of *Aphelodoris karpa*. F. Rhinophore of *Aphelodoris karpa*, posterior view. a, ampulla; bc, bursa copulatrix; bm, muscular portion of buccal mass; dvd, distal vaginal duct; e, esophagus; ej, ejaculatory duct; fgm, female gland mass; u, uterine duct; ot, oral tube; pe, penial sheath; p1, first portion of prostate; p2, second portion of prostate; rs, receptaculum seminis; sg, salivary glands; v, vagina; vg, vestibular gland. Scale bar = 5mm.

intensity between species, with irregular, broken white or cream patches around the notal margins and on the dorsal midline. Some specimens are white with brown pigment speckled over the dorsum. There is often a major tubercle or swelling just anterior to the gills, and rarely a few smaller tubercles are present close to the major one. These tubercles may be tipped in orange pigment. The notal margin consists of a thin white or transparent edge that is always present, and a wider, orange submarginal border that is often present.

The rhinophoral pockets are raised cylindrical sheaths, and the branchial pocket has a slightly raised rim. The rhinophoral and branchial pockets also have a thin white or transparent border and often, a submarginal border in orange. The rhinophores have approximately 28–34 lamellae. The club is chocolate brown with a white line up the anterior side (Figure 3D), and a white tip which also may show some orange pigment. Some lamellae, or parts thereof, are white, and this is more prominent on the posterior side of the rhinophore club (Figure 3F). The rhinophoral stalks are brown, with a broad, translucent white stripe up the anterior side of the stalk and a fine line down the posterior side. The demarcation between the gills is not always clear, and this species tends to have five to seven tripinnate gills. The gills are chocolate brown and white, and may be tipped in cream or orange. The relative amount of each pigment varies greatly between individuals. In all living specimens (7), the anal papilla was translucent white with some orange pigment on the rim.

The foot often has an orange border, and the sides of the foot are translucent white or cream with varying amounts of brown pigment present. The oral tentacles are laterally grooved in both live and preserved specimens, and typically have orange pigment on at least half the length.

Buccal mass

The muscular portion of the buccal mass is about half the length of the oral tube (Figure 3A). The salivary glands are flattened and paddle-like in shape closest to their junction with the buccal mass, while distally they become thin and elongate. The radula is yellow and the first few rows are often disproportionally shorter than the remaining rows. The radular formulae below are based on counts made on the widest part of the radula.

<u>Specimen</u>	<u>Radular formula</u>
WAM S12625	35 x 87.0.98 (asymmetrical radula)
WAM S12626	31 x 95.0.95
WAM S12652	34 x 90.0.97 (asymmetrical radula)
WAM S24940	34 x 91.0.91

All the radular teeth are hamate. The innermost lateral tooth is short with a narrow cusp. There is a small ridge along the back of the tooth up to the apex of the hook (Figure 4A). The next few inner lateral teeth (approximately 27) gradually become larger and tend to have shorter hooks than the innermost tooth. The hooks curve laterally toward the centre of the radula. The rest of the middle lateral teeth are the largest (Figure 4B), and the hook extends to about midway down the tooth. All the lateral teeth have the same ridge as the innermost tooth, which becomes higher and shorter toward the outer lateral teeth. The four outermost teeth are reduced slightly in size (Figure 4C). Two of the radulae examined here were asymmetrical and the radula from WAM S12652 lacked the innermost lateral tooth on the left side (Figure 4A). The labial cuticle is smooth, and free of armature, although appears somewhat ‘honeycombed’ under examination with SEM (Figure 4D).

Reproductive system

The base of the penial bulb is often folded and obscured by a vestibular gland (Figure 3B).

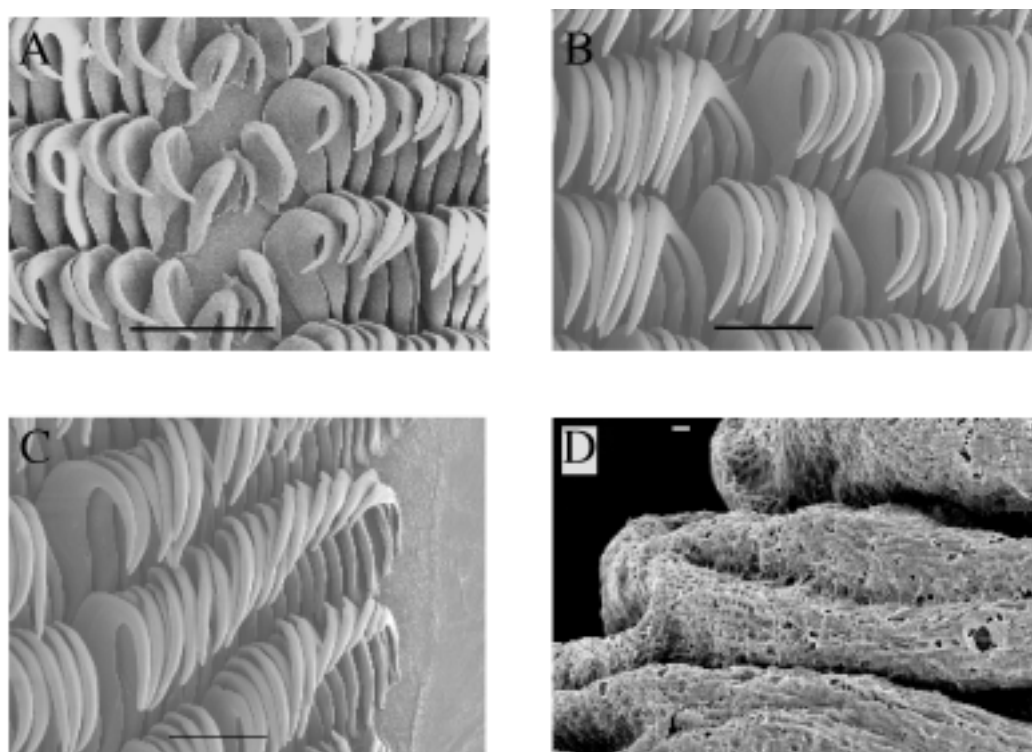


Figure 4 SEM photographs of the radula and jaws of *Aphelodoris gigas*. A. Central region, showing single inner lateral tooth (WAM S12625). Scale bar = 100 μ m. B. Middle lateral teeth (WAM S24940). Scale bar = 100 μ m. C. Outer lateral teeth (WAM S24940). Scale bar = 100 μ m. D. Surface of labial cuticle (WAM S12625). Scale bar = 10 μ m.

This gland is slightly lighter in colour, is tough but not muscular, and while it can appear as a separate pouch in some specimens, it most often overlays and covers the base of the penial bulb. When the vestibular mass is removed (Figure 3C), the penial bulb extends in length. The demarcation of the penial bulb from the ejaculatory duct is not always clearly defined. The ejaculatory duct is highly muscularized, extremely long and coiled upon itself. In some specimens the ejaculatory duct forms a neatly coiled mound, in others, a rather tangled knot which may be intertwined with the retractor muscles joining to the oral tube.

