

***Dendrodoris arborescens* (Collingwood, 1881) (Mollusca: Nudibranchia): larval characteristics reveal a masked porostome species**

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Abstract – The nudibranch *Dendrodoris arborescens* (Collingwood, 1881) has hitherto been (1) mistaken for the sympatric species *D. nigra* (Stimpson, 1855), which is similar in appearance; or (2) considered as a colour form of *D. fumata* (Rüppell and Leuckart, 1828). However, our investigations linking larval form, morphology and colour pattern, clearly show that *D. arborescens* is a separate valid species with its own set of unique characteristics. This result increases the number of *Dendrodoris* species known from Australian to thirteen, highlights the useful nature of larval features to species identification and contributes to the resolution of a previously confusing species complex.

INTRODUCTION

For many years the genus *Dendrodoris* Ehrenberg 1831 has been the subject of considerable scientific interest. This results from its relatively common occurrence (Valdés *et al.* 1996; Brodie *et al.* 1997), internal (*de novo*) production of toxic chemicals (Cimino *et al.* 1983; Karuso 1987, Fontana *et al.*, 1999), unique functional biology and microstructure (Klussmann-Kolb and Brodie 1999; Wägele *et al.* 1999), suctorial feeding method (Young 1969) and intriguing phylogenetic placement (Valdés and Gosliner 1999; Wägele and Willan 2000; Wollscheid-Lengeling *et al.* 2001; Valdés 2003). Several widespread members of the genus e.g., *Dendrodoris nigra* (Stimpson 1855) and *D. grandiflora* Rapp, 1827 are also well known for their considerable colouration variation (see Edmunds 1971; Thompson 1975; Rose 1985; Valdés *et al.* 1996). In Australia, this latter attribute in *D. nigra* and its sibling species *D. fumata* have masked the existence of a less common but very similar looking sympatric species *D. arborescens*.

Rose (1985) studied in detail the egg masses and embryonic development of “colour variants” of the nudibranch *D. nigra* along the coast of New South Wales. He documented at least two forms that were clearly distinguished by larval morphology. However, no anatomical or morphological investigation of the colour variants was undertaken nor were associated voucher specimens designated, making re-evaluation difficult.

Several years later Brodie *et al.* (1997) investigated the external morphology and internal anatomy of *D. nigra* and separated it from the sympatric species *D. fumata* (Rüppell and Leuckart, 1828) on the basis

of external appearance (colour pattern, gill structure) and features of the reproductive system (vestibular gland). Three colour forms of *D. fumata* (grey, black and orange/red) were identified during their study (see Brodie *et al.* 1997). Although these forms were found to be externally dissimilar, they were published together under the name *D. fumata* because of their indistinguishable internal anatomy and apparent overlapping egg mass characteristics.

The current manuscript will thus provide (a) additional evidence for the continued separation of *D. nigra* and *D. fumata*, (b) clear evidence for the separation of the grey and black forms of *D. fumata*, and (c) recognition that *D. fumata* (black form) is a junior synonym of *D. arborescens*. Data from Rose (1985) and Brodie *et al.* (1997) are reassessed and combined with new data obtained from fresh observations of the colour pattern, morphology and larval features of living specimens. This is undertaken for the three species (and forms) discussed above, except for the orange form of *D. fumata* that was not found during fieldwork for the present manuscript in Spring 2003. As discussed by Brodie *et al.* (1997: 414–5) the black form of *D. fumata* is a synonym for *D. arborescens* Collingwood (1881) and we now follow their statement that “if the black form of *D. fumata* is clearly distinguished as a valid species we have no hesitation in using Collingwood’s name for it”. Two good colour photographs of *D. arborescens* are found in popular books (Willan and Coleman 1984 photograph 128 page 41; Wells and Bryce 1993 photograph 182 page 141) under the name *D. nigra*, and they both depict specimens from Western Australia.

Traditionally in opisthobranch taxonomy, as in

other invertebrate groups, both external morphology and internal anatomy has been the primary basis used to separate species. However, several studies (e.g., Eckelbarger and Grassle, 1987, Calado and Urgorri, 2002) highlight the useful nature of information such as size and shape of eggs and larval characteristics to species resolution. The following study ensues that lead.

