

New records of marine flatworms (Platyhelminthes: Polycladida: Cotylea) from Singapore

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Abstract. Five *Cotylea* species found during the Comprehensive Marine Biodiversity Survey (CMBS) between 2010 and 2015 are illustrated as new records for Singapore. They are: *Thysanozoon nigropapillosum*, *Thysanozoon nigrum*, *Pseudobiceros bajae*, and *Pseudoceros microcelis* of the family Pseudocerotidae, and *Cycloporous venetus* of the family Euryleptidae. Also for the first time, *Thysanozoon nigrum* is recorded in the Indo-Pacific region, *Pseudobiceros bajae* beyond the eastern Pacific, and *Pseudoceros microcelis* beyond the eastern Indian Ocean.

Key words. Pseudocerotidae, Euryleptidae, *Pseudobiceros*, *Thysanozoon*, *Cycloporous*, Singapore

INTRODUCTION

This is a continuation on the study of polyclads found in Singapore during the Comprehensive Marine Biodiversity Survey, a five-year project from 2010–2015 that was a collaboration between the National Parks Board of Singapore and the National University of Singapore. An estimated 76 cotylean species are presently known from Singapore (unpublished data), including 16 species recently featured by Bolaños et al. (2016). Here, we describe and illustrate five species that are new to Singapore. These include four pseudocerotids: *Thysanozoon nigropapillosum*, *Thysanozoon nigrum*, *Pseudobiceros bajae*, and *Pseudoceros microcelis*, and one euryleptid: *Cycloporous venetus*. All are classified in the clade Rhabditophora, order Polycladida, suborder Cotylea and superfamilies Euryleptoidea and Pseudoceroidea (Lang, 1884; Faubel, 1984).

In the family Pseudocerotidae, the genera *Pseudobiceros* and *Pseudoceros* form the majority of the species found in Singapore waters. In this report, members of the genus *Thysanozoon* are also featured. These are less commonly encountered and seen only at subtidal regions. The diagnosis of *Thysanozoon* is dependent on two key morphological features: 1) a papillate dorsal surface and 2) the presence of two male gonopores. Similar looking members of the genus *Acanthozoon* also have papillated dorsal surfaces, but have only one male gonopore. To distinguish between the two genera, specimens need to be examined ventrally for the number of gonopores (Faubel, 1984). The apparent rarity of members of the genus *Thysanozoon* could be that they have been overlooked owing to the animals' cryptic colouration and small size. The generally high turbidity of Singapore waters also tends to make observations of these flatworms challenging.

Many colourful members of the family Euryleptidae can easily be confused with some species of *Pseudoceros*. The morphology of the pharynx is an important character to distinguish them. Those of *Pseudoceros* are complex, branch-like, whereas the euryleptids have a tubular, cylindrical pharynx (Faubel, 1984; Newman & Cannon, 1994, 2002). Although the generally small size of euryleptids tends to make them difficult to find, *Cycloporous venetus* (featured herein) seems to be common in Singapore.

MATERIALS & METHODS

During the Comprehensive Marine Biodiversity Survey, specimens were collected from various locations around Singapore Island and its offshore islands. The flatworms were hand collected with a soft paintbrush from various habitats in the intertidal and subtidal zones. Specimens were kept in individual containers for transportation. In the laboratory, they were placed in glass petri dishes, measured, and photographed with either a Nikon D800 digital SLR camera with 60 mm macro lens and speedlight SU-800 flash system, or a Canon EOS 5D digital SLR camera with MP-E65 macro lens and speedlite 430EX II flash system.

For preservation, the flatworms were coaxed onto a piece of filter paper immersed in sea water, and then transferred onto a block of frozen 10% buffered formalin together with the filter paper (protocol modified from Newman & Cannon, 1995). The specimens were left in the fixative for 24–48 hours before they were transferred to 70% ethanol. Under 'Material

examined', polyclad specimens with ZRC.PLA catalogue numbers were deposited in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum at the National University of Singapore. Material listed carrying numbers with the prefixes SUB (subtidal), INT (intertidal), and OTC (outside of the regular CMBS survey sites) have not been preserved, but photographs of these specimens are available. Dimensions of each specimen are given in length × width in millimetres.

All material listed under 'Material examined' have been collected from Singapore. The specimens were carefully examined in life as important morphological characters, such as colour, pattern, shape of pseudotentacles, type of pharynx, are often lost or become distorted after preservation. Identification was based mainly on the colour and pattern following the system established by Newman & Cannon (1994, 1997, 1998). As the internal reproductive anatomy of pseudocerotids is extremely homogeneous (Newman & Cannon, 1994), it is not used in this study.

TAXONOMY

Family Euryleptidae

Cycloporus venetus Newman & Cannon, 2002 (Fig. 1)

Cycloporus venetus Newman & Cannon, 2002: 293 (type locality: North Exmouth, West Australia).

Cycloporus venetus — Newman & Cannon, 2003: 66; Sreeraj & Raghunathan, 2015: 85.

Material examined. 1 ex. (ZRC.PLA.0146): 4 × 3 mm, subtidal, Little Sister's Island, 16 November 2013; 1 ex. (SUB-0547, not preserved): 5 × 3 mm, subtidal, Pulau Hantu, 26 May 2014; 1 ex. (ZRC.PLA.0145): 6 × 4 mm, subtidal, Pulau Sudong, 25 January 2015.

Diagnosis. Dorsal surface ranging from vivid opaque blue to pale light blue (sometimes almost fading to white medially) with a narrow white median line. Wide yellow marginal band with a narrow colourless or transparent rim. Yellow margin becomes deep orange at the region of the pseudotentacles (Fig. 1A, B). Ventral surface of similar colour and marginal bands as the dorsal side (Fig. 1C, D). Simple, small marginal pseudotentacles without obvious folds. Cerebral eyespots in an elongated oval shape. Numerous, scattered pseudotentacular eyes, more concentrated at the base of the pseudotentacles (Fig. 1E).

