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COLOUR PATTERN VARIATION IN THE TROPICAL FLATWORM, *PSEUDOCEROS* (PLATYHELMINTHES: POLYCLADIDA), WITH DESCRIPTIONS OF THREE NEW SPECIES

L. J. Newman and **L. R. G. Cannon**

ABSTRACT. - The majority of *Pseudoceros* can be distinguished by their distinct colour patterns (Newman & Cannon, 1994a). However, three new species all show a remarkable similarity in colour to *P. bimarginatus* Meixner, 1907, and close examination revealed distinct, though subtle, differences between these species. We describe the importance of accurate documentation of colour patterns in Pseudocerotidae and discuss the biological significance of colour patterns in polyclads.

INTRODUCTION

Although free-living polyclad flatworms are conspicuous inhabitants of coral reefs (Hyman, 1954; 1959; Prudhoe, 1985; 1989; Newman & Cannon, 1994a, b) there are few comprehensive taxonomic accounts of these worms from tropical waters. The most flamboyantly coloured marine flatworms belong to the genera *Pseudoceros* and *Pseudobiceros* which are commonly found in waters of the Great Barrier Reef, Australia and eastern Papua New Guinea (Newman & Cannon, 1994a, b).

Newman & Cannon (1994a) determined that from the over 150 *Pseudoceros* species recognised by Faubel (1984) and Prudhoe (1985, 1989) only 26 can reliably be placed. We have since described a further 18 new species bringing the total number of *Pseudoceros* species to 44 (Newman & Cannon, 1994a, Newman et al., 1994). Intraspecific homogeneity of the reproductive structures was found in *Pseudoceros* alone, and colour patterns can be used to separate species (Newman & Cannon, 1994a).

In the past pseudocerotid polyclads have been poorly represented in collections and, since most animals loose their colour and pattern on fixation, reliable documentation of colour patterns has been rare. Newman & Cannon (1995) designed a new fixation technique for pseudocerotids which preserves the specimens intact and partially retains their colour patterns.

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This study describes three new species of *Pseudoceros* from the Great Barrier Reef and eastern Papua New Guinea which closely resemble a common species, *P. bimarginatus*. The colour of the marginal bands, however, varies. The importance of accurate colour documentation and its biological significance in Pseudocerotidae is discussed.

MATERIALS AND METHODS

Polyclads were hand collected from under boulders at the reef crest or under ledges on the reef slope by SCUBA from Heron Island (23°27'S, 151°55'E) and One Tree Island (23°30'S, 152°05'E) southern Great Barrier Reef (GBR) and Madang (5°14'S, 145°45'E) eastern Papua New Guinea. Flatworms were kept in separate sampling containers and brought back to the laboratory live, and retained in 1 litre plastic ice cream containers. Worms were photographed in situ or in the laboratory (see Newman & Cannon, 1994a). Unless otherwise stated all animals were collected during the day and photographed by L. J. Newman and A. E. Flowers.