The prostate gland consists of two parts. The first part (P1) that connects to the ejaculatory duct is rather flattened, translucent whitish, and glandular in appearance. In contrast, the second half (P2) appears thin-walled, convoluted, cream-coloured, and is more homogeneous in texture. The two parts are joined by only a very thin membrane in places, and cannot be easily disentangled from each other or the female gland mass during dissection. The ampulla is long and sinuous and is mostly covered by the prostate.

The short, wide vagina is muscular, and narrows as it reaches the bursa copulatrix that is large and spherical. The distal vaginal duct is long and usually looped. It is reasonably wide at first, and tapers as it reaches the receptaculum seminis, which is small and pyriform. The uterine duct joins the distal vaginal duct not far from the receptaculum seminis and enters the female gland mass close to the ampulla.

***Aphelodoris karpa* sp. nov.**

Figures 1B, 3E, 5

Aphelodoris sp. 'red-spotted' Coleman, 2001, p 54–55.

Material examined*Holotype*

One specimen, south-west West Lewis Island, Dampier Archipelago, Western Australia, 20°36.249'S, 116°35.710'E, 7 m, 27/7/00, live length 100 mm, preserved length 52 mm, coll. N. Wilson, WAM S12619.

Paratypes

One specimen, dissected, south-west West Lewis Island, Dampier Archipelago, Western Australia, 20°36.249'S, 116°35.710'E, 7 m, 27/7/00, live length 100 mm, preserved length 33 mm (contracted), coll. P. Hutchings, WAM S12716; one specimen, Enderby Island, Dampier Archipelago, Western Australia, coll. C. Bryce, AM C204865.

Other material

One specimen, dissected, Castle Point, Dorre Island, Shark Bay, Western Australia, 25°07.54'S, 113°06.96'E, 13/5/95, preserved length 62 mm, coll. S. Slack-Smith and M. Hewitt, WAM S24924; one specimen, dissected, NE corner Trimoville Island, Monte Bellos Islands, Western Australia, 20°23'S, 115°33'E, preserved length 35 mm (contracted), coll. C. Bryce and F. Wells, WAM S12653; one specimen, photo only, Cassini Island, Kimberley, Western Australia, coll. C. Bryce.

Distribution

Aphelodoris karpa has been found from Shark Bay, Western Australia, to the Gove Peninsula in the Northern Territory (Coleman, 2001).

Etymology

The species name is taken from the Yindjibarndi language, spoken by indigenous people in the Pilbara region where the Dampier Archipelago is located. It means to ascend, rise up, and fly up and away. It can also mean to carry, transport or take along which describes well the escape response of these animals- swimming up, and being carried along in the current.

Natural History

This species is often present in silty, high current areas. It also is active during the day and can employ the same swimming escape response as *A. gigas*.

External morphology Figure 1B

The body is very soft and fleshy, broadly ovate, with a wide mantle overlap. Living specimens reached a maximum live crawling length of 100 mm. The notum is typically medium to dark brown; sometimes small, dark brown patches are also visible submarginally. The notum usually has one or two major tubercles anterior to the gills in the dorsal midline, and there are always many smaller tubercles present on the rest of the notum. The tubercles

are lighter in colour and usually tipped in orange pigment. The notal margin consists of a thin white or transparent edge that is always present, and a wider, orange submarginal border that is often present.

The rhinophoral pockets are raised cylindrical sheaths, and the branchial pocket has a slightly raised rim. The rhinophoral and branchial pockets also have a thin white or transparent border and usually a submarginal border in orange. The rhinophores have approximately 27–39 lamellae. The club is chocolate brown with a white line up the anterior and posterior sides, and a white tip. Brown pigment is present on the posterior side of the rhinophoral stalks which are translucent white. The five tripinnate gills are chocolate brown and white, and may be tipped in cream or orange pigment. The relative amount of each pigment varies enormously between individuals. In the two living specimens, the anal papilla rim was white, although brown specks were often present on the sides.

The foot often has an orange border that can be solid or broken, and the sides of the foot are translucent white with varying amounts of brown pigment present. The oral tentacles are laterally grooved in both live and preserved specimens, and are usually tipped with orange pigment.

Buccal mass

The muscular portion of the buccal mass is about equal in length to the oral tube. The salivary glands are flattened and paddle-like in shape closest to their junction with the buccal mass, while distally they become thin and elongate. The radula is yellow and the first few rows are often disproportionately shorter than the remaining rows. The radular formulae below are based on counts made on the widest part of the radula.

<u>Specimen</u>	<u>Radular formula</u>
WAM S12619	39 x 98.0.93 (asymmetrical radula)
WAM S24924	36 x 98.0.98
WAM S12653	40 x 110.0.110

All the radular teeth are hamate. The innermost lateral tooth is short with a long, narrow cusp (Figure 5A). It also has a small ridge that projects out of the apex of the hook. The next few inner lateral teeth (approximately 12) gradually become larger, with a somewhat shorter cusp that curves laterally inward (Figure 5D). The rest of the middle lateral teeth are the largest, and the hook extends to about midway down the tooth (Figure 5B). All the lateral teeth have a slight ridge at the apex of the hook that becomes higher and shorter toward the outer lateral teeth. The four outermost teeth are reduced slightly in size (Figure 5C). Occasionally, an aberrant tooth may have a bifid tip (Figure 5C).

Reproductive system Figure 5E

The base of the penial bulb is often folded and obscured by a vestibular gland (Figure 3B). This gland is slightly lighter than the penial bulb in colour, tough, and while it can appear as a separate pouch in some specimens, it most often overlays and covers the base of the penial bulb. The demarcation of the penial bulb from the ejaculatory duct is not always clearly defined, but often forms a small loop where it tapers and becomes less muscular.

The prostate gland consists of two parts. The first part (P1) that connects to the ejaculatory duct is rather flattened, translucent whitish, and more glandular in appearance. The second half (P2), appears more thin-walled and convoluted, cream in colour, and is more homogeneous/uniform in texture. The two parts are joined by only a very thin membrane in places, and cannot

