

***Pseudoceros magangensis*: a new species of
pseudocerotid flatworm (Platyhelminthes: Polycladida)
from Taiwan**

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Abstract

A new species of pseudocerotid flatworm, *Pseudoceros magangensis*, is described herein. This species is most abundant in the northeastern coast of Taiwan. Its background color pattern is mottled cream with pink hue, and it possesses reddish-purple, unevenly sized and spaced spots on its margin. Its mottled cream color and marginal spots pattern is similar to the congeners *Pseudoceros indicus*, *P. goslineri*, and *P. concinnus*. However, *P. indicus* has cream white background coloration and blue marginal spots, *P. goslineri* has orange, pink, and brick red dots, and *P. concinnus* has a royal blue medium line. Further anatomical differences between these four species are described in this work. Notably, the male organ develops first in *P. magangensis*, followed by that of the female, and the mating season for this species seems to be between April to July.

Keywords: Polyclad flatworm, *Pseudoceros*, new species

Introduction

Polyclads are free-living turbellarians commonly found in tropical and sub-tropical coral reefs (Bolanos et al., 2007). Pseudocerotids are amongst the most diverse and beautiful flatworms (Newman & Cannon, 2003). The majority of species belonging to the family Pseudocerotidae Lang, 1884 are classified under two genera: *Pseudoceros* Lang, 1884 and *Pseudobiceros* Faubel, 1984 (Hyman, 1954, 1955, 1959; Prudhoe 1985, 1989; Rawlinson & Litvaitis, 2008). The former genus has at least 164 species, of which 129 have been validated (Tyler et al., 2015). *Pseudoceros* are distinguished primarily by their external color patterns (Hyman, 1954, 1959; Prudhoe, 1985, 1989). Cladistic analyses utilizing morphological data have revealed that overall color and color patterns increase the resolution of pseudocerotid genera cladograms (Rawlinson and Litvaitis, 2008).

Newman and Cannon (1994) divided the *Pseudoceros* and *Pseudobiceros* genera found within the Indo-Pacific, which is the region with the highest polyclad diversity, into six groups based on color patterns; such divisions have proven useful for intra- and inter-generic comparisons. Group 1 consists of species with an even background color, Group 2 species have distinct marginal bands, Group 3 worms have longitudinal stripes, Group 4 (the most species group) consists

of species with spots, dots, and/or mottled color patterns, Group 5 species have maculate, and Group 6 species have transverse streaks or stripes. Although it is somewhat arbitrary, 13 species were listed under Group 4, including *Pseudoceros indicus* Newman and Schupp, 2002, *Pseudoceros concinnus* Collingwood, 1876, and *Pseudoceros goslineri* Newman and Cannon, 1994. All three species possess cream mottling color patterns, making them difficult to discern *in situ* (Newman and Schupp, 2002).

On a higher taxonomic level, polyclad flatworms are distinguished by the structure of their reproductive systems (Faubel, 1983, 1984). Faubel (1984) isolated the genus *Pseudobiceros* from *Pseudoceros* based on the finding that *Pseudobiceros* has dual male reproductive systems. However, many pseudocerotids are known for their remarkably uniform reproductive anatomy and hence species diagnosis is based primarily on color patterns (Bolanos et al., 2007), especially in the *Pseudoceros* and *Pseudobiceros* genera (Newman and Cannon, 1994, 1995, 1996, 1998).

All polyclad flatworms are hermaphroditic. Michiel and Newman (1998) first described the copulatory behavior of *Pseudoceros bifurcus in situ* and in the laboratory. During copulation, two animals move toward each other. After a short encounter, they simultaneously evert their penises with

stylets and stab each other to exchange spermatophores through hypodermic insemination, either reciprocally or unilaterally, in a display of behavior known as “penis fencing” (Michiels and Newman, 1998). However, little work has been done on attempting to determine when they mate *in situ*, as well as how much their populations change in density over an annual cycle. In this study, a new polyclad flatworm species, *Pseudoceros magangensis* is described, and its mating behavior in a tide pool was documented. New insight into polyclad flatworm reproductive system development was also obtained upon sectioning three differently sized of *P. magangensis* specimens.

Materials and Methods

This study was mainly conducted within a tide pool at Magang (25°01'N, 121°59'E), which had been formed by erosion of the northeastern coastline of Taiwan (Fig. 1). It is one of the biggest tide pools in in Magang erosion platform, measuring about 15, 10, and 1.5 m in length, width, and depth, respectively. *Pseudoceros magangensis* specimens were also collected from the intertidal and subtidal zones of Wanlitong (21°59'N, 120°42'E) and Hejie (21°57'N, 120°42'E), both in southwestern Taiwan (Pingtung County) (Table 1). *Pseudoceros goslineri*, and *P. concinnus* were also collected and photographed from Magang tide pool. *Pseudoceros indicus* was collected from



Fig. 1. The study areas and collection sites of the newly described flatworm species *Pseudoceros magangensis* new sp.