Distribution. West Australia, Indonesia, Japan (Newman & Cannon, 2005), India (Sreeraj & Raghunathan, 2015).

Taxonomic remarks. Species determination amongst members of the genus *Cycloporus* is mostly based on colour and pattern, given that the internal reproductive anatomy is similar between species (Newman & Cannon, 2002). Distinguishing species of *Cycloporus* can be challenging since these animals tend to be transparent and the colour greatly depends on the content of the intestinal branches. However, *Cycloporus venetus* has a unique and diagnostic colour and pattern. The specimens from Singapore show some colour variation from the original description, ranging from sky blue to having a slight blue tint instead of bright blue. The yellow and colourless marginal bands remain consistent.

Family Pseudocerotidae

Thysanozoon nigropapillosum (Hyman, 1959) (Fig. 2)

Acanthozoon nigropapillosum Hyman, 1959: 581 (Type locality: Ifaluk Atoll, Micronesia).

Thysanozoon nigropapillosum – Faubel, 1984: 213; Gosliner et al., 1996: 111; Newman et al., 2003: 198; Dixit & Raghunathan, 2013: 168; Jie et al., 2014: 764.

Thysanozoon sp. 1 – Newman & Cannon, 2005.

Material examined. 1 ex. (ZRC.PLA.0174): 9 × 7 mm, intertidal, Big Sister's Island, 16 September 2014; 1 ex. (OTC-0254, not preserved): 25 × 20 mm, intertidal, Lazarus Island, 16 June 2014.

Diagnosis. Dorsum black with numerous yellow-tipped blunt papillae of various size, scattered randomly (Fig. 2D); and distinct broad, white marginal band (Fig. 2A). Simple, folded pseudotentacles. Cerebral eyespots in a clear, oval-shaped area (Fig. 2C). Ventral side blackish with white marginal band (Fig. 2B).

Distribution. This species seems to be widely distributed in the Indo-Pacific region according to 11 photographic records from Newman & Cannon (2003, 2005) and one from Gosliner et al. (1996). Known localities include Ifaluk Atoll in Micronesia, Taiwan, Nicobar Islands, Fiji, Indonesia, Japan, Marshall Islands, Papua New Guinea, and the Red Sea.

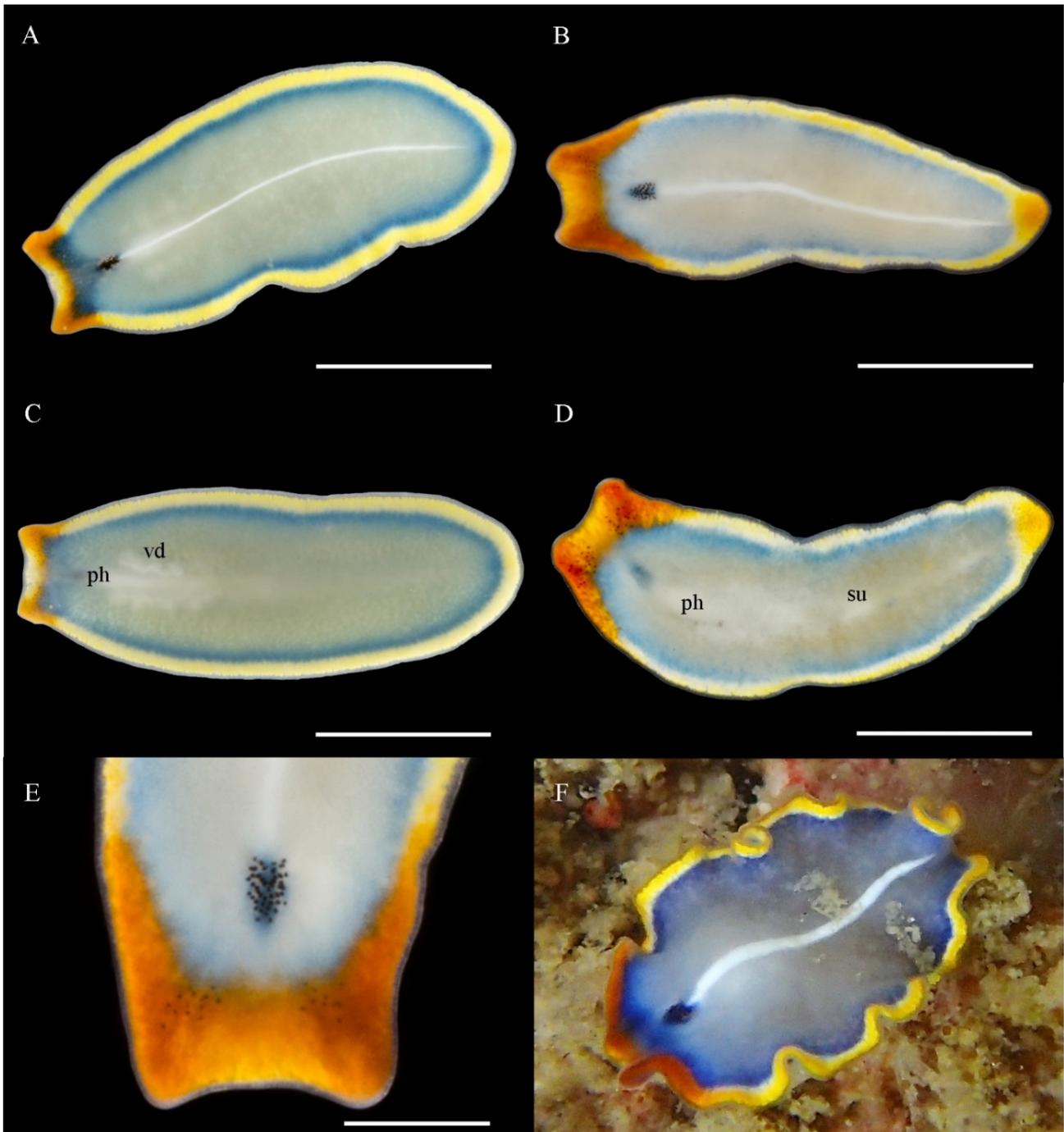


Fig. 1. *Cycloporus venetus*, living animal. A (ZRC.PLA.0145) and B (SUB-0547), dorsal view of live animal, showing colour variation and the cerebral eyespots; C (ZRC.PLA.0145) and D (SUB-0547), ventral view of live animal, showing the pseudotentacular eyespots, pharynx, vas deferens and sucker; E (SUB-0547), anterior region showing marginal pseudotentacles, cerebral eyespot and pseudotentacular eyes; F, in-situ photograph of animal, Pulau Hantu. Scale bars = 5 mm [A–C]; 1 mm [E]. ph, pharynx; su, sucker; vd, vas deferens. (Photographs by: Rene S. L. Ong, Figs. 1A–E; photograph by: Toh Chay Hoon, Fig. 1F).