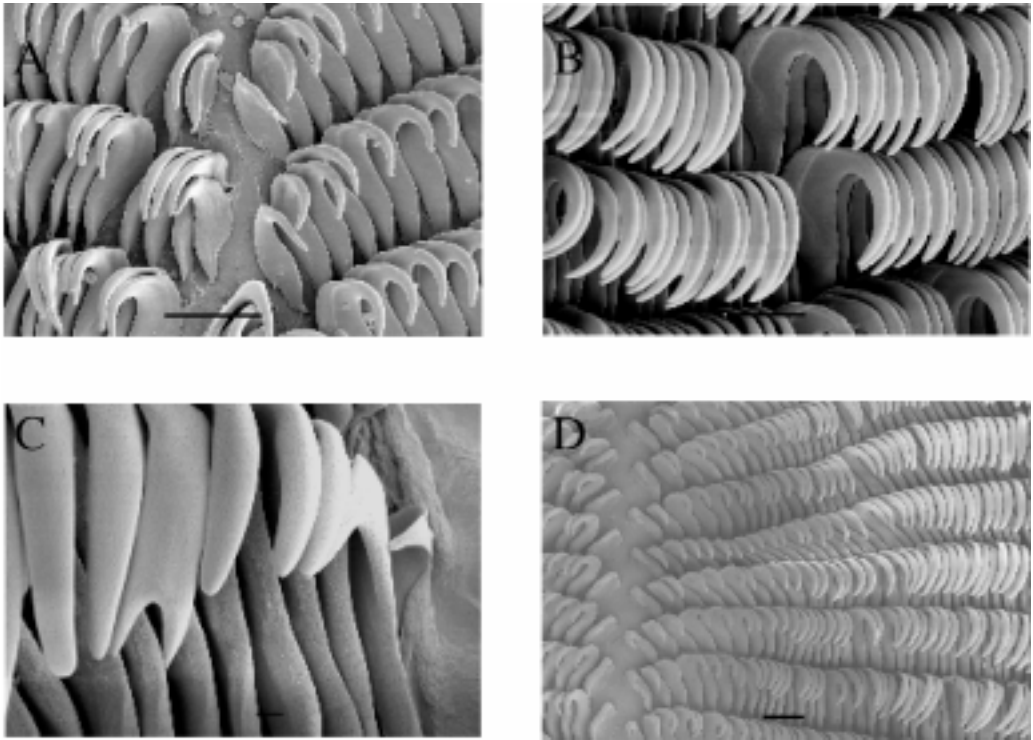


Figure 5 SEM photographs of the radula and jaws of *Aphelodoris karpa*. A. Central region, showing inner lateral teeth (WAM S24924). Scale bar = 100 μ m. B. Middle lateral teeth (WAM S12619). Scale bar = 100 μ m. C. Outer lateral teeth, including an aberrant bifid tooth (WAM S12619). Scale bar = 10 μ m. D. Half row of radula showing an increase in tooth size (WAM S12619). Scale bar = 100 μ m.

be easily disentangled from each other or the female gland mass during dissection. The ampulla is long and sinuous and is mostly covered by the prostate.

The short, wide vagina is also muscular, and leads to the large and spherical bursa copulatrix. The distal vaginal duct is short, and narrows just prior to its connection to the very small, pyriform receptaculum seminis. The uterine duct joins the distal vaginal duct approximately midway between the bursa copulatrix and the receptaculum seminis, and enters the female gland mass close to the ampulla.

Remarks

Both *Aphelodoris gigas* and *A. karpa* have variable external colouration but can be readily separated on the structure of the mantle (heavily pustulose mantle in *A. karpa*, less so in *A. gigas*) and colouration of the notum (no white markings on *A. karpa*, white markings present on *A. gigas*). Internally, differences include the lengths of the ejaculatory duct (very long in *A. gigas* and short in *A. karpa*) and the distal vaginal duct (long in *A. gigas* and short in *A. karpa*). There are also some differences in the proportions of the radula with *A. gigas* having fewer rows and less teeth per row than *A. karpa*.

Both *Aphelodoris gigas* and *A. karpa* have more radular teeth in a half-row and are much larger in body length than any other *Aphelodoris* species. They also lack a swelling at the end of the distal vaginal duct (=large uterine duct of Burn). The only other species that similarly lack this swelling are *A. antillensis* and *A. brunnea*. The morphology of the distal vaginal duct is unknown for *A. luctuosa*, as no drawings of the reproductive system exist.

The presence of a vestibular gland in *Aphelodoris* was explicitly labelled on drawings of the reproductive system for *A. juliae*, *A. greeni*, *A. berghi* and *A. varia* in Burn (1966) but the structure was not discussed. It also appears possible that it is present in *A. rossquickii*, although only a fold in the base of the penial sheath was described. Bergh (1907) illustrated the same structure in his description of *A. brunnea*, although did not identify it as such. A vestibular gland appears absent (no mention in relevant literature) in *Aphelodoris antillensis*, *A. lawsae* and is to date undetermined in *A. luctuosa*. The structure that has previously been identified as a vestibular gland is present in *Aphelodoris gigas* and *A. karpa*, although the morphology is slightly variable. It most often overlays a fold at the base of the penial bulb, although can sometimes form a distinct pouch.

O'Donoghue (1924) recorded *Aphelodoris affinis* Eliot, 1907 from the Houtman Abrolhos Islands in Western Australia. This identification is unlikely to be correct, as Eliot's species is a synonym of the New Zealand endemic, *A. luctuosa* Cheeseman (Burn, pers. comm.). The large size differences between O'Donoghue's specimens and *Aphelodoris gigas* and *A. karpa*, as well as differences in radular formulae suggest they are not conspecific. O'Donoghue's species is most likely one of the presently undescribed *Aphelodoris* from the south coast of Western Australia.

Aphelodoris greeni Burn, 1966

Figures 2B, 6, 7, 9.

Aphelodoris greeni Burn, 1966:342–343, figures 15–17.

Material examined

One specimen, dissected, "The Rock", Bicheno, Tasmania, 14 m, 13/2/00, live length 45 mm, preserved length 28 mm, coll. N. Wilson; two specimens, photo only, Point Puer, Port Arthur, Tasmania, 12 m, 18/2/00, coll. N. Wilson and S. Litherland.

Distribution

This species has previously been recorded only from the Tamar River estuary, Kelso, in northern Tasmania, Australia (Burn, 1966). It is reported here from Bicheno and Port Arthur, eastern and south-eastern Tasmania respectively (Figure 6B).

External morphology

The body is soft, long and has a wide mantle brim. The body colour is cream and there are medial patches of reddish brown (Figure 6A). One specimen from Port Arthur had the majority of the notum covered with reddish brown (Figure 2B). The notal margin consisted of small blocks of brown pigment, alternating with the cream body colour that became white towards the edge. The blocks of brown pigment tended to be darker at the edge with a lighter, more orange pigment toward the centre of the notum. The notum surrounding the medial patches has pale brown reticulate markings (Figure 6A).