Table 1. *Pseudoceros magangensis* examined from Taiwan.

| Specimen number | Length | Width | Habitat | Location | Collector | Date | Status |
|------------------------|-------------------------|-----------------------|--------------------|-------------------|-----------|------------------|-----------|
| Holotype | | | | | | | |
| TMPL000009 | 43 mm | 14 mm | tide pool | Magang, Taiwan | WBJ | 21 April 2012 | mature |
| Paratype | | | | | | | |
| TMPL000012 | 33 mm | 15 mm | tide pool | Magang, Taiwan | WBJ | 18 December 2011 | mature |
| TMPL000013 | 27 mm | 18 mm | tide pool | Magang, Taiwan | WBJ | 24 March 2012 | mature |
| TMPL000014 | 41 mm | 10 mm | tide pool | Magang, Taiwan | WBJ | 21 April 2012 | mature |
| Other material | | | | | | | |
| TMPL000010 | 32 mm | 9 mm | tide pool | Magang, Taiwan | WBJ | 27 November 2011 | mature |
| TMPL000011 | 45 mm | 14 mm | tide pool | Magang, Taiwan | WBJ | 27 November 2011 | mature |
| TMPL000015 | 25 mm | 10 mm | Intertidal zone | Wanlitong, Taiwan | SCK | 1 February 2013 | immature |
| TMPL000016 | | | tide pool | Magang, Taiwan | WBJ | 14 April 2012 | dead body |
| TMPL000017 | 11 mm | 4 mm | tide pool | Magang, Taiwan | WBJ | 1 November 2014 | immature |
| TMPL000018 | 15 mm | 6 mm | tide pool | Magang, Taiwan | WBJ | 1 November 2014 | immature |
| TMPL000019 | 21 mm | 9 mm | tide pool | Magang, Taiwan | WBJ | 1 November 2014 | immature |
| TMPL000020 | 35 mm | 11 mm | tide pool | Magang, Taiwan | WBJ | 1 November 2014 | immature |
| average Mature size | 37 mm (2.7~45 mm) | 13 mm (9~18m m) | | | | | |

Note. TMPL = Specimens of National Taiwan Museum for Polyclad.

Table 2. *Pseudoceros indicus* examined from Singapore.

| Specimen number | Length | Width | Habitat | Locality | GPS coordinates | Date |
|-----------------|--------|-------|-----------------|---------------------------|---------------------------|------------------|
| ZRC.PLA.0061 | 14 mm | 7 mm | intertidal zone | Chek Jawa, Singapore | 1° 24.27'N 103° 59.564'E | 19 October 2012 |
| ZRC.PLA.0062 | 21 mm | 6 mm | intertidal zone | Pulau Sekudu, Singapore | 1° 24.283'N 103° 59.302'E | 15 June 2014 |
| ZRC.PLA.0063 | 13 mm | 6 mm | subtidal zone | Lazarus Island, Singapore | 1° 13.317'N 103° 51.170'E | 23 May 2013 |
| ZRC.PLA.0064 | 15 mm | 7 mm | subtidal zone | Pulau Pawai, Singapore | 1° 11.215'N 103° 42.952'E | 29 November 2014 |
| ZRC.PLA.0066 | 15 mm | 4 mm | subtidal zone | Pulau Senang, Singapore | 1°10.201'N 103° 44.421'E | 25 January 2015 |

Note. ZRC.PLA. = The Zoological Reference Collection of Platyhelminthes in Lee Kong Chian Natural History Museum, Singapore

various localities in Singapore (Table 2). Specimens were photographed, either *in situ* or in the laboratory, and underwater photographs were taken while snorkeling in the Taiwanese tide pools. The *P. magangensis* population from Magang tide pool was quantified at least twice each month from November 2012 to December 2013, and their feeding habit and mating behavior was observed from 2012 to 2015.

All specimens were fixed on 10% frozen formalin (diluted with seawater) for at least two days. Specimens were then transferred into 70% ethanol prior to histology. All of the specimens collected from Taiwan were deposited in the National Taiwan Museum (TMPL), Taipei, Taiwan. while *P. indicus* specimens collected from Singapore were deposited in the Zoological Reference Collection

(ZRC. PLA) of Lee Kong Chian Natural History Museum at the National University of Singapore.