Taxonomic remarks. *Thysanozoon nigropapillosum* (Hyman, 1959) was originally described as *Acanthozoon nigropapillosum* distinguished by a black body, pale yellow margin with white-tipped papillae and two male gonopores. It is based on the number of male gonopores that Faubel (1984) transferred the species to the genus *Thysanozoon*. A formal re-description of *Thysanozoon nigropapillosum* by Jie et al. (2014) indicates that Hyman (1959) had based her colour description of the species on preserved material, thereby causing the discrepancy in the colour description. Reported as '*Thysanozoon* sp.1' in Newman & Cannon (2005), this specimen is in fact *Thysanozoon nigropapillosum* though it was not recognised as such due to the colour difference from Hyman's (1959) original description of the type.

Thysanozoon raphaeli Bolaños, Quiroga & Litvaitis (2007) and *Thysanozoon flavotuberculatum* Hyman (1939) are two congeners that also have yellow papillae. However, both species lack the distinctive white margin that is present in

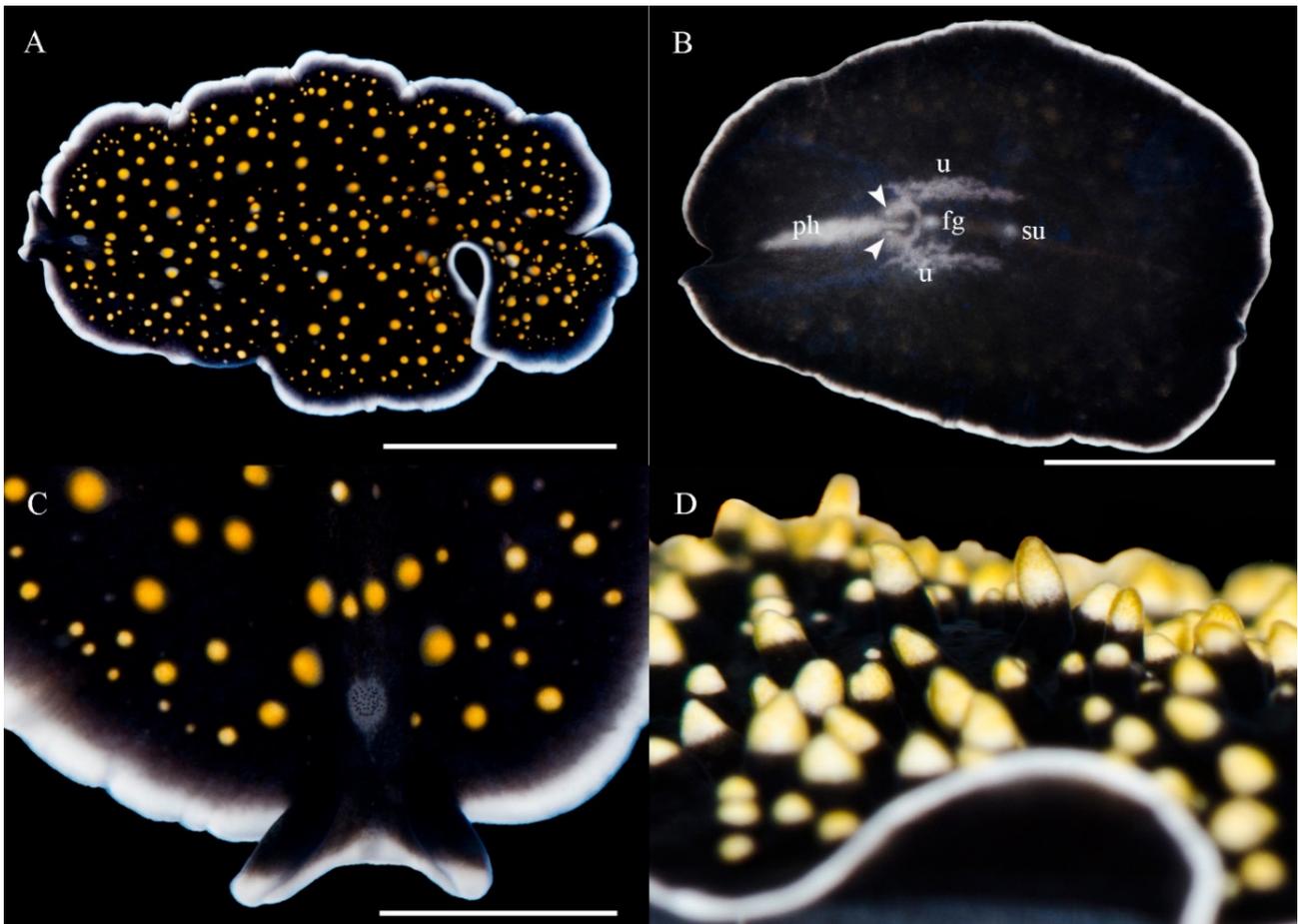


Fig. 2. *Thysanozoon nigropapillosum*, living animal (OTC-0254). A, dorsal view of live animal; B, ventral view of live animal, showing the pharynx, male gonopores (arrowhead), female gonopore, uteri, and sucker; C, anterior region showing simple-folded pseudotentacles and cerebral eyespots in a clear area; D, close-up view of the papillae, showing the yellow tip and black base. Scale bars = 10 mm [A, B], 5 mm [C]. fg, female gonopore; ph, pharynx; su, sucker; u, uteri. (Photographs by: Rene S. L. Ong).