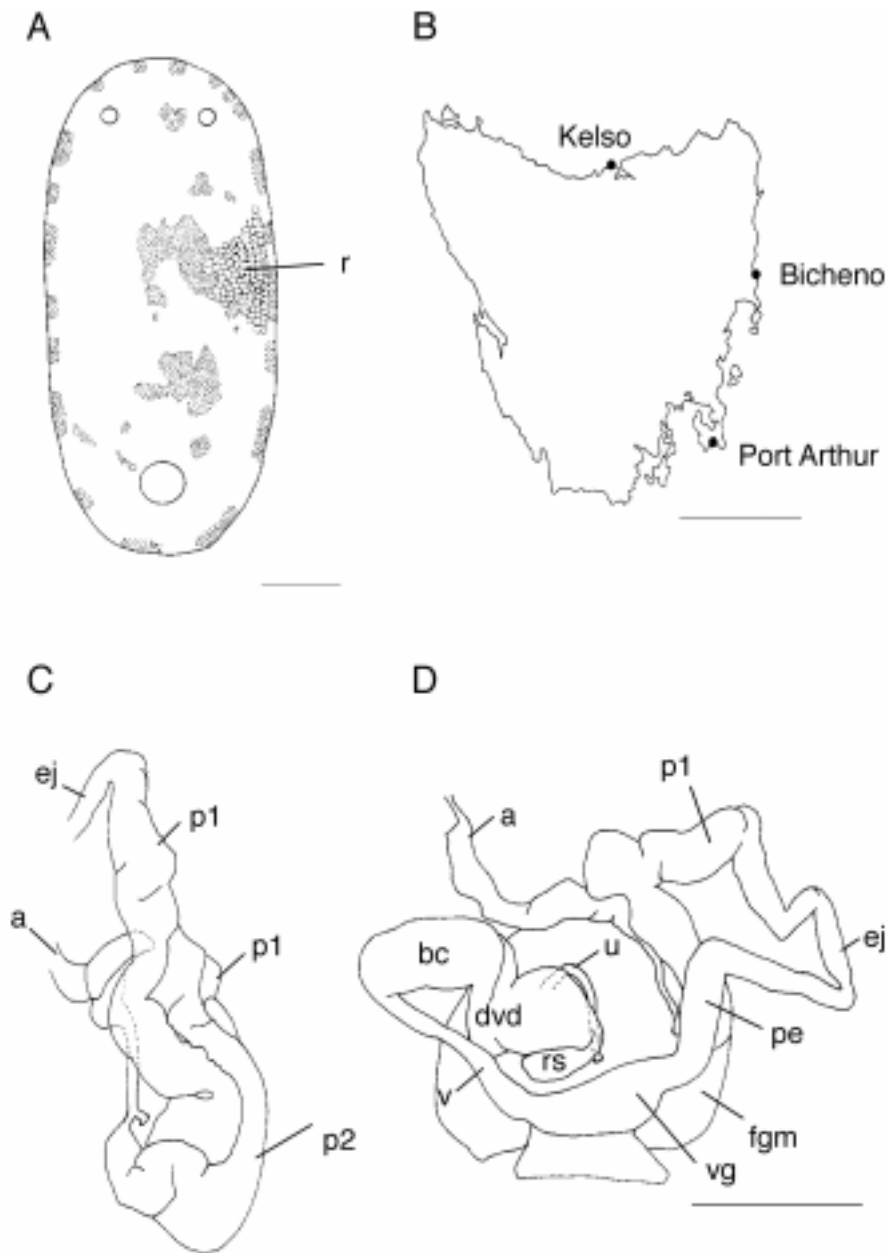


Figure 6 *Aphelodoris greeni* (AM C203862). A. Stylised picture showing distribution of brown pigment on notum; r, represents an example of the reticulation found on the rest of the notum. Scale bar = 5mm. B. Distribution of *A. greeni*, Tasmania, Australia. Scale bar = 100km. C. Arrangement of the two part prostate. Scale bar = 5mm. D. Reproductive system of *A. greeni*. Scale bar = 5mm.
a, ampulla; bc, bursa copulatrix; dvd, distal vaginal duct; ej, ejaculatory duct; fgm, female gland mass; u, uterine duct; pe, penial sheath; p1, first portion of prostate; p2, second portion of prostate; rs, receptaculum seminis; v, vagina; vg, vestibular gland.

The oral tentacles are short, blunt and laterally enrolled, and the gills are situated posteriorly. Both the branchial and rhinophoral pockets have a raised brim, the latter being higher. The pockets are all edged with a fine, white line. The rhinophores have approximately 18 lamellae. The rhinophoral stalks are cream with some light brown pigment overlaid mostly on the posterior side. The clubs are brown with a white line running up the middle of the anterior side, and a light tip. The lamellae are edged in white, giving them a bluish tinge. The gills are also brown but white on the outer face, similarly producing a bluish tinge.

Buccal mass

The salivary glands are long and yellow-brown in colour. They are flattened and paddle-like closest to the buccal bulb but elongate distally. The radula is a reddish brown colour, and in the examined specimen, had a formula of $23 \times 64.0.64$ in the widest part. The innermost lateral tooth has a prominent hump at the apex of the cusp (Figure 7A) and the innermost ten teeth in a half row are reduced in size. The remaining teeth in the row are consistent in size and shape (Figure 7B), with the outermost three or four teeth being slightly reduced in size (Figure 7C). There is a consistent thin, pinched, longitudinal ridge at the base of the teeth, most prominent on the middle lateral teeth (Figure 7D), and absent on the eight outermost teeth.

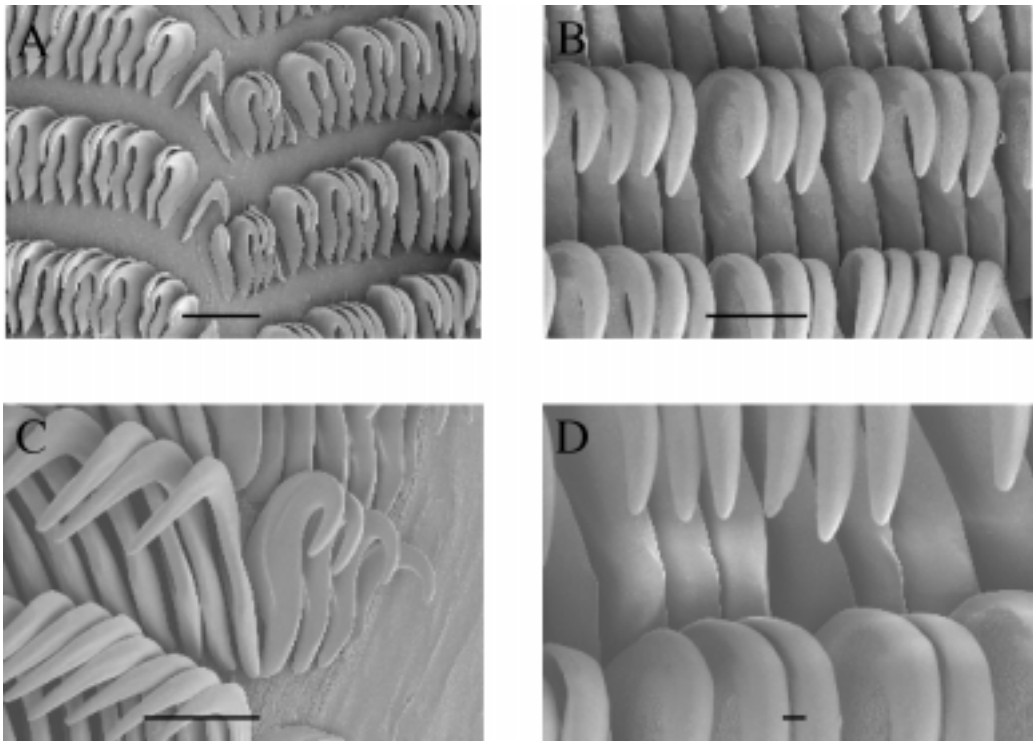


Figure 7 SEM photographs of the radula and jaws of *Aphelodoris greeni* (AM C203862). A. Central region, showing inner lateral teeth. Scale bar = 100 μ m. B. Middle lateral teeth. Scale bar = 100 μ m. C. Outer lateral teeth. Scale bar = 100 μ m. D. Pinched ridge on base of middle lateral teeth. Scale bar = 10 μ m.