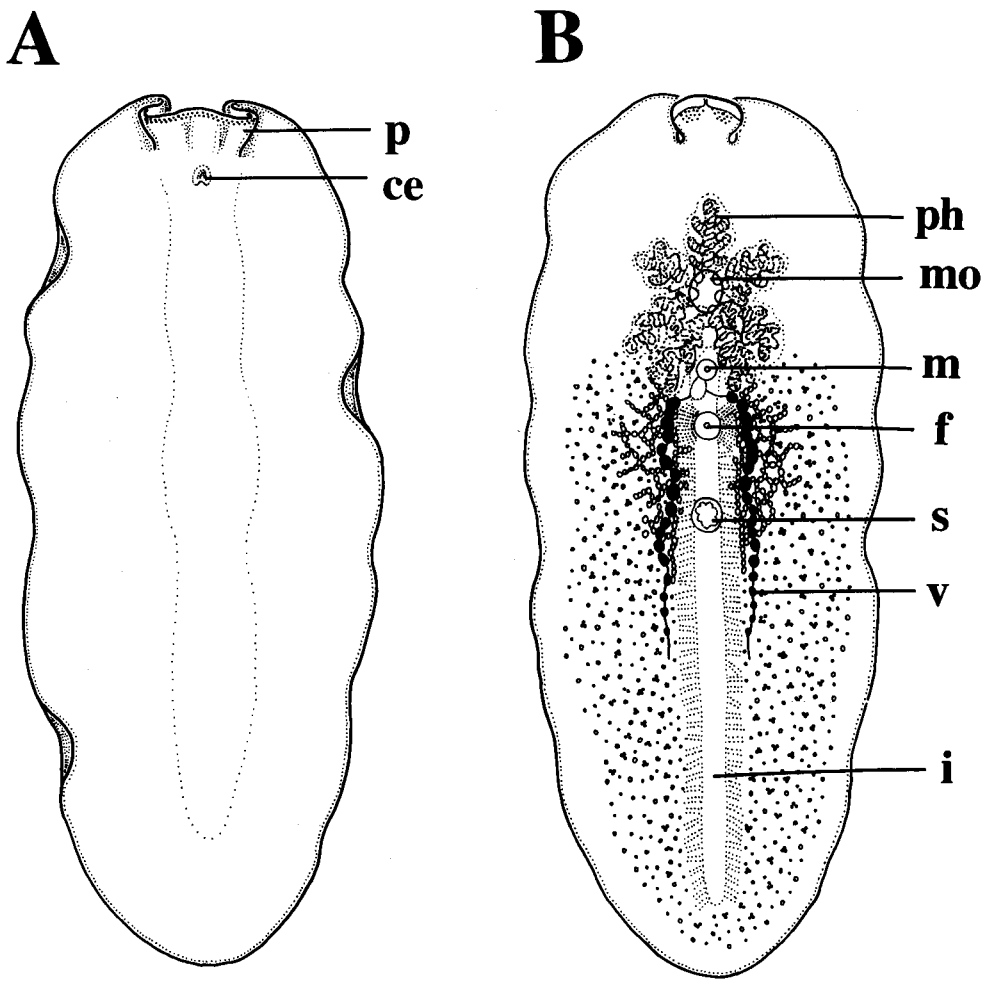

Fig. 1. Morphology of *Pseudoceros*. A, dorsal surface; B, ventral surface (ce-cerebral eyespot, f - female pore, i - intestine, m - male pore, mo - mouth, p - pseudotentacles, ph - pharynx, s - sucker, v - vas deferens, after Newman & Cannon, 1994a).

Specimens were fixed on frozen polyclad fixative (formaldehyde calcium acetate - propylene glycol, propylene phenoxytol) for at least 48 hours then preserved in 70% EtOH for histological preparation (Newman & Cannon, 1995). Whole mounts were prepared by first staining with Mayer's Haemalum, dehydrating in graded alcohols and mounting in Canada balsam. Longitudinal serial sections of the reproductive region were prepared by embedding tissue in 56°C Paraplast, cutting at 6-8 μm, and staining with haematoxylin and eosin.

All material is lodged at the Queensland Museum: wholemounts are designated (WM), serial sections (LS) and wet specimens (S). Descriptions of colours are based on the living animals and the colour descriptions have numbers (in brackets) which refer to Pantone Colors Series U by Letraset 1989.

DESCRIPTION

Pseudoceros confusus, new species
(Figs. 2A-C, 5B)

Material examined. - Holotype - WM (QM G210483), reef crest, One Tree Island, Australia, 15 Sep.1992.

Paratype - LS (QM G210478), reef crest, Heron Island, 28 Aug.1992.

Other material: - WM (QM G210477), reef crest, Heron Island, 23 Aug.1992; WM (QM G210479), 06 Sep.1992; WM (QM G210480), 08 Sep.1992; S (QM G210485, 05 Feb.1993; LS (QM G210481), reef crest, One Tree Island, 12 Sep.1992; WM (QM G210482), 13 Sep.1992; S (QM G210484), 15 Sep.1992; S (QM G210495), 13.08.1993; S, 2 spec., (QM G210496), 14 Aug.1993; WM (QM G210498), 19 Aug.1993.

Description.- Background colour cream-white with 5 marginal bands, 3 bands are distinct; from inside to outside: narrow opaque white; granular black; orange (137); granular black; and narrow, yellow at rim. Faint narrow medial white line starting posteriorly to the cerebral eyespot ending prior to posterior margin. Ventrally cream-white or light orange with yellow rim.

Pseudotentacles simple folds. Relatively small horseshoe shaped cerebral eyespot with about 20 eyes. Pseudotentacular eyes obscured due to black marginal band. Size: 18 x 9 mm (immature) to 30 x 18 mm (mature).

Vas deferens unbranched. Seminal vesicle oblong and large (475 μm long), ejaculatory duct relatively long and coiled. Prostate rounded oval (150 μm long). Stylet short (115 μm long). Male and female antrum deep.

Etymology.- From *confusor* (L.) = confusing, for its similarity to *P. bimarginatus*.

Distribution. - Common from the reef crest at Heron Island and One Tree Island, southern Great Barrier Reef, Australia.