Thysanozoon nigropapillosum. In addition, *Thysanozoon raphaeli* is translucent black with minute white spots around the margin, and *Thysanozoon flavotuberculatum* is greyish with scattered black flecks.

Thysanozoon nigropapillosum looks similar to an unidentified *Acanthozoon* species that is commonly sighted in Singapore (pers. obs.). However, they can be distinguished by the white marbled patches present on *Acanthozoon* sp. Also, the papillae of *Acanthozoon* sp. are yellow-tipped with a white base, and cover the entire body while those on *Thysanozoon nigropapillosum* are much fewer and do not reach the margin, leaving a papillae-free area prior to the white band. In addition, the ventral side of *Acanthozoon* sp. is whitish and not black as on *Thysanozoon nigropapillosum*.

Thysanozoon nigropapillosum can also be easily confused with *Pseudoceros microcelis* Prudhoe (1989) as they share a similar colour and pattern. *Pseudoceros microcelis* has only one male gonopore and does not have papillae on its dorsal surface (see taxonomic remarks for *Pseudoceros microcelis*).

Thysanozoon nigrum Girard, 1851 (Fig. 3)

Thysanozoon nigrum Girard, 1851: 137 (type locality: Coast of Florida, United States of America).

Thysanozoon brochii var. *nigrum* – Lang, 1884: 535.

Thysanozoon nigrum – Hyman, 1939: 15; Hyman, 1940: 484; Hyman, 1952: 196; Hyman, 1955a: 263; Hyman, 1955b: 137; Verrill, 1901: 41; Marcus & Marcus, 1968: 69.

Material examined. 1 ex. (ZRC.PLA.0177): 55 × 20 mm, subtidal, Pulau Senang, 29 November 2014; 1 ex. (ZRC.PLA.0175): 60 × 25 mm, subtidal, Pulau Salu, 15 January 2015; 1 ex. (ZRC.PLA.0176): 50 × 20 mm, subtidal, Pulau Salu, 15 January 2015.

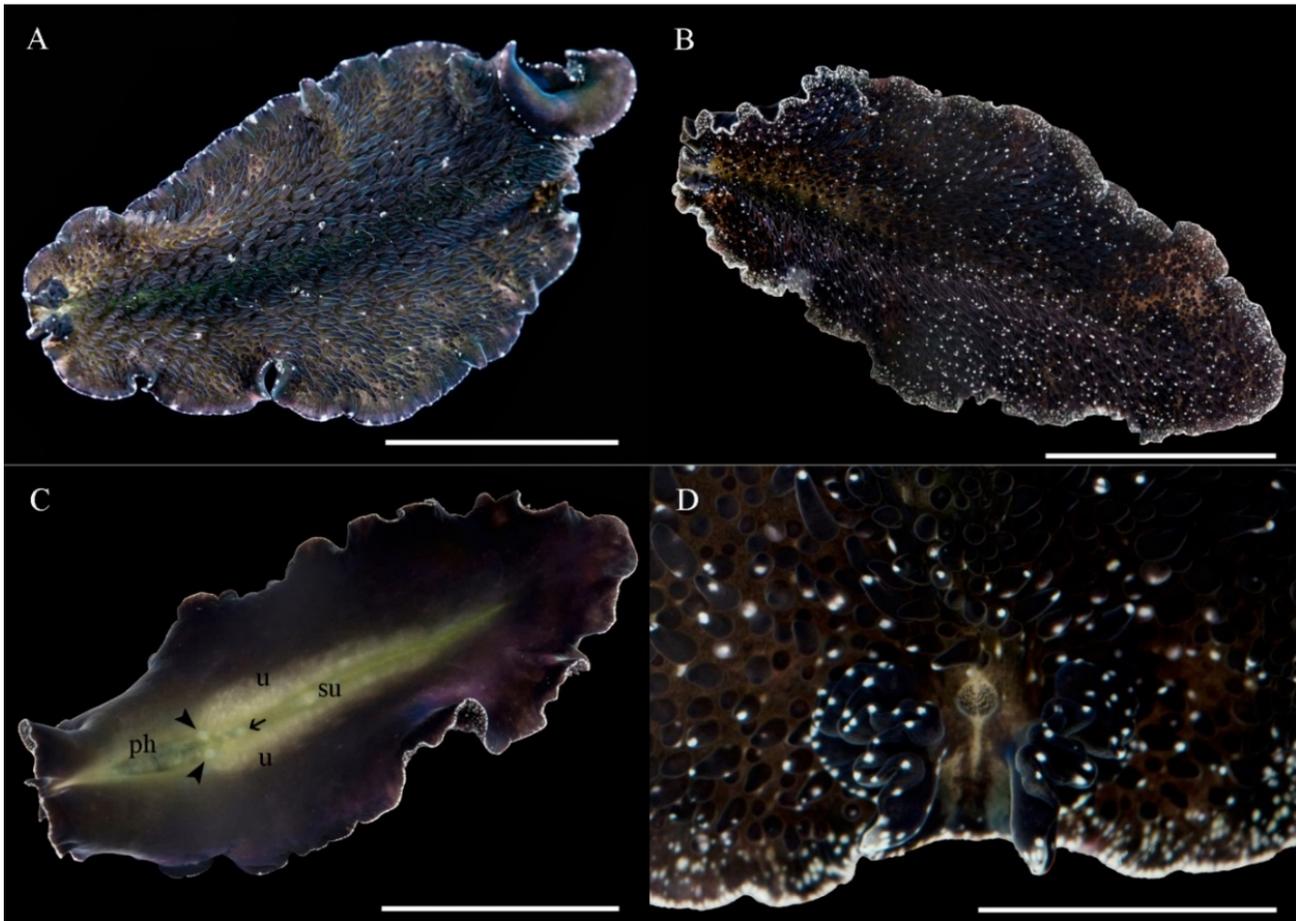


Fig. 3. *Thysanozoon nigrum*, living animal. A (ZRC.PLA.0177) and B (ZRC.PLA.0175), dorsal view of live animals, showing the different intensity of white speckles; C (ZRC.PLA.0175), ventral view of live animal, showing the pharynx, male gonopores (arrowhead), female gonopore (arrow), uteri, and sucker; D (ZRC.PLA.0175), anterior region showing the ruffled, square-like pseudotentacles and cerebral eyespots in a clear area. Scale bars = 20 mm [A–C], 10 mm [D]. ph, pharynx; su, sucker; u, uteri. Photographs by Rene S. L. Ong.