Reproductive system Figure 6D

A vestibular gland overlays the base of the penial bulb. The penial bulb is long and muscular and graduates into the short ejaculatory duct. The prostate gland consists of two parts. The first part (P1) that connects to the vas deferens is flattened, pinkish and slightly more glandular in appearance than the second half (P2), which appears slightly more thin-walled and convoluted than P1, and is a light orange colour. The two parts of the prostate could be disentangled from each other and the female gland mass during dissection (Figure 6C). The ampulla is long, reasonably thin and partially covered by the prostate.

The vagina is narrower than the ejaculatory duct, and is not muscular. It leads to the oblong-shaped bursa copulatrix. The distal vaginal duct leading from the bursa copulatrix to the receptaculum seminis is greatly swollen and flattened into a pouch towards the latter end. The swelling is a similar size to the bursa copulatrix. The swelling narrows greatly as it approaches the pyriform receptaculum seminis. The uterine duct joins the mid-part of the swelling and enters the female gland mass a short distance from the ampulla.

Remarks

The material of *A. greeni* examined here closely matches the original description (Burn, 1966). However, there appears to be some variation in the morphology of the vestibular gland. Here it is recorded as a swelling at the base of the penial bulb, whereas the original illustration (Burn, 1966) showed a pouch extending off to the side of the penial sheath. The same variation was recorded within *Aphelodoris gigas* and *A. karpa*. The radular formula of *A. greeni* examined here closely corresponds to Burn's original description. The range extension reported here is of some interest as it indicates that the two species recorded in Tasmania (*A. greeni* and *A. juliae* Burn) are not geographically isolated as reported by Burn (1989). Burn (1966) differentiated these species on colouration, shape of the salivary glands, the size difference of the swelling of the duct leading to the receptaculum seminis, and slight radular differences. Rudman (2000) suggested these species are probably conspecific but provided no evidence. However, the swelling of the distal vaginal duct leading to the receptaculum seminis in *A. greeni* is quite striking and contrasts with the condition described for *A. juliae*. For the present, *Aphelodoris greeni* and *A. juliae* should continue to be treated as separate, distinct species until re-investigation of latter species is carried out.

Burn (1966) indicated that the six species occurring in southern Australia could be separated into two groups according to external colouration. The first group shared a notal reticulate pattern (*A. lawsae*, *A. rossquicki*, *A. varia*) while the other had variable median blotches instead of lines on the notum (*A. julia*, *A. greeni*, *A. berghi*). The specimens of *A. greeni* examined here had both medial blotches and a faint surrounding reticulum of pale brown pigment. While external appearance is useful to help identify live animals, it should be treated with caution for *Aphelodoris* species until the full range of colour variation is well-documented (see Hamann, 1992 for wide variation in *A. antillensis*).

Sperm Ultrastructure

Aphelodoris gigas sp. nov., *A. greeni* and *A. varia* exhibit spermatozoan features characteristic of most heterobranch gastropods: an acrosomal complex (a membrane-bound acrosomal vesicle and a nonmembrane-bound pedestal), a helically ornamented nucleus, a complex, elongate, helical midpiece (consisting of modified mitochondrial derivative – paracrystalline material + matrix material – that envelopes a central axoneme with a glycogen

helix) and an annulus and annular accessory body separating the midpiece from the terminal glycogen piece.

***Aphelodoris gigas* sp. nov.**

Figure 8

Acrosomal complex

The acrosomal vesicle of *A. gigas* is large and spherical, measuring 0.21–0.25 μm ($n=6$) in diameter. It rests in a shallow depression at the anterior end of the short, conical, partially striated, acrosomal pedestal that measures 0.45–0.7 μm in length ($n=6$) (Figure 8A, B). Longitudinal sections through the pedestal reveal fine, parallel striations orientated at approximately 35° relative to the transverse plane (Figure 8B inset). There is a short region of overlap between the pedestal and the nuclear apex.

Nucleus and neck

The nucleus measures 4.90 μm in length ($n=1$) and is rectangular-like in transverse section (Figure 8A, D, G). This may not be a true reflection of the morphology as the nucleus is noticeably fibrous and inflated. There are no obvious helices on the nucleus (Figure 8A), although there are slight bulges that may be interpreted as vestigial helices; their morphology obscured by nuclear inflation. The coarse fibres that surround the axoneme are transversely striated, repeating at 51–57 nm ($n=4$) (Figure 8F). Where the axoneme penetrates the nuclear invagination, the doublets remain distinct from the coarse fibres but in contact with them. This invagination is relatively deep and pointed, and is mostly filled with centriolar derivative (Figure 8A, D, F).

Midpiece

The length of the midpiece is approximately 382 μm ($n=9$, light microscopy). The matrix component of the mitochondrial derivative forms the principal component of two secondary helices both of which diminish in size posteriorly (Figure 8C, E, I). There is one glycogen helix present which is initially small (Figure 8C). However, it enlarges rapidly, then follows a pattern of gradual reduction in size again towards the terminal region of the midpiece (Figure 8E, H, J, L).

Annular complex

The annular complex is composed of the annulus (located immediately posterior to the end of the mitochondrial derivative), the annular accessory body (adjacent to the inner edge of the annulus) and the glycogen piece (following the annular accessory body) (Figure 8K). The axoneme appears to degenerate a short distance prior to the annulus. The glycogen piece measures 1.73 μm in length ($n=1$), excluding the annulus. The glycogen piece is long and conical, and appears to have a higher concentration of glycogen granules in the periphery (Figure 8K, L).