Remarks. - This species is very similar in colour pattern to *P. bimarginatus* but it possesses one extra black marginal band.

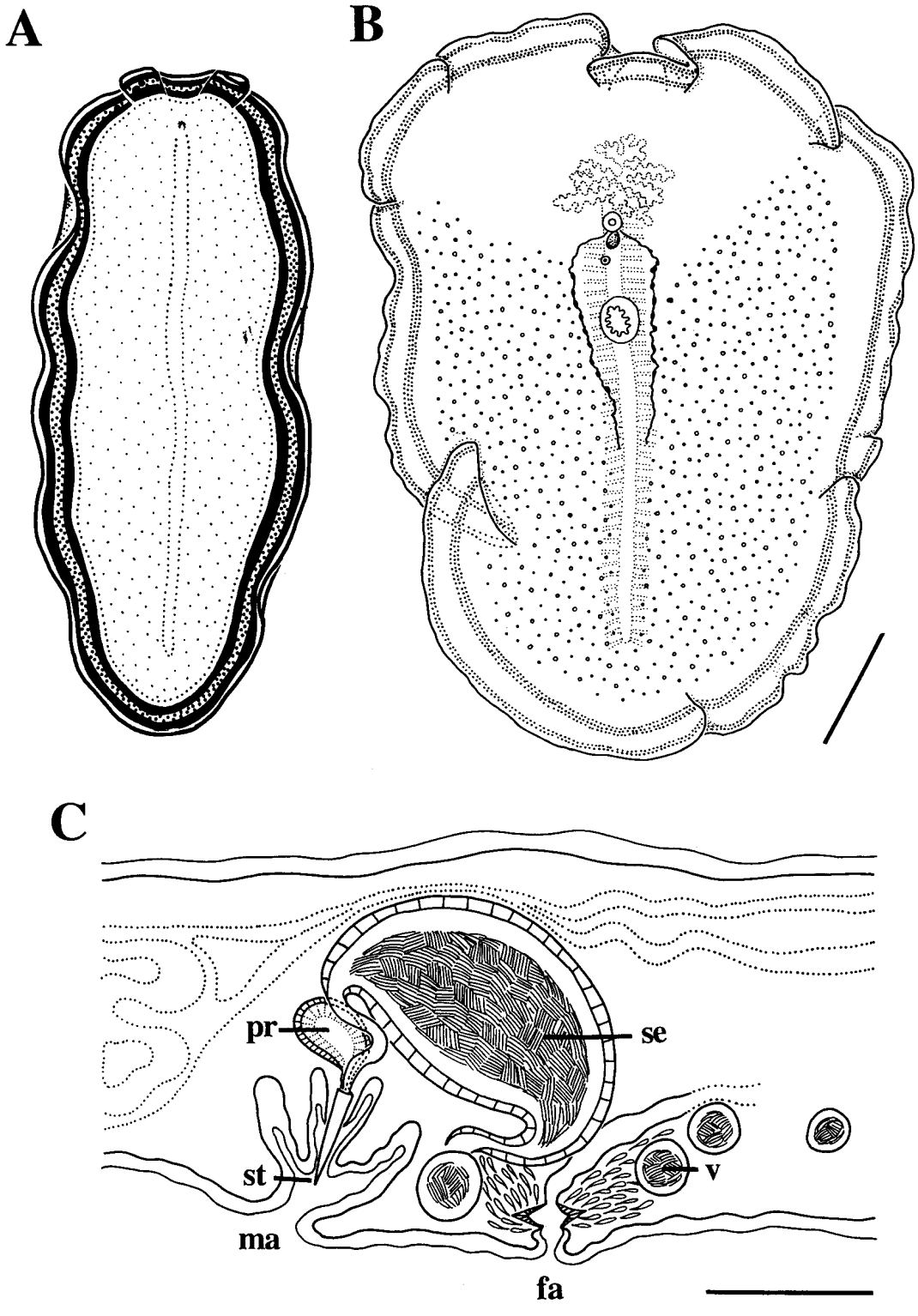

Fig. 2 *Pseudoceros confusus*, new species. A, diagram of the dorsal colour pattern; B, wholemount showing the ventral surface morphology (QM G210483); C, reconstruction of the reproductive anatomy (QM G210478). Scales: B, 5 mm; C, 250 μm (fa - female antrum, ma - male antrum, pr - prostate, se - seminal vesicle, st - stylet, v - vas deferens).

Pseudoceros contrarius, new species
(Figs. 3A - C, 6A)

Material examined. - Holotype - LS (QM G210638), out on rubble, reef slope, 3 m depth, Magic Passage, Madang, Papua New Guinea.