Diagnosis. Black or dark greyish body covered with numerous black, elongated and pointy papillae. Numerous or scarce white speckles or spots may be present. Narrow, white interrupted rim formed by small white dots randomly spaced around the entire body (Fig. 3A, B). Ventral side of lighter colour than the dorsal but without white speckles or spots (Fig. 3C). Laterally ruffled, square-like pseudotentacles with white tips that are formed by a cluster of dots. Horseshoe-shaped cerebral eyespots in a clear oval area that has a short line projecting anteriorly and posteriorly (Fig. 3D).

Distribution. Sub-tropical western Atlantic along the coast of Florida, Texas, Bermuda, Bahamas. This seems to be the first record from the Indo-Pacific.

Taxonomic remarks. *Thysanozoon nigrum* Girard (1851) is the only species in this genus that has an entirely black body with black papillae. It was observed that in some specimens, numerous white speckles may be present, while in others, such white pigmentation is almost absent. Of all the worms examined, one particular specimen (ZRC.PLA.0175) has white spots densely packed around its margin, so closely arranged that it appeared to have a white rim. Therefore, the presence and arrangement of the white speckles or spots may vary from one worm to another. During photography, it was also observed that depending on the light intensity and background colour of where the animal is being photographed, the worm may appear brown or light grey instead of black. It is hereby important to note that when exposed to strong light intensity and on light background, one can mistake the worm as brown, hence misidentifying as another species, such as *Thysanozoon brocchii*. We believe that *Thysanozoon nigrum* has a circumtropical distribution, and it is possible that some specimens in the Indo-Pacific region could have been misidentified as *Thysanozoon brocchii*.

***Pseudobiceros bajae* (Hyman, 1953) (Fig. 4)**

Pseudoceros bajae Hyman, 1953: 365 (type locality: Gulf of California, Mexico).

Cryptobiceros bajae—Faubel, 1984: 215.

Pseudobiceros bajae—Newman & Cannon, 1994: 240; Marquina et al., 2015: 373; Bahia & Schrodler, 2016: 109.