***Aphelodoris varia* (Abraham, 1877)**

Figure 9B, D, H, J, K

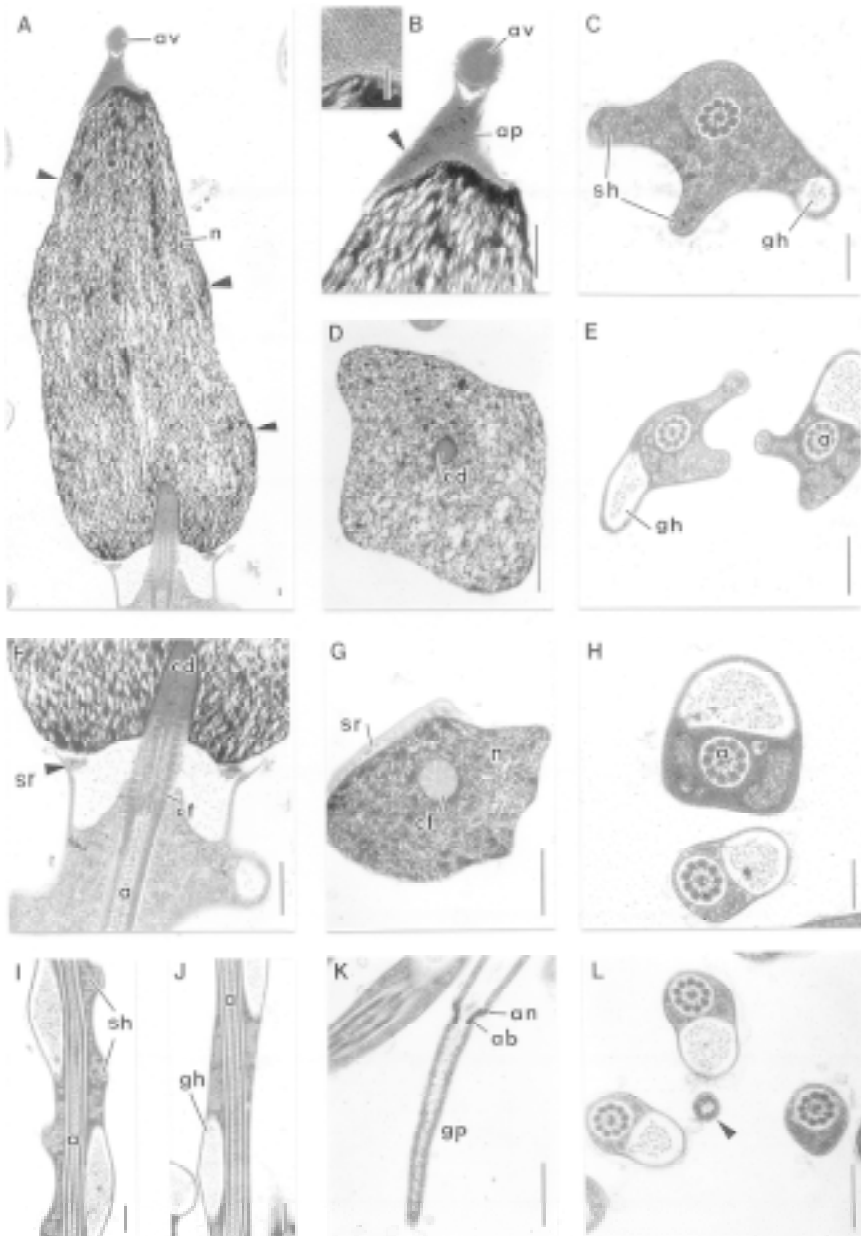


Figure 8 Sperm ultrastructure of *Aphelodoris gigas* sp. nov. (WAM S12652). A. Longitudinal section (LS) of entire acrosome and nucleus. B. LS acrosomal vesicle and pedestal, arrow highlighting angular striations shown in inset. C. Transverse section (TS) of midpiece showing two secondary helices and one glycogen helix. D. TS through nucleus and axonemal invagination. E. TS midpiece. F. LS nucleus/midpiece junction. G. TS nucleus showing axonemal arrangement. H. TS midpiece. I. LS midpiece. J. LS midpiece. K. LS terminal region of spermatozoan. L. LS midpiece and glycogen piece (arrow).

a, axoneme; ab, annular accessory body; an, annulus; ap, acrosomal pedestal; av, acrosomal vesicle; cd, centriolar derivative; cf, coarse fibres; gh, glycogen helix; gp, glycogen piece; n, nucleus; sh, secondary helix; sr, subnuclear ring.

Acrosomal complex

The vesicle of *Aphelodoris varia* is spherical, measuring 0.15 μm ($n=3$) (Figure 9B). It rests in a shallow depression at the anterior end of the short, conical pedestal that measures 0.23–0.28 μm in length ($n=6$) (Figure 9B). Longitudinal sections through the pedestal reveal a region of fine striations orientated at approximately 46° relative to the transverse plane. There appears to be almost no overlap between the pedestal and the nuclear apex (Figure 9B).

Nucleus and neck

The nucleus is 6.40 μm in length ($n=1$) and has a single angular keel although appears circular in transverse section (Figure 9D). The coarse fibres that surround the axoneme are laterally striated, repeating at 51–56 nm ($n=1$). Where the axoneme penetrates the nucleus, there is a relatively deep, pointed invagination that is mostly filled with centriolar derivative.

Midpiece

The length of the midpiece is approximately 153 μm ($n=9$, light microscopy). The matrix component of the mitochondrial derivative forms a secondary helix over a short region of the midpiece (proximal to the nucleus) (Figure 9H). This part of the midpiece also has the matrix arranged in a lamellate condition. There is one glycogen helix present, and it reduces in size and disappears towards the terminal region of the midpiece (Figure 9K).

Annular complex

The glycogen piece consists of a short stub at the terminal region of the spermatozoan (Figure 9K). It measures 0.12–0.15 in length ($n=4$) and just covers the annular accessory body that projects towards the centre of the glycogen piece.

Aphelodoris greeni Burn, 1966

Figure 9A, C, E–G, I

Acrosomal complex

The oblong acrosomal vesicle of *A. greeni* is 0.1–0.13 μm high by 0.18 wide ($n=2$). It rests in a very shallow depression at the anterior end of the acrosomal pedestal. The pedestal is conical in shape and measures 0.38–0.4 μm in length ($n=3$). Longitudinal sections through the pedestal reveal fine, parallel striations orientated at approximately 31° relative to the transverse plane. There appears to be almost no overlap between the pedestal and the nuclear apex.

Nucleus and neck

The nucleus is 6.20 μm in length ($n=1$) and has a single angular keel although it appears circular in transverse section (Figure 9A, C). The coarse fibre periodicity was not determined. Where the axoneme penetrates the nucleus, there is a relatively deep, pointed invagination that is mostly filled with centriolar derivative (Figure 9F, G).

Midpiece

The length of the midpiece is approximately 156 μm ($n=8$, light microscopy). Secondary helices, formed by the matrix component of the mitochondrial derivative, are present over a short portion of the midpiece (proximal to the nucleus) (Figure 9I). This part of the midpiece