Description. - Background colour cream with three distinct marginal bands; inner narrow, bright yellow (393); middle narrow black, and wide orange (137) at rim. Bright yellow line extends from the pseudotentacles posteriorly down the median line ending prior to the posterior margin. Black triangle between the pseudotentacles extending to the cerebral eyespot. Ventral surface cream with two marginal bands; inner narrow black, orange at rim.

Pseudotentacles simple folds. Cerebral eyespot with about 30 eyes. Dorsal pseudotentacular eyes obscured due to black marginal band. Size: 15 x 5 mm (mature).

Vas deferens unbranched. Seminal vesicle rounded oblong (443 µm long), ejaculatory duct relatively long and coiled. Prostate oval (122 µm long). Stylet 144 µm long. Male and female antrum deep.

Etymology. - From *contrare* (L.) = opposite, for its opposite colour pattern when compared to *P. bimarginatus*.

Distribution. - Only one specimen is known from the reef crest, Madang, Papua New Guinea.

Remarks. - The colour pattern of the marginal bands of this species is the opposite to that of *P. bimarginatus* since the marginal bands, from outside to inside, are yellow, black and orange in *P. bimarginatus* and orange, black and yellow in *P. contrarius* (see also Newman & Cannon, 1994b). The black triangle between the pseudotentacular eyes is absent in *P. bimarginatus*, but is also found in *P. intermittus*, new species (see below).

Pseudoceros intermittus, new species
(Figs. 4A - C, 6B)

Material examined. - Holotype - WM (QM G210372), reef crest, Heron Island, 17 Aug.1989.

Paratype - LS (QM G210379), reef crest, Heron Island, 22 Feb.1992.

Other material: - WM (QM G210373), reef crest, Heron Island, 17 Aug.1989; WM (QM G210645), 18 Jan.1992; WM (QM G210378), 29 Jul.1992; WM (QM G210380), 02 Aug.1992; LS (QM G210640), 04 Feb.1993; SS (QM G210642), 05 Feb.1993; LS (QM G210639), 17 Feb.1993; WM (QM G210647), 23 Feb.1993. One Tree Island- S (QM G210641), 16 Aug.1993; WM (QM G210646), 17 Aug.1993.

Description. - Background colour white or white-cream with three distinct, usually interrupted, marginal bands: inner wide, orange-brown (151); middle narrow, black; bright yellow-green (386) at rim. Distinct black triangle and white spot between the marginal tentacles extending to the cerebral eyes. Ventrally pink-orange (135), same marginal bands.

Body oval with only a few, if any, marginal ruffles. Pseudotentacles simple folds. Cerebral eyespot with about 30 eyes. Dorsal pseudotentacular eyes obscured due to the black marginal band. Size: 10 x 8 mm (immature) to 14 x 8 mm (mature).