MATERIALS AND METHODS

Adult specimens of *D. nigra* (23 specimens), *D. fumata* [= *D. fumata* (grey form) of Brodie *et al.*, 1997] (6 specimens) and *D. arborescens* [= *D. fumata* (black form) of Brodie *et al.*, 1997] (7 specimens) were collected intertidally from two sites, Rowes Bay, Townsville (146°50', 19°10') and Cockle Bay, Magnetic Island, Townsville (146°48', 19°12'), Queensland, Australia, between September and November 2003. Animals were transferred to the laboratory and kept in 2.6 L containers on a 4,500 L continuous-flow closed water system at 26–27°C with a photoperiod 13h light/11h dark. *Ad libitum* feeding was provided with the sponge *Tedania* sp., collected from the same sites, attached to small boulders or pieces of dead coral. Nudibranchs were inspected daily and when spawning occurred, egg masses were detached from the substrata and transferred to isolated beakers with Millipore

filtered seawater. Once hatched, larvae were raised at a concentration of 5 larvae/ml and fed on a microalgal mixture of 1:1 *Isochrysis* sp. and *Pavlova* sp. (about 5000 cells/ml). Whenever undivided zygotes were found in fresh laid spawn masses, a sample was taken to measure their average diameter. Measurements on ten different zygotes were taken using a high-powered microscope and calibrated stage micrometer. The same measurement procedures were undertaken for the larval shells during different periods of development. Additionally, observations on larval behaviour (> 10 for each species) were also recorded.

The thirty-six adult specimens used to produce larvae were compared and matched to the species and form descriptions of Brodie *et al.* (1997). In addition, one specimen of *D. arborescens* from Rowes Bay (~ 60 mm live length) collected intertidally by John Collins on August 14th 1994 was examined by resin histology (see Wägele *et al.* 1999 and Brodie 2001 for details of the methodology used) to provide confirmation of reproductive system components. A voucher specimen of *D. arborescens* will be lodged in the Museum of Tropical Queensland (MTQ), Townsville. An additional specimen (QM MO18868) was lodged at MTQ, under the name *D. fumata* (black form), by Brodie *et al.* (1997).