For histological analysis, the region of the polyclads containing the reproductive organs was removed, dehydrated with a series of increasing concentrations of ethanol (70, 80, 90, 95, and 99%), embedded in 56°C Paraplast wax (Leica Microsystems), serial sectioned longitudinally at 5–8 µm thickness, and stained with haematoxylin and eosin. The resulting serial sections of *P. magangensis* were deposited in the National Taiwan Museum in Taipei, with those of *P. indicus* deposited in the Lee Kong Chian Natural History Museum.

Results

Family Pseudocerotidae Lang, 1884 偽角扁蟲科

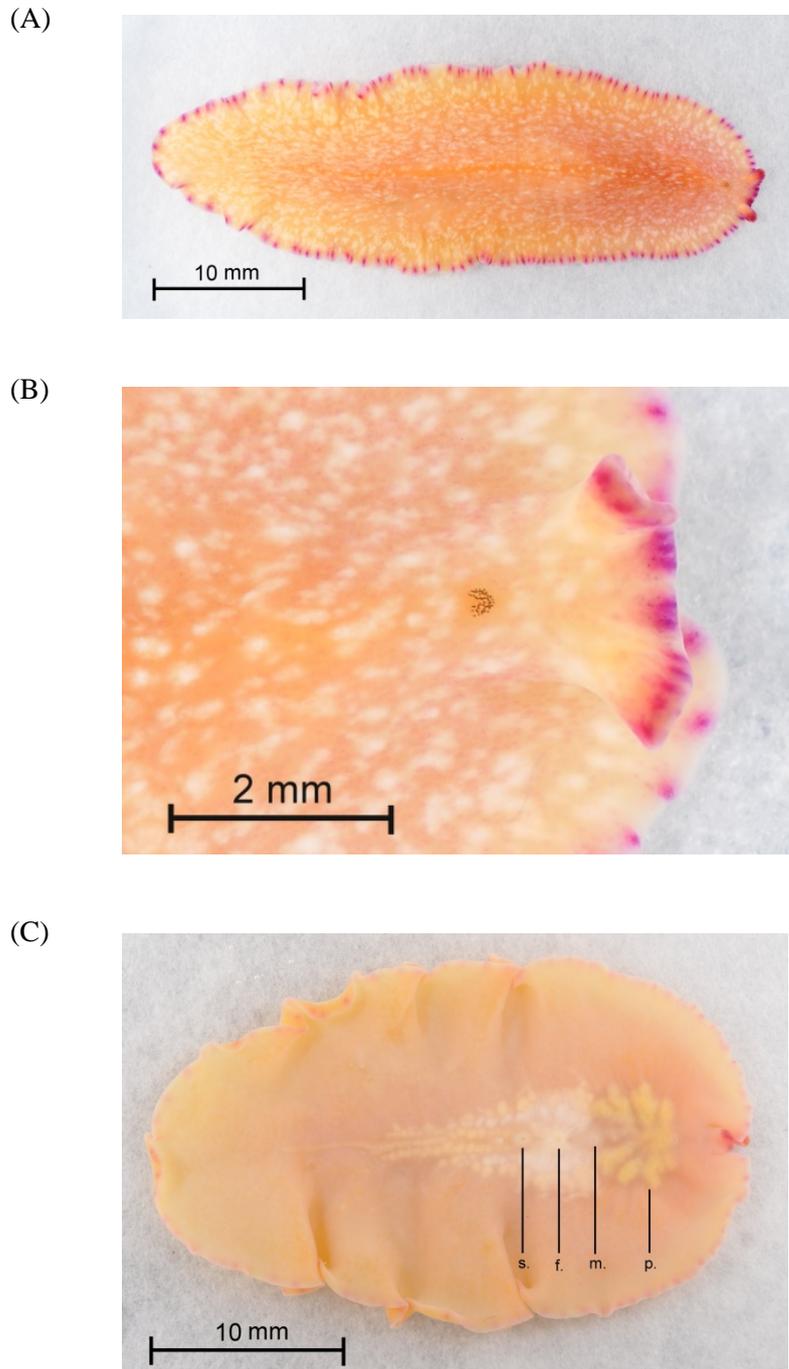


Fig. 2. *Pseudoceros magangensis* new sp. Anterior end to the right. (A) Dorsal view, holotype (TMPL000009). (B) Eyespots (TMPL000014). (C) Ventral view (TMPL000009). f= female pore; m=male pore; p=pharynx; s= sucker.