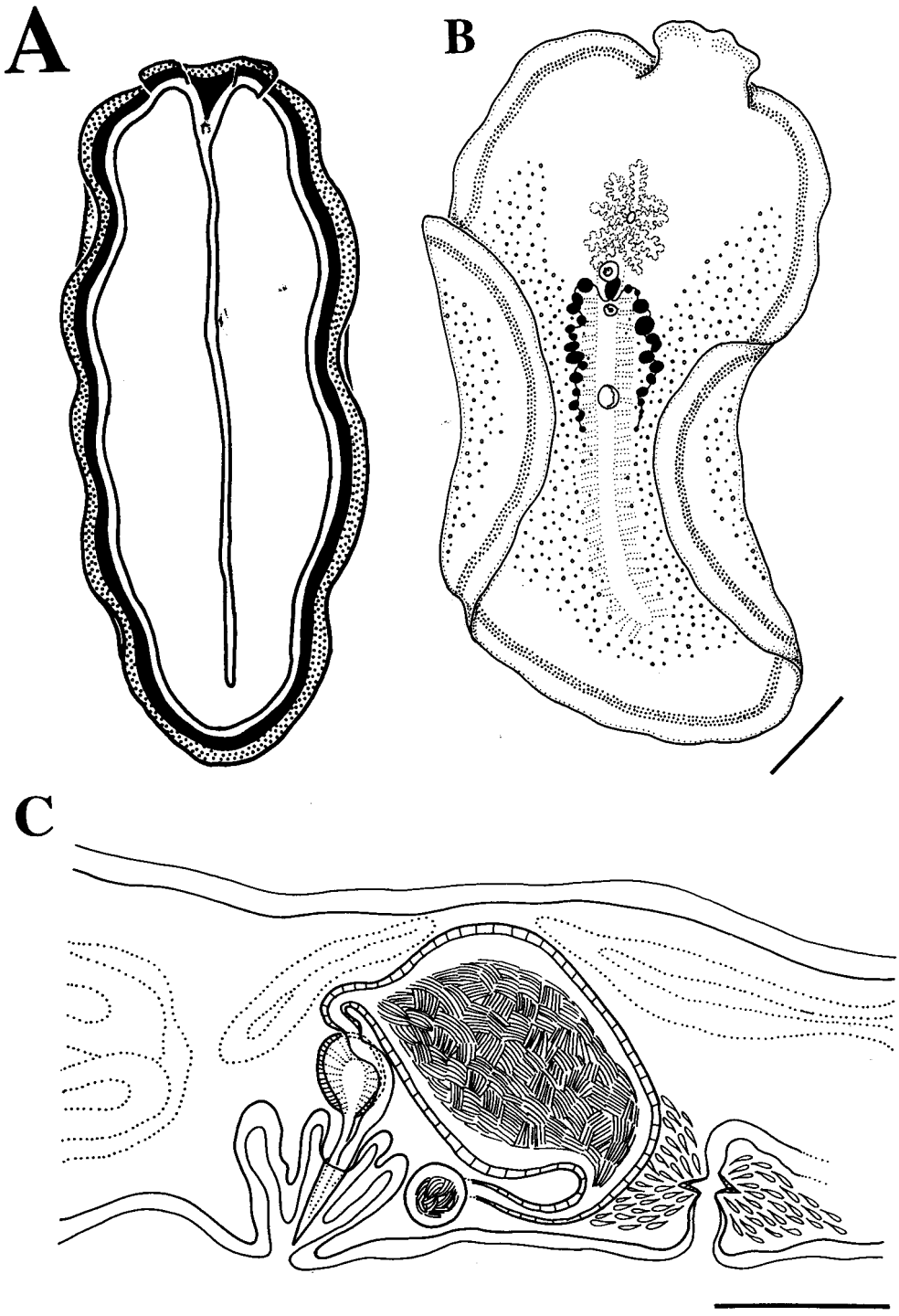

Fig. 3. *Pseudoceros contrarius*, new species. A, diagram of the dorsal colour pattern; B, wholemount showing the ventral surface morphology (QM G210638); C, reconstruction of the reproductive anatomy (QM G210638). Scales: B, 2.5 mm; C, 250 μm.