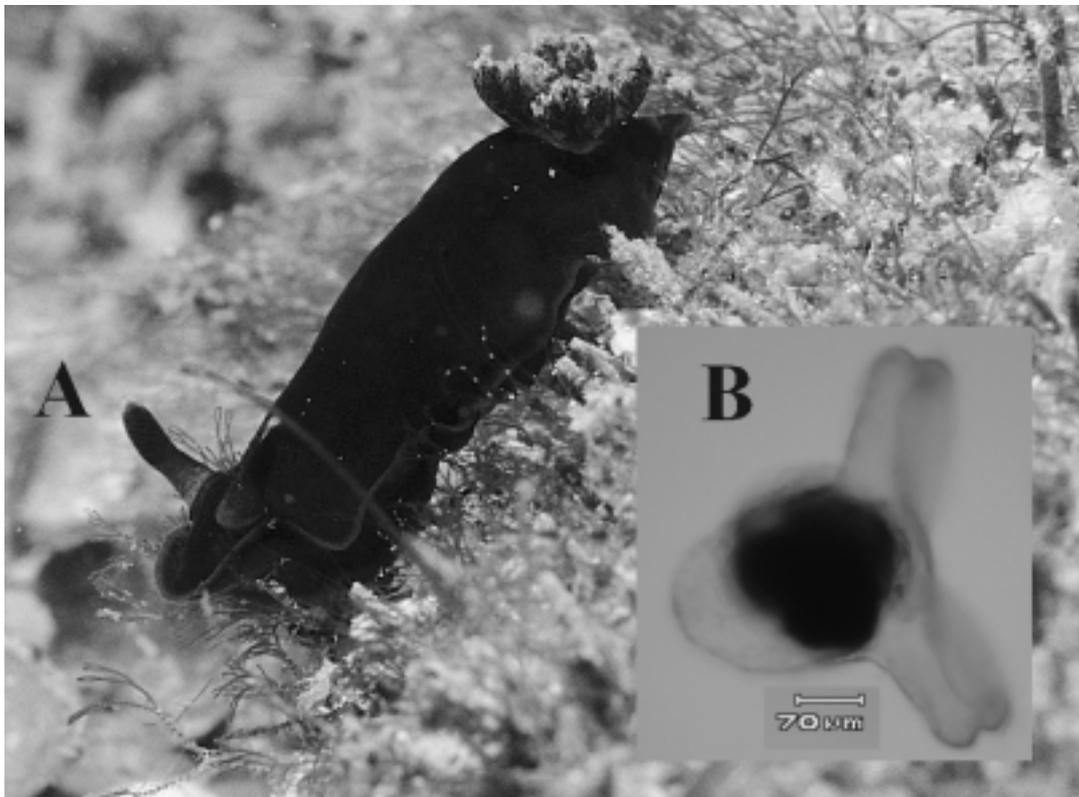


Figure 1 A, living specimen of *D. arborescens* showing the low body profile, elongate body, smooth notum with wavy margins and pale apices to gills. Note that the small pale dots on the notum are artefacts (grains of sediment), photograph by Clay Bryce; B, a living veliger larvae of *D. arborescens*.

RESULTS

Colour pattern and morphology

All specimens examined conformed to the previous, species and form descriptions of Brodie *et al.* (1997) and these details plus the extensive associated synonymies will not be repeated here. However, it is useful to reiterate the confirmed external appearance of *D. arborescens* (that is consistent irrespective of size) and repeat several contrasting features of *D. nigra* because the two species can be so easily mistaken. Individuals of *D. arborescens* are uniformly black, have a low body profile, an elongate body and a smooth notum often with wavy margins (Figure 1A). The dorsum lacks white spots (note dots in Figure 1A are artefacts) or any other significant markings except for a marginal red border (rather than a submarginal band) to the notal edge. The black or dark grey gills always have pale apices and the gill plumes spreads outward in resting individuals. In contrast the individuals of *D. nigra* used in the current study, were more variable in colour with a red sub-marginal band to the notum sometimes but not always present and white spots to the notum predominately but not always present (see Brodie *et al.* 1997 Figures 1A and B). Additionally in living, non-stressed, resting individuals the individual gill plumes are cupped inwards.

Reproductive system anatomy

The histological analysis of the reproductive system of *D. arborescens* found a vestibular gland located close to the distal oviduct. This gland is embedded in the body wall. The vestibular gland was found to have the same unusual cellular structure as already well documented for other members of the genus.

Spawn and egg size

A comparison of spawn and egg size characteristics, as determined in the current study,

is provided in Table 1. *Dendrodoris nigra* is by far the species with the smallest eggs (74–81 μm), followed by *D. fumata* (117 μm). Accordingly, *D. arborescens*, with bigger eggs (126–137 μm), has the smallest number of eggs per mass (~6,500 eggs/mass). The three species present a similar time to hatching (5–8 days) at the studied temperatures, but again they can be ranked by the size of the larval shell at hatching.

Larval characteristics

The larvae of *D. arborescens* (Figure 1B) have a densely pigmented, but still translucent, cup-like shell (Figure 2A), deprived of operculum. The pigmentation gives the shell a brownish tonality. The bilobed velum is very broad, about 260–280 μm wide, and cannot be retracted into the shell. The larval shell is discarded by the veliger larvae about 6 days after hatching however at this stage individuals still maintain a planktonic behaviour and can be seen to ingest provided microalgae. Larvae of this species were kept alive in the laboratory for up to 30 days. Eye development was visible quite late in the larval development sequence but this could be an artefact, since the larva is very opaque.

In contrast to *D. arborescens*, both *D. fumata* and *D. nigra* have a transparent, spirally coiled, typical type 1 (*sensu* Thompson, 1961) larval shell (Figures 2B and 2C1 respectively) with an operculum. The velum of an individual larva can be totally retracted into the shell in both species. Veliger larvae of *D. nigra* hatch 4–5 days prior to eyespot development. When larvae were fed, microalgae were easily visible in the larval gut and considerable shell growth (135 to 269 μm) was observed in the 30 days they survived in the laboratory (Figure 2C2) (growth rate = 4.5 $\mu\text{m}/\text{day}$). In comparison, veligers of *D. fumata* were larger than those of *D. nigra* at hatching and already had eyespots. Larvae of this species were also fed and kept alive for 30 days, however during this period no significant growth

Table 1 A comparison of the spawn, egg size and larval shell characteristics found among the three species of *Dendrodoris* examined in the current study.