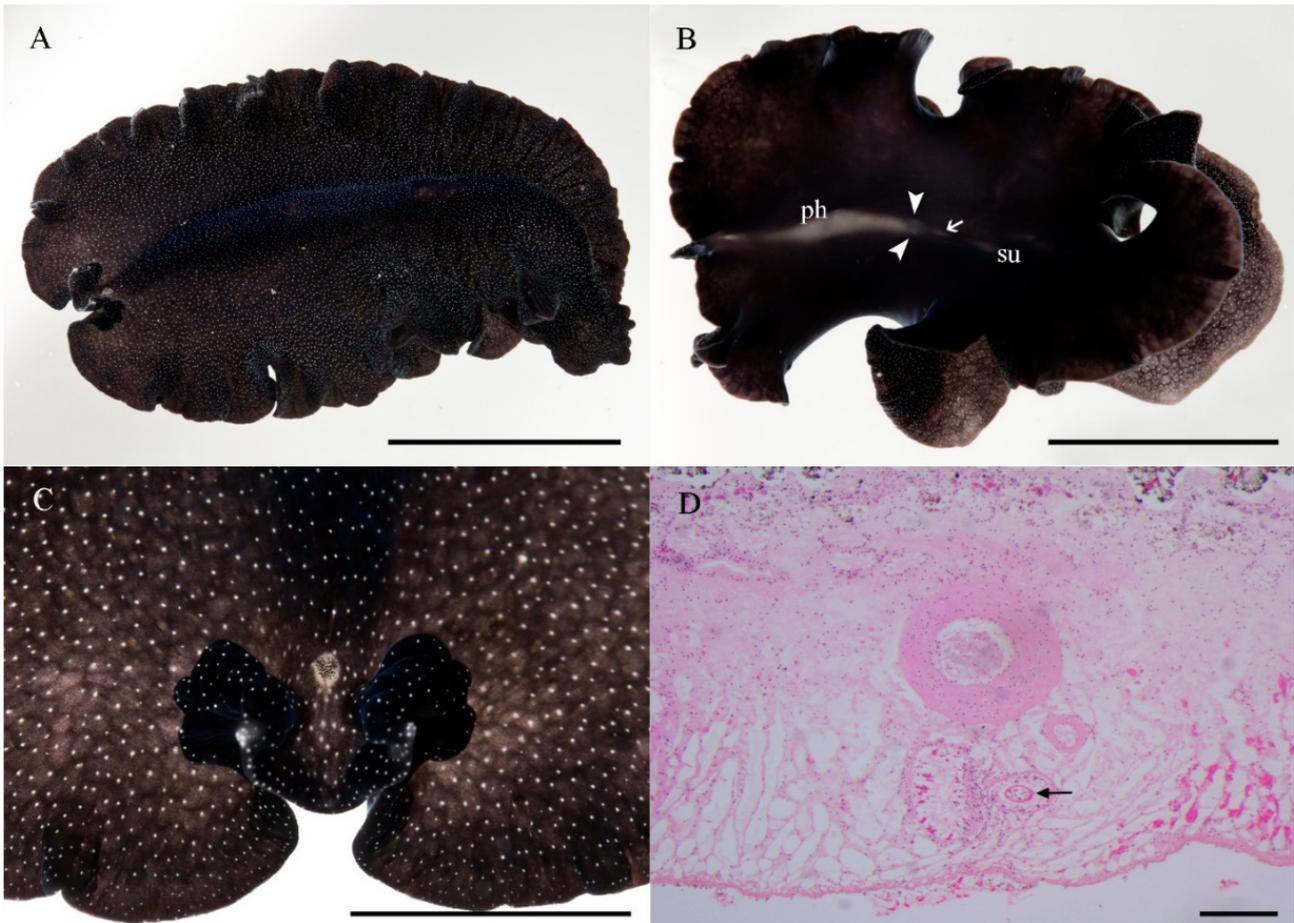


Fig. 4. *Pseudobiceros bajaе*, living animal (ZRC.PLA.0197). A, dorsal view of live animal; B, ventral view of live animal, showing the pharynx, male gonopores (arrowhead), female gonopore (arrow), and sucker; C, anterior region showing the ruffled, square-like pseudotentacles and cerebral eyespots in a clear area; D, sagittal histological section of one male copulatory system pointing at the stylet (arrowhead). Scale bars = 20 mm [A, B], 10 mm [C], 200 μm [D]. ph, pharynx; su, sucker. (Photographs by: Rene S. L. Ong).

Material examined. 1 ex. (ZRC.PLA.0197): 65 × 20 mm, intertidal, Terumbu Berkas, 10 August 2014; 1 ex. (OTC-0423, not preserved): 45 × 30 mm, intertidal, Terumbu Semakau, 28 April 2013.

Diagnosis. Black dorsal surface with small white dots covering the entire body (Fig. 4A). Ventral side of similar colour without any white dots (Fig. 4B). Laterally ruffled, square-like pseudotentacles with white tips. Cerebral eyespots in a clear oval-shaped area (Fig. 4C).

Distribution. Gulf of California, Mexico. This seems to be the first record from the Indo-west Pacific.

Taxonomic remarks. Faubel (1984) erected the genus *Cryptobiceros* which is distinguished from *Pseudoceros* on the basis of “penis without any cuticular elements”, while the other key characteristic remained the same as *Pseudoceros*. Newman & Cannon (1994) indicated that this genus is not valid, and made the new combination *Pseudobiceros bajaе*, which is followed here. We agree with this new classification since there should be more than a key character to significantly warrant the creation of a new genus. In addition, from the sagittal histological section of the male copulatory system (Fig. 4D) of *Pseudobiceros bajaе*, the stylet (the only cuticular or sclerotised element of pseudocerotids) can clearly be seen. This further shows that the criteria that Faubel originally erected is not a valid key.

Pseudobiceros bajaе and *Pseudobiceros stellae* Newman & Cannon (1994) are the only two species in the genus *Pseudobiceros* with black background and small white dots over the dorsal surface. *Pseudobiceros stellae* Newman & Cannon (1994) has a black or brown dorsal surface with white speckles and dots forming clusters of different sizes, and an interrupted, narrow white rim formed by white spots. The median area is darker and raised, with white speckles and dots sometimes forming a faint, narrow line (Fig. 5A, B). The ventral surface is light greyish-brown with white dots (Fig. 5C).

The two species can be distinguished by the arrangement of the dots over the dorsal surface and the shape of the pseudotentacles. *Pseudobiceros bajaе* has evenly spaced, individual dots whereas *Pseudobiceros stellae* has groups of minute dots clustered together. In addition, the white dots on *Pseudobiceros bajaе* do not form a white rim. Furthermore,