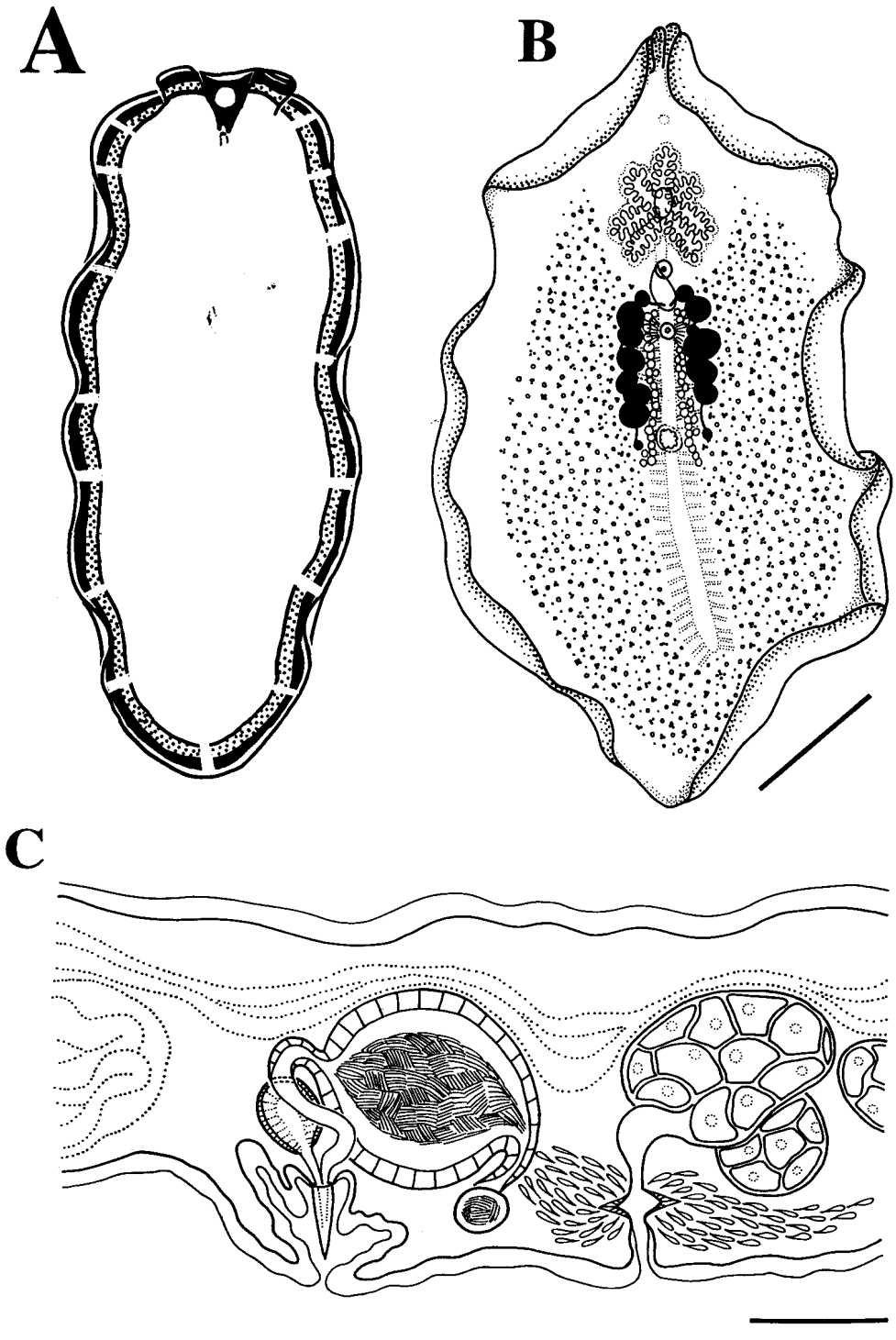

Fig. 4. *Pseudoceros intermittus*, new species. A, diagram of the dorsal colour pattern; B, wholemount showing the ventral surface morphology (QM G210372); C, reconstruction of the reproductive anatomy (QM G210379). Scales: B, 2.5 mm; C, 250 μm.