Species	<i>D. arborescens</i>	<i>D. fumata</i>	<i>D. nigra</i>
Uncleaved egg diameter range (μm) (number of eggs) (number of spawn masses)	126 \pm 4.6 – 137 \pm 4.7 (n = 30) (n = 15) (3)	117 \pm 4.8 (n = 7) (1)	74 \pm 2.8 – 81 \pm 1.7 (n = 10) (n = 30) (8)
Estimated no. eggs/mass (number of spawn masses)	6,528 – 7,296 (2)	54,945 (1)	8,448 – 169,344 (23)
Time to hatch in days (26–27°C) (number of spawn masses)	6–7 (3)	6–8 (3)	5–7 (6)
Early hatched larval shell length (μm) (number of larvae)	151 \pm 8.4 (6)	220 \pm 13.9 (11)	135 \pm 6.0 (13)

Table 2 A comparison of colour pattern, gill structure, egg mass details and larval characteristics recorded from previous studies in comparison to the current study.

SPECIES	FEATURE	Gohar and Soliman 1967	Rose 1985	Brodie <i>et al.</i> 1997	CURRENT STUDY
<i>D. arborescens</i>	Colour pattern	–	As <i>D. nigra</i> Form 2 jet black or red-rimmed mantle	As <i>D. fumata</i> (black form) uniform black, no notal markings except marginal red border to mantle margin. Gill apices pale.	Uniform black, no notal markings except marginal red border to mantle margin. Gill apices pale.
	Reproductive (vestibular gland)	–	not dissected or sectioned	Not seen	Present, embedded in body wall
	Gill structure	–	not recorded	Individual plumes spread outwards in resting individual	Individual plumes spread outwards in resting individual
	Larval shell features	–	dark pigmentation on shell, no operculum	–	larval shell with brown pigment; no whorl; no operculum
	Time for hatching (Days) [temperature °C]	–	9.0 – 9.6 [22–23]	–	6–7 [26–27]
	Shell length at hatching (µm)	–	jet black – 153 red-rimmed – 144	–	151 (6)
	Eggs per spawn mass	–	67,584 (average)	8,500 – 25,500 (4)	6,528 – 7,296 (2)
	Capsule size (µm)	–	jet black – 163 red-rimmed – 140	166.2 – 224.0 (4)	–
	Egg size (µm)	–	121 – 129	133.6 – 159.0 (3)	126 – 137 (3)
<i>D. fumata</i>	Colour pattern	adults – translucent grey	–	Translucent grey	Translucent grey
	Reproductive (vestibular gland)	Organ system dissected but vg not seen	–	Not seen	Present embedded in body wall, see Brodie 2001.
	Gill Structure	semi-circular collar open posteriorly	–	Individual plumes spread outwards in resting individual	Individual plumes spread outwards in resting individual

	Larval Shell features	Transparent smooth larval shell (Type B) and operculum	-	-	Larval shell transparent; with whorls and operculum
	Time for hatching (Days) [temperature °C]	5.5 [28] – 17 [17]	-	-	6–8 [26–27]
	Shell length at hatching (µm)	157	-	-	220 (11)
	Eggs per spawn mass	3,344 – 165,490	-	11,000 – 122,500 (9)	54,945 (1)
	Capsule Size (µm)	110 – 135	-	154.1 – 184.4 (9)	-
	Egg size (µm)	100 (average)	-	125.2 – 150.9 (6)	117 (1)
<i>D. nigra</i>	Colour pattern	-	As <i>D. nigra</i> Form 1 white-speckled, brown-black or grey	Very variable –generally black or dark, often with white spots, sometimes with a red sub-marginal band to the notal edge. Gill plume uniformly dark in colour.	Body black to dark grey, often with white spots, sometimes with a red sub-marginal band to the notal edge. Gill plumes uniformly dark in colour.
	Reproductive (vestibular gland)	-	not dissected or sectioned	Present, annexed to distal oviduct	Present, annexed to distal oviduct
	Gill structure	-	not recorded	Gill plumes cupped inwards	Gill plumes cupped inwards
	Larval shell features	-	transparent larval shell	-	Larval shell transparent with whorls and operculum
	Time for hatching (Days) [temperature °C]		9.0 – 9.6 [22–23]		5–7 [26–27]
	Shell length at hatching (µm)	-	114	-	135 (13)
	Eggs per spawn mass	-	120,000 – 242,000	13,500 – 199,000 (34)	8,448 – 169,344 (23)
	Capsule size (µm)	-	121	98.7 – 137.8 (36)	-
	Egg size (µm)	-	75	66.3 – 93.4 (35)	74 – 81 (8)