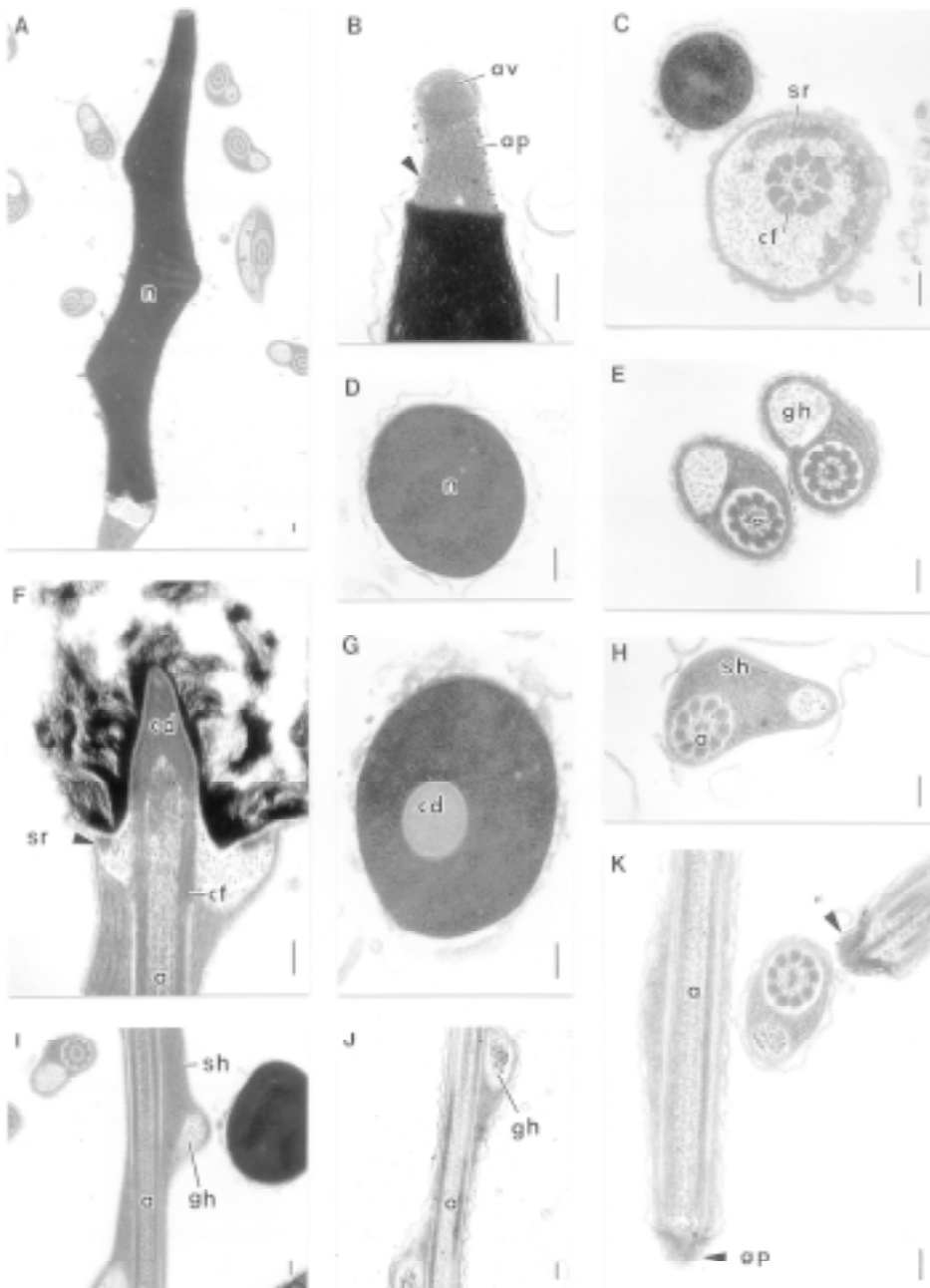


Figure 9 A, C, E-G, I. Sperm ultrastructure of *Aphelodoris greeni* (AM C203862). B, D, H, J, K. Sperm ultrastructure of *Aphelodoris varia* (AM C203863). A. Longitudinal section (LS) through nucleus. B. LS acrosomal complex. C. Transverse section (TS) through nucleus and neck region. D. TS nucleus. E. TS midpiece. F. LS nucleus/midpiece junction. G. TS nucleus and axonemal invagination. H. TS midpiece. I. LS midpiece. J. LS midpiece. K. LS terminal region of spermatozoan, glycogen piece marked by arrows. a, axoneme; ap, acrosomal pedestal; av, acrosomal vesicle; cd, centriolar derivative; cf, coarse fibres; gh, glycogen helix; gp, glycogen piece; n, nucleus; sh, secondary helix; sr, subnuclear ring.

also has the matrix arranged in a lamellate condition. There is a single glycogen helix that reduces in size towards the terminal region of the midpiece (Figure 9E).

Annular complex

This region was not observed.

Remarks

While in all respects these *Aphelodoris* species showed sperm features common to many heterobranchs, there were a number of characteristics shared by all three species that may prove to be widespread in the group. These features include: (1) the presence of a short, conical acrosomal pedestal which is partially striated with fine, angular striations, (2) a deep, pointed invagination of the axoneme into the nucleus, and (3) secondary helices present on the midpiece. While there was few differences between the sperm ultrastructure of *Aphelodoris greeni* and *A. varia*, there were a number of differences between these two species and *Aphelodoris gigas*.

The size of the acrosomal vesicle in *A. greeni* and *A. varia* was very similar while in *A. gigas* it was almost doubled. *Aphelodoris gigas* and *A. varia* shared the spherical shape of the vesicle, while *A. greeni* had a more oblong vesicle. *Aphelodoris gigas* and *A. greeni* showed an acrosomal pedestal similar in length, while that of *A. varia* was shorter. The striations present in the pedestal were always more pronounced in *A. gigas* than in either *A. varia* or *A. greeni*.

The overlap between the acrosomal pedestal and the nucleus was very shallow in *A. gigas*, and negligible in *A. greeni* and *A. varia*. The nuclear morphology of *A. greeni* and *A. varia* is almost identical. It was not possible to ascertain some nuclear details for *A. gigas* examined in the present study, as all nuclei were always fibrous and inflated. The cause of this phenomenon is unknown, but is most likely linked with fixation and spermatozoan age (see Healy and Willan, 1991 for discussion).

The midpiece length of *Aphelodoris varia* and *A. greeni* was almost identical, while *A. gigas* had a midpiece that was more than double these in length. While all species possessed secondary helices, they were present for only a small portion on *A. greeni* and *A. varia* (proximal to the nucleus). This same region exhibited the matrix component arranged in a lamellate condition in *A. varia* and *A. greeni* that was absent in *A. gigas*. In *A. gigas*, these secondary helices were very pronounced and persisted further along the midpiece. The midpiece secondary helices on *A. gigas* are the most prominent known so far for all the Nudibranchia. To date, out of all the genera examined in the Doridina (17), only three chromodorid genera that have been examined possess secondary helices (*Chromodoris*, *Hypselodoris* and *Risbecia*) (Wilson and Healy, 2002), while most aeolids and arminids investigated to date have such structures (Healy and Willan, 1991).

The glycogen piece of *Aphelodoris gigas* was much longer and developed than that recorded for *A. varia*. Unfortunately, this structure was not observed in *A. greeni* for comparison. The length of the glycogen piece varies widely between genera in the Chromodorididae (Wilson and Healy, 2002) although comparisons within genera are yet to be made.

DISCUSSION

The discovery of a two-part prostate in the genus *Aphelodoris* supports its inclusion in the Halgerdidae; all known species of *Halgerda* and *Asteronotus* share a two-part prostate (Fahey and Gosliner, 2001). As this feature never been reported in previously described *Aphelodoris*

species, it prompted investigation of a species from a different geographical region to the two new species. Upon re-examination, *A. greeni* from southern Australia also exhibited a two-part prostate. However, the degree of prostate differentiation in this species was limited to differential pigmentation, and the glandular differences between the two prostate parts was not nearly as evident as that described above for *A. gigas* and *A. karpa*. The differential pigmentation is more easily detectable in material that has been preserved in glutaraldehyde than in formalin, as the former preservative tends to retain more tissue colour. All but the most basal *Halgerda* species have a prostate that clearly shows two distinct glandular types, and this is considered to be an apomorphic trait (Fahey and Gosliner, 1999). Other features that *Aphelodoris* and *Halgerda* share are the presence of dark pigment on the posterior side of the rhinophore stalks, and the tendency of pigment to be easily scratched off the notal surface (*Halgerda*- Shireen Fahey, pers. comm.; *Aphelodoris*- Eliot, 1907; O'Donoghue, 1923; Burn, 1962; pers. obs.)