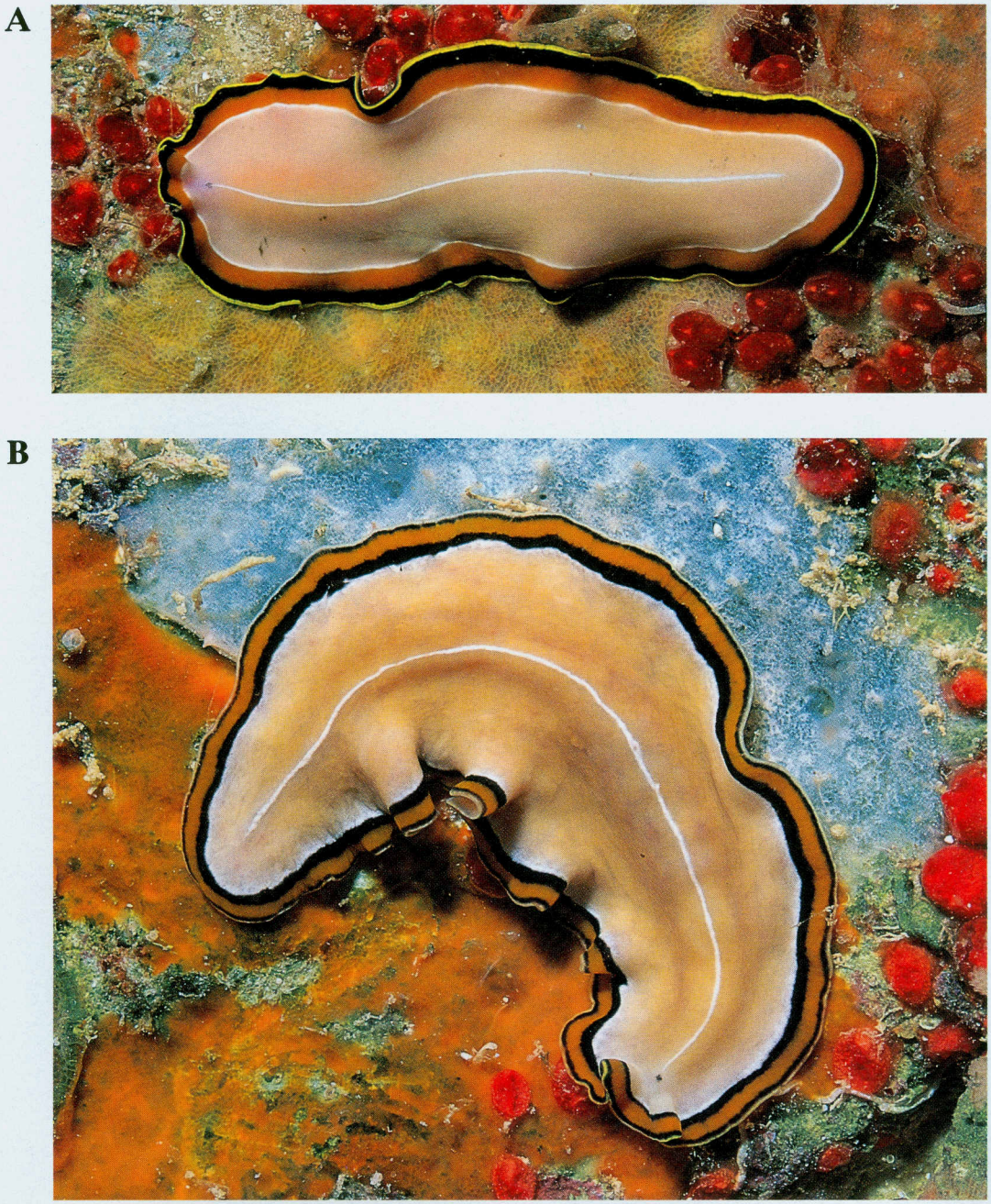

Fig. 5. A, *Pseudoceros bimarginatus* Meixner, 1907, Heron Island, GBR; B, *P. confusus,* new species, Heron Island, GBR.

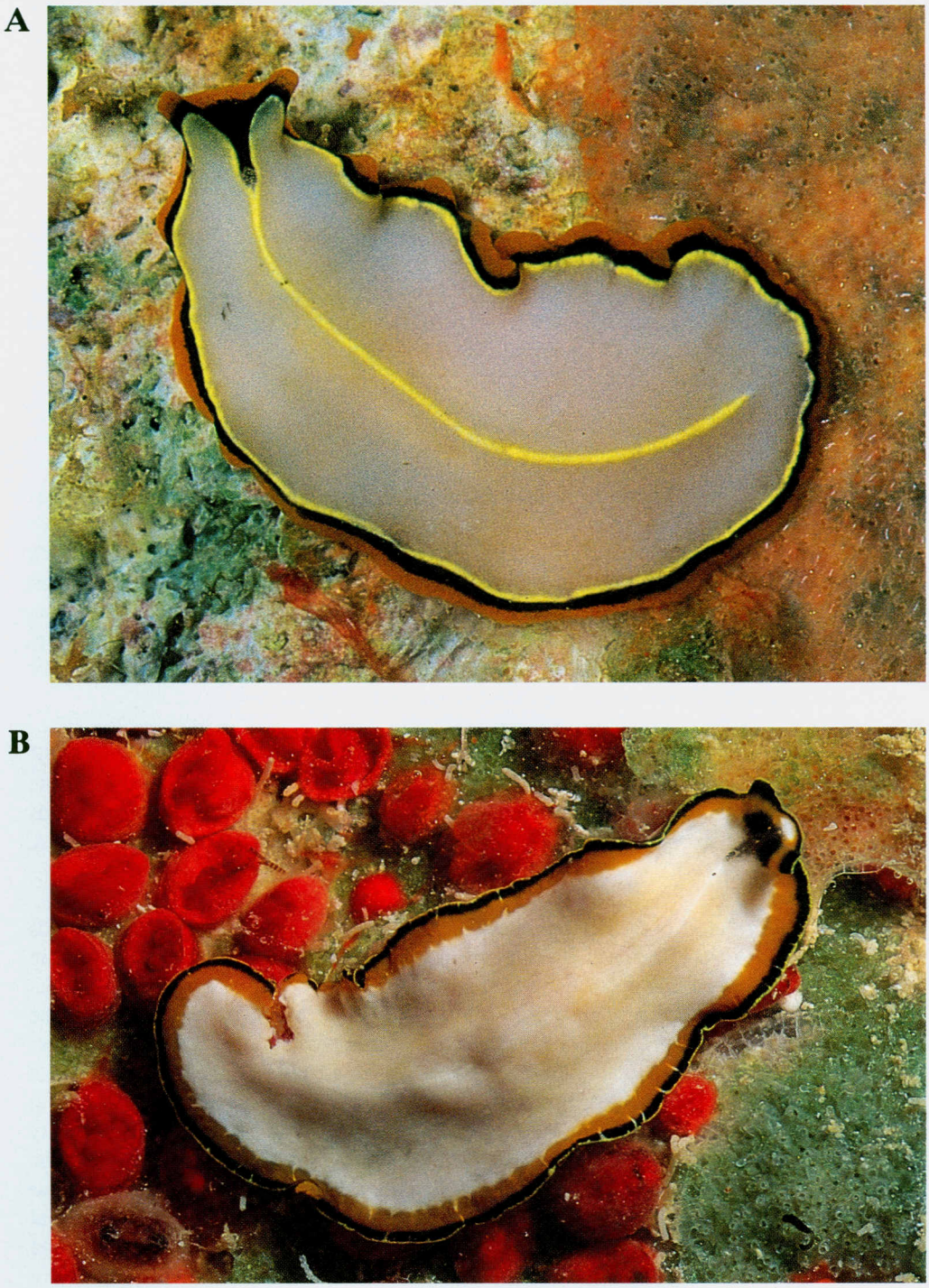

Fig. 6. A, *B. contrarius*, new species, Madang, PNG; D, *P. intermittus*, new species, Heron Island, GBR.

Vas deferens unbranched. Seminal vesicle rounded, oblong (442 μm long), ejaculatory duct relatively long and coiled. Prostate oval (196 μm long). Stylet 167 μm. Male and female antrum deep.

Etymology. - From *intermittum* (L.) = broken, for its intermittent marginal bands.

Distribution. - Common from reef crest and usually found on white encrusting colonial ascidians, Heron Island, Southern Great Barrier Reef, Australia.

Remarks. - This species has the same marginal band pattern as *P. bimarginatus* with the exception that these bands are intermittent: there is a distinctive black triangle between the pseudotentacles as seen in *P. contrarius*, but this species lacks the white spot between the pseudotentacles as found in *P. intermittus*. Newman & Cannon (1994a) incorrectly named the type of this new species *P. bimarginatus*.

DISCUSSION

The three species *P. confusus, P. contrarius* and *P. intermittus* possess marginal bands of the same colour as *P. bimarginatus* and without careful examination, these four species could easily be confused. In comparison to *P. bimarginatus, P. confusus* has one extra black marginal band; *P. contrarius* has the opposite coloured marginal banding and also a black triangle between the pseudotentacles; *P. intermittus* also possesses a black triangle between the pseudotentacles but lacks the white spot and, as well, it has intermittent marginal banding. Newman & Cannon (1994a) determined that colour pattern alone could differentiate species of *Pseudoceros*. No differences were found in the male reproductive structures.

Pseudoceros bimarginatus is a conspicuous and common species from the southern GBR (Newman & Cannon, 1994a). It is often found out during the day, moving over coral rubble in shallow reef waters. Its vibrant orange and yellow and contrasting black marginal bands suggest that this polyclad is displaying aposematic or warning colouration (Newman & Cannon, 1994a; 1994c).

Despite the brilliance of their colours and patterns, like opisthobranchs molluscs, polyclads are not always obvious in their natural environment. Since polyclads have no means of forming a visual image, their colours and patterns are logically the result of selection against predation. Patterns may be cryptic against an appropriate background or conversely may advertise the worms presence. Bright colours alone may be sufficient to provide a predator with a longer time to 'consider' before striking (Guilford, 1986), a clear advantage to prey. In the case of species described here, however, the predominance of yellow, orange and black bands, typical aposematic colours (Cott, 1940), suggests these worms are warning of their unpalatability (Newman et al., 1994). Although most work on aposematic colouration has been done in the terrestrial environment, Sundberg (1979) demonstrated that the nemertean, *Tubulanus annulatus,* which is white with red stripes was protected from predation after the fish learnt to recognise this worm as unpalatable; and Rosenberg (1989) gave details of the tropical reef gastropod, *Cymphoma gibbosum,* which is white with orange and black markings, i.e. not dissimilar to the worms described here, which is similarly abroad in daylight and is presumed to show aposematic colouration.

The worms clearly exhibit brilliant colours, but without experimental evidence of

unpalatability or predator learning we cannot irrefutably claim that these colours are aposematic. It may prove that one of these worms is unpalatable and the others not (Batesian mimicry), or all may be unpalatable (Müllerian mimicry). We do know that when fish were offered polyclads they always spat them out, that damaged worms are often encountered and, further, that worms are remarkably able to repair torn tissue (Flowers & Newman, unpublished). We suggest these worms will prove to be exhibiting mimicry and/or simply convergence and that individual selection would be an adequate explanation here for its evolution.

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