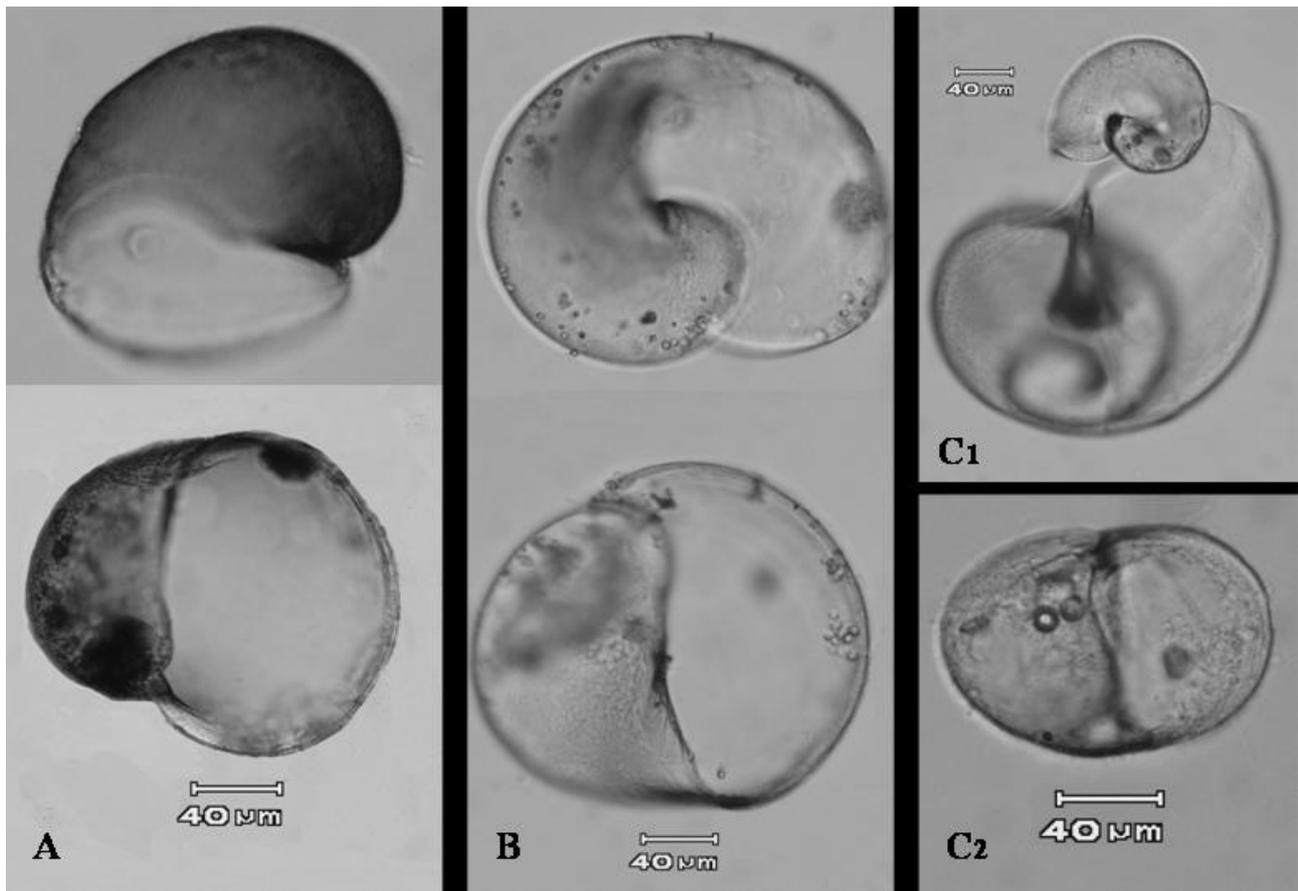


Figure 2 A, the pigmented larval shell of *D. arborescens* photographed from two different angles. B, the transparent larval shell of *D. fumata* photographed from two different angles. C, the transparent larval shell of *D. nigra* also photographed from two different angles plus also showing (in the top photograph) two different stages of development; C1 = the shell of a recently hatched veliger compared to the shell of a veliger fed for 30 days, C2 = the shell of a recently hatched veliger from a different angle. Note the different magnifications used between C1 and C2.

in larval shell length was observed even though microalgae were visible in the gut.

DISCUSSION

Contrary to the findings of Brodie *et al.* 1997, which did not employ histological analyses, a vestibular gland was found to be present in the reproductive system of *D. arborescens*. However, this gland was embedded in the body wall, which explains its previous lack of detection by dissection alone. The vestibular gland of *D. arborescens* was found to have the same unusual cellular structure as already well documented for *D. nigra* and *D. fumata* (grey form and orange/red form) by Wägele, *et al.* (1999, Figure 4A and B), Klussmann-Kolb and Brodie (1999, Figure 1A) and Brodie (2001: 102).

As also discussed by Brodie *et al.* (1997), under the name *D. fumata* (black form), adults of *D. arborescens* are easily confounded with *D. nigra*. Nevertheless, when one looks at the larval morphology, as already pointed out by Rose (1985), these two species are easily distinguishable (Table

2). Rose (1985) provides a detailed description of the larval development of what he called forms 1 and 2 of *D. nigra*. Form 2 is further divided into “red-rimmed mantle” and “jet-black” animals and we wonder why this author did not go the obvious step further of revalidating the specific name *D. arborescens*. All of the main features of spawn masses and larval characteristics presented by Rose (1985) match our results, except in respect to time to hatching and the fact that we did not find, and therefore did not investigate, any “jet-black” animals. A