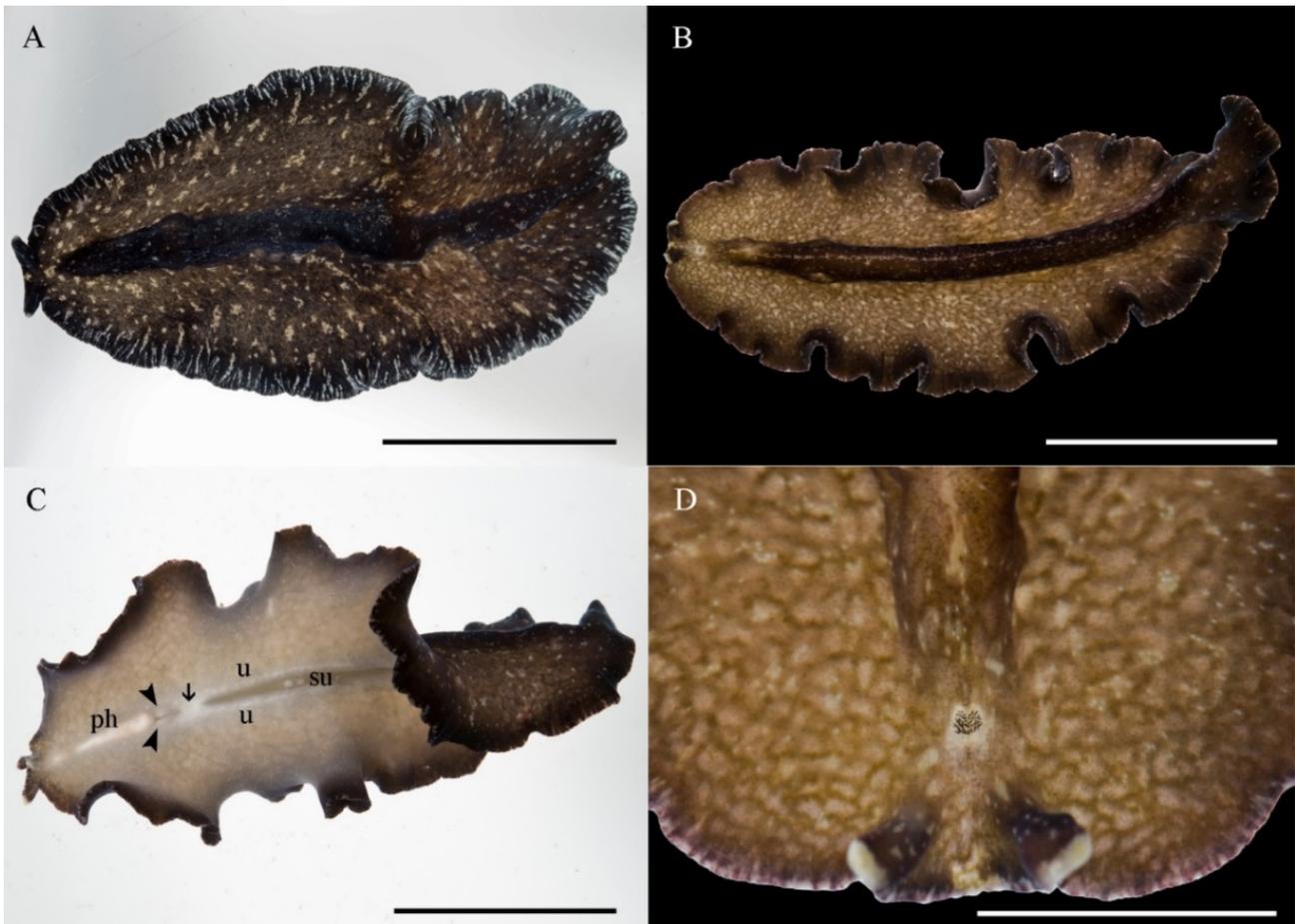


Fig. 5. *Pseudobiceros stellae*, living animal. A (OTC-0605) and B (INT-0005), dorsal view of live animals, showing the colour variation; C (INT-0005), ventral view of live animal, showing the pharynx, male gonopores (arrowhead), female gonopore (arrow), and sucker; D (INT-0005), anterior region showing the simple-folded pseudotentacles and horseshoe-shaped cerebral eyespots in a clear area. Scale bars = 20 mm [A–C], 10 mm [D]. ph, pharynx; su, sucker; u, uteri. (Photographs by: Rene S. L. Ong).

Pseudobiceros stellae has simple, folded pseudotentacles (Fig. 5D) whereas *Pseudobiceros bajae* has ruffled, square-like pseudotentacles.

Pseudobiceros stellae has been recorded from India, Papua New Guinea, and Australia at the Great Barrier Reef and Coral Bay (Newman & Cannon, 2005). In Singapore, it is known from the intertidal zones of Cyrene Reef, Kusu Island and Seringat Kias where aggregations, possibly associated with breeding and abundance of food, have been observed (Ong et al., 2015).

***Pseudoceros microcelis* Prudhoe, 1989 (Fig. 6)**

Pseudoceros microcelis Prudhoe, 1989: 85 (type locality: Inhaca Island, Mozambique).

Material examined. 1 ex. (ZRC.PLA.0179): 20 × 15 mm, subtidal, Pulau Biola, 20 August 2013; 1 ex. (photographic record, not preserved), subtidal, Big Sister’s Island, March 2015; 1 ex. (photographic record, not preserved), subtidal, Pulau Hantu, March 2013.

Distribution. Inhaca Island, Mozambique. This may be the first record of the species from beyond the type locality.

Diagnosis. Black dorsal surface with numerous yellow spots of varying sizes (Fig. 6A). The yellow spots are slightly raised (Fig. 6D), which may give a false impression of presence of papillae. A marginal band of greyish-white surrounded the body, followed by an outer narrow yellow rim, including the pseudotentacles. Ventral surface of greyish black, with the same rim and marginal band (Fig. 6B). Rounded cerebral eyespots on a clear area, and pseudotentacles of simple folds (Fig. 6C).