Bergh (1879, 1880) described and figured a denticle on the inner side of the first tooth in each half row of *Aphelodoris antillensis*. This has never been reported in any subsequent redescriptions (Meyer, 1977; Marcus and Marcus, 1963, 1967; Thompson, 1980; Edmunds and Just, 1985; Hamann, 1992) nor found in any other *Aphelodoris* species. Bergh's (1880) drawings clearly show an inner denticle; therefore in agreement with Burn (1966) I also conclude that Bergh's specimen possessed an aberrant radula. Similar to other *Aphelodoris*, the two new species described in the present account have a prominent ridge or flange along the inside edge of all the teeth. In these species, the ridge is most obvious in the innermost tooth, less obvious in the lateral teeth close to the middle, although becomes more prominent closer to the outer lateral teeth.

Although the sperm morphology of only relatively few dorids is known to date, the data collected here for *Aphelodoris* suggests some similarity to the sperm of *Asteronotus cespitosus* and *Sclerodoris* cf *apiculata* (Healy and Willan, 1991). Both these species have an acrosomal complex that resembles *Aphelodoris* in size and shape although neither has been reported to possess striations in the pedestal. *Sclerodoris* cf *apiculata* also has weak nuclear keels that correspond to those seen in *Aphelodoris varia* and *A. greeni*. The nucleus of *Asteronotus* was recorded as appearing fibrous and inflated, similar to the condition reported here for *A. gigas*. Interestingly, Healy and Willan (1991) also reported that the matrix component of midpiece in *Sclerodoris* cf *apiculata* was arranged in a lamellate condition proximal to the nucleus. This was also reported in the present study for *A. varia* and *A. greeni*. Aspects of sperm morphology appear to have great potential for phylogenetic characters, although many more dorid genera need to be investigated before specific hypotheses can be generated.

While relatively little is known about the ecology of *Aphelodoris*, swimming has been reported previously in *Aphelodoris antillensis* (Thompson, 1980), and *A. brunnea* (Gosliner, 1987), and in an undescribed species from Victoria, Australia (Burn, pers. comm.). Both *A. gigas* and *A. karpa* also swim vigorously when provoked, but will habituate to stimulus quickly. This suggests swimming is primarily used as a defensive/escape response although it may also aid dispersal. It is not certain whether other species of *Aphelodoris* also swim. *Aphelodoris varia* (Abraham) is commonly encountered on the east coast of Australia but no swimming responses have ever been recorded.

Edmunds and Just (1985) reported that the egg mass of *Aphelodoris antillensis* is a cone shaped, descending spiral with two rows of eggs. From their diagram, the eggs appear approximately 400 µm in diameter and are certainly large enough to suggest direct

development. In Puerto Rico, Marcus and Marcus (1970) counted about 320 eggs in a spiral spawn mass of *Aphelodoris antillensis*. The egg mass of an undescribed *Aphelodoris* sp. from tropical Western Australia is a spiral egg mass that contains orange ova (Coleman, 2001, p28). It too, contains few large eggs, and this may be typical of the genus. If direct development is present, the resulting restricted dispersal may help explain some of the external variation present. *Aphelodoris varia* has been noted to engage in mating congregations that can involve up to twenty individuals (Coleman, 1981), although no other details of its reproductive biology is known.

The genus *Aphelodoris* shows several species-groups in Australia and appears to have undergone relatively large speciation events in this region, compared with the rest of the world. Only *Aphelodoris antillensis*, *A. brunnea*, and *A. luctuosa* are found outside Australian waters, with the latter being found in nearby New Zealand. This indicates that 72% of described species are endemic to Australia. The group also predominates in temperate waters – the only tropical representatives being *Aphelodoris antillensis* and the two species described here, *A. gigas* and *A. karpa*.

The following species of *Aphelodoris* Bergh are currently recognized as valid:

Aphelodoris antillensis Bergh, 1879. Bermuda and the Caribbean.

Bergh, 1879: 108. Type by monotypy. Bergh, 1880: plate XVI, figures 12–18; Marcus and Marcus, 1963:32, figures 40–42; Marcus and Marcus, 1967:92, figures 120–121; Marcus and Marcus, 1970:67, figure 117; Meyer, 1977: 301; Thompson, 1980: 89, figures 11b, 12a–b; Edmunds and Just, 1985:55, figure 3; Hamann, 1992.

Chromodoris bistellata Verill, 1900; Clark, 1984; Jensen and Clarke, 1985.

A. varia (Abraham, 1877). Southern QLD, NSW and VIC, Australia.

Burn, 1966:347, figure 21–22, 33–34; Coleman, 1981:95, 35 (bottom); Coleman, 1989:23; Debelius, 1998:258; Coleman, 2001:54.

Doris variabilis Angas, 1864:44, plate 4, figure 1; non Kelaart, 1859.

Doris varia Abraham, 1877:209.

Doris praetenara Abraham, 1877:258, plate 30, figures 10–12.

Archidoris varia Burn, 1957:20.

Asteronotus varius Iredale and McMichael, 1962:93.

A. luctuosa Cheeseman, 1882. New Zealand.

Cheeseman, 1882:213; Odhner, 1934:269; Coleman, 2001:54.

A. cheesemani Eliot, 1907:342.

A. affinis Eliot, 1907:343.

A. brunnea Bergh, 1907. Cape Point to East London, South Africa.

Bergh, 1907:60, plate 12, figure 1; Gosliner, 1987:66.

A. berghi Odhner, 1924. VIC and TAS, Australia.

Odhner, 1924:53; Burn, 1962:102, figures 6–7; Burn, 1966:343, figures 18–20, 32; Burn, 1989:768, plate 53.1; Coleman, 2001:53.

A. luctuosa Bergh, 1905:75, plate 5, figures 26–32, plate 6, figures 1–2, non *Doris luctuosa* Cheeseman, 1882.

A. lawsae Burn, 1966. Nuyts Archipelago to St Vincent Gulf, SA, Australia.

Burn, 1966:337, figures 7–9, 30; Burn, 1989:768, plate 53.2–3; Coleman, 1989:24; Coleman, 2001:54.

- Archidoris varia* Basedow and Hedley, 1905:150, plate 5, non *Doris varia* Abraham, 1877:209; Cotton and Godfrey, 1933:100, plate 2, figures 6–7, plate 4, figures 11–13.
- A. rossquicki* Burn, 1966. Cape Otway to Wilsons Promontory, VIC, Australia.
Burn, 1966:339, figures 10–11, 31.
- Archidoris varia* Odhner, 1934:270, non Abraham, 1877:209.
- A. juliae* Burn, 1966. Huon Channel, south-east TAS, Australia.
Burn, 1966:341, figures 12–14.
- A. greeni* Burn, 1966. Kelso to Port Arthur, TAS, Australia.
Burn, 1966:342, figures 15–17.
- A. gigas* sp. nov. Dampier Archipelago to Kimberleys, WA, Australia.
Aphelodoris sp. Wells and Bryce, 1993:96–97 (112); Debelius, 1998:258.
- A. karpa* sp. nov. Shark Bay, WA, to Gove, NT, Australia.
Coleman, 2001: 54 (lower right), 55 (upper left).

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