considerably longer hatching time for both forms is found in the work of Rose (1985) and this difference is certainly due to the lower temperature conditions found in New South Wales (central eastern Australia), which were also adopted in the laboratory (22–23°C). Form 1 is the true *D. nigra* whereas form 2 should be attributed to *D. arborescens*. Nevertheless, both “red-rimmed mantle” and “jet-black” sub-forms have the same, very distinctive, larval shell, differing only in some meristic characters. Besides that, the larval data for form 2 “red-rimmed mantle” were obtained only

from a single egg mass. Rose (1985) also refers to the resemblance of this unusual larval shell to that of the dorid *Aegires punctilucens* as described by Thiriot-Quievreux (1977). More recently, the same type of shell was found in *Aegires albopunctatus* by Goddard (2001). Goddard (2001) and Gibson (2003) also discuss the presence of this type of larval shell in the notaspideans, especially the lack of operculum. In addition, we are not aware of any other species that sheds its shell and then lives for so long as a shell-less larva.

Although limited, the data that we were able to obtain for *D. fumata* are in the same range of those presented by Gohar and Soliman (1967) and Brodie *et al.* (1997), the latter as *D. fumata* grey form. Like Brodie *et al.* (1997), we did not observe any opaqueness in the larval shell of *D. fumata* as previously documented for Red Sea individuals by Gohar and Soliman (1967). The larval shells of *D. fumata* and *D. nigra* were always transparent in the current study.

Based on external features, colour pattern, gill structure and larval characteristics, we conclude that this species is *D. arborescens*, whose type locality is Hanin Strait, in Formosa Strait, China, and which was previously not known to be found along the Australian coastline. Its recognition here raises the number of known members of *Dendrodoris* in Australia (see Brodie 2004) to thirteen and strongly reinforces the useful nature of larval biology to the resolution of confounded species complexes.

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