***Pseudoceros* Lang, 1884 偽角扁蟲屬**
***Pseudoceros magangensis* (new species)**
馬崗偽角扁蟲 (Figs. 2-3, 8A)

Examined materials

Holotype: TMPL000009. The details of holotype and the other examined specimens are listed in Table 1.

Description. Body elongate, oval and leaf-like in shape (Fig. 2A). Background mottled cream with pink hue in median region; pink hue fades towards posterior end. Along its margin are reddish-purple spots, uneven in size and spacing between the spots. Pseudo-tentacles made of simple folds in the middle of the anterior end. Cerebral eyes arranged in a horseshoe-shaped cluster behind the pseudo-tentacles (Fig. 2B). Ventrally cream with a tinge of pink (Fig. 2C). Pharynx highly ruffled and branched. Mouth opening located in the middle of the pharynx. Single male pore posterior to the pharynx, followed by the female pore.

Sucker located posterior to the female pore. Body with an average size of 37 x 13 mm when mature (N = 6, standard deviation = 7.2 and 3.3 mm, respectively).

Reproductive organs. TMPL000009, vas deferens unbranched, seminal vesicle rounded and oblong (1,071 μ m length). Ejaculatory duct long and coiled. Prostate gland round (429 μ m wide). Stylet long and narrow (405 μ m long x 60 μ m wide); stylet length: width ratio = 1:6.75. Male and female antra deep (Fig. 3).

Etymology. Named after the locality where the holotype was found.

Ecology. *Pseudoceros magangensis* is amongst the most abundant flatworms in Magang tide pool (2008-2015, personal data). They were observed to feed on an undetermined species of colonial ascidian (Fig. 4A), which was abundant throughout the year. During feeding, *P. magangensis* typically laid motionlessly over its food. They stretched out its highly ruffled and

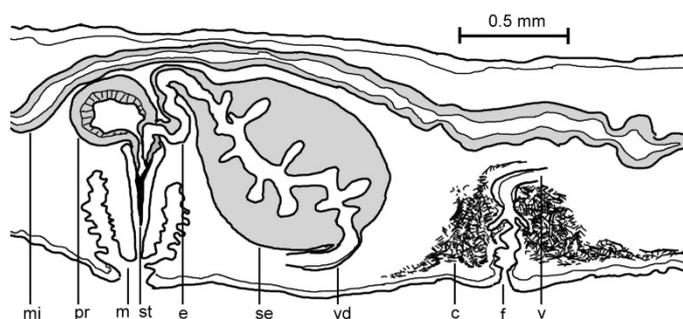


Fig. 3. Diagrammatic representation of the reproductive anatomy of the newly described species *Pseudoceros magangensis* new sp. Anterior end to the left. c=cement gland; e=ejaculatory duct; f=female pore; mi=intestine; m=male pore; pr=prostate gland; se=seminal vesicle; st=stylet; v=vagina; vd=vas deferens.

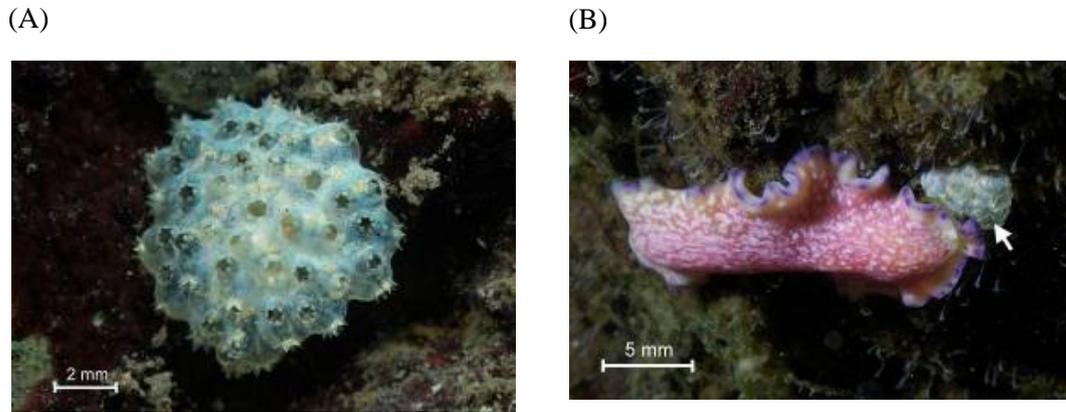


Fig. 4. (A) Unidentified colonial ascidian (photographed in a Magang tide pool) that serves as the main source of food. (B) A *Pseudoceros magangensis* new sp. was feeding colonial ascidian.