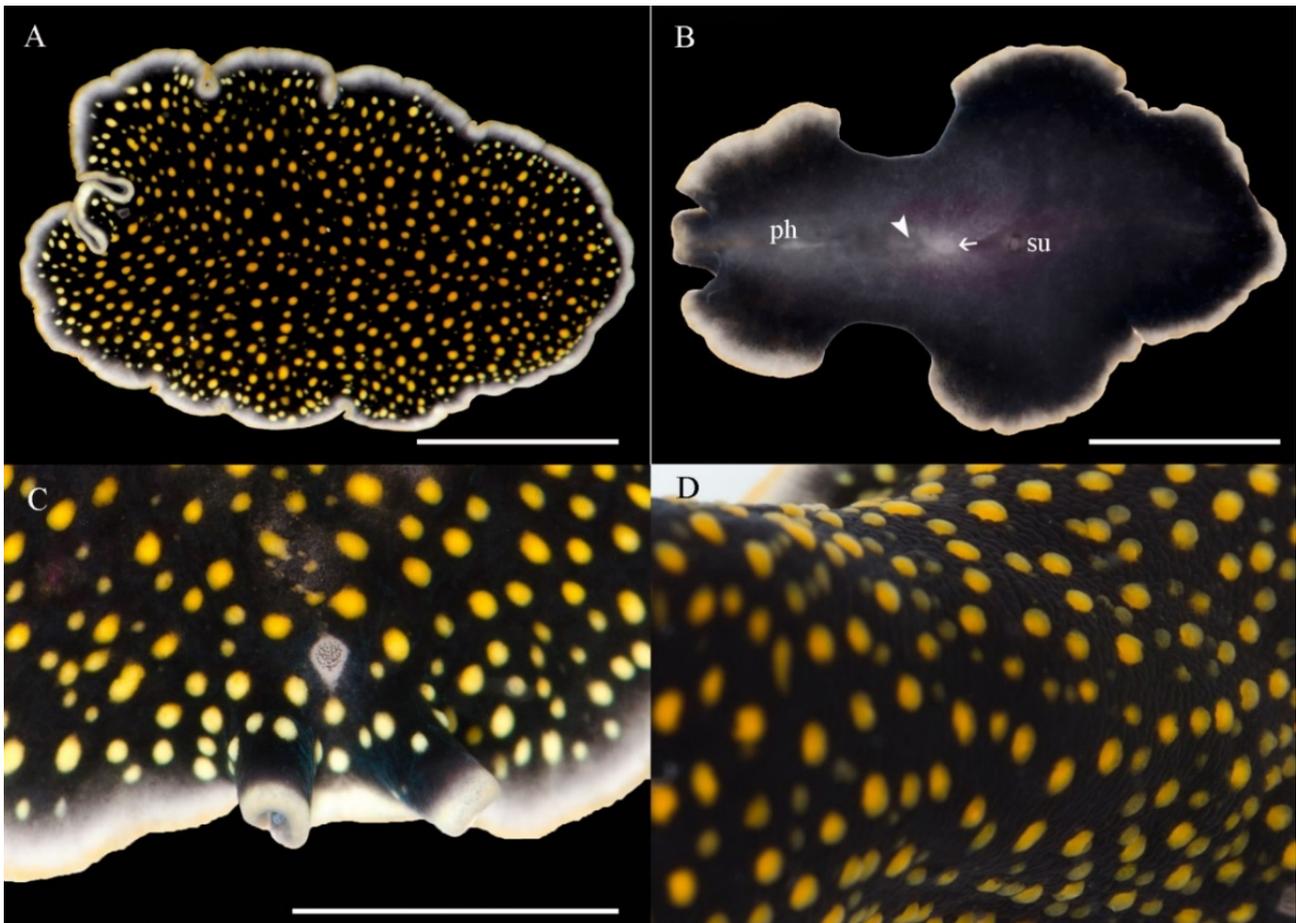


Fig. 6. *Pseudoceros microcelis*, living animal (ZRC.PLA.0179). A, dorsal view of live animal; B, ventral view of live animal, showing the pharynx, male gonopore (arrowhead), female gonopore (arrow), and sucker; C, anterior region showing the simple-folded pseudotentacles and cerebral eyespots in a clear area; D, close-up view of the raised spots on the dorsal surface. Scale bars = 5 mm [A–C]. ph, pharynx; su, sucker. (Photographs by: Rene S. L. Ong).

Taxonomic remarks. *Pseudoceros microcelis* was originally reported by Prudhoe (1989) from a single preserved specimen. The colour of the living animal was described from a watercolour painting as black dorsal surface with numerous yellow spots, bordered by a grey band which is encircled by yellow. Prudhoe (1989: 85, fig. 33) also provided an illustration of this species and enough description of the reproductive organs to correctly place this taxon as a *Pseudoceros*. It is unclear why Newman & Cannon (1994, 1998) did not include *Pseudoceros microcelis* in their lists of valid *Pseudoceros* species. It is possible they could have overlooked it. *Pseudoceros microcelis* is the only *Pseudoceros* species with distinct yellow spots over a black dorsal background and a white margin and yellow rim.

Pseudoceros microcelis can easily be mistaken for *Thysanozoon nigropapillosum* as they share a similar colour and pattern. *Pseudoceros microcelis* has only one male gonopore and lacks papillae on its dorsal surface. However, the latter character needs to be carefully examined because the yellow spots are raised and can give the false impression that these are papillae (see Fig. 6D).

DISCUSSION

The cotyleans are possibly the largest and most diverse group of flatworms found in Singapore waters. The relatively high diversity could be attributed to the wide variety of estuarine and marine habitats found in Singapore. In the north, the Johor Strait contains estuarine environs, with mangroves and mudflats, interspersed with sandbars and rocky shores (Tan et al., 2015). In the south, the Singapore Strait, a channel that connects the Malacca Strait to the South China Sea (Tan et al., 2016) features diverse and often interspersed marine habitats such as sandbars, mudflats, seagrasses, coral reefs, rocky and coral rubble areas. These may have provided the different environments and/or food sources that allow for high species diversity. Accessibility to many of the sites are however, limited and restricted. Submerged reefs can only be accessed during extreme low tides. While at the subtidal regions, the high turbidity of our waters reduced visibility to around 2 m (Guest et al., 2016). This, along with high water current, makes finding and studying polyclads fairly challenging. Nonetheless, we have, during the five-year Comprehensive Marine Biodiversity Survey, managed to sample and document numerous species for a small geographic area.

We are reporting for the first time, the presence of *Thysanozoon nigrum* in the Indo-Pacific region, *Pseudobiceros bajae* beyond the eastern Pacific, and *Pseudoceros microcelis* beyond the eastern Indian Ocean. It is possible that many of the polyclad species are much more wide-spread than reported, and some species may have a cosmopolitan distribution. Furthermore, cotylean larvae are known to be planktonic (Lang, 1884; Prudhoe, 1985; Rawlinson, 2014) which could explain the wide distribution of some species. In addition, Singapore, with its strategic maritime location and heavy shipping traffic, could be a hot-spot for alien organisms transported by ocean-going vessels from other parts of the world. It is possible for some polyclads, such as *Thysanozoon nigrum*, to be non-native to the Indo-Pacific. However, owing to the general neglect in the study of this group, poor recording and limited availability of literature on this taxon, particularly in the Indo-Pacific region, the geographic distribution of many species has not been properly documented. It is therefore not appropriate to make assumptions.

It has been observed that, of the six species described in this report, only *Pseudobiceros stellae* is seasonally abundant (Ong et al., 2015) and often encountered at the intertidal zone, whereas *Pseudobiceros bajae* was encountered at submerged reefs. The sightings of *Pseudobiceros bajae* were far and few possibly because of its preferred habitat, the submerged reefs where we could only access during extreme low tides. *Cycloporous venetus*, *Thysanozoon nigropapillosum*, *Thysanozoon nigrum*, and *Pseudoceros microcelis* had only been recorded and sighted at subtidal zones so far. It is believed that *Pseudoceros microcelis* could probably be more common than recorded as it can be easily mistaken as *Thysanozoon nigropapillosum*.

With these five new records, the estimated number of cotylean species known from Singapore is now 81. However, a large number of these cotyleans have not been identified to the species level. They are being studied and we hope to report on them in the near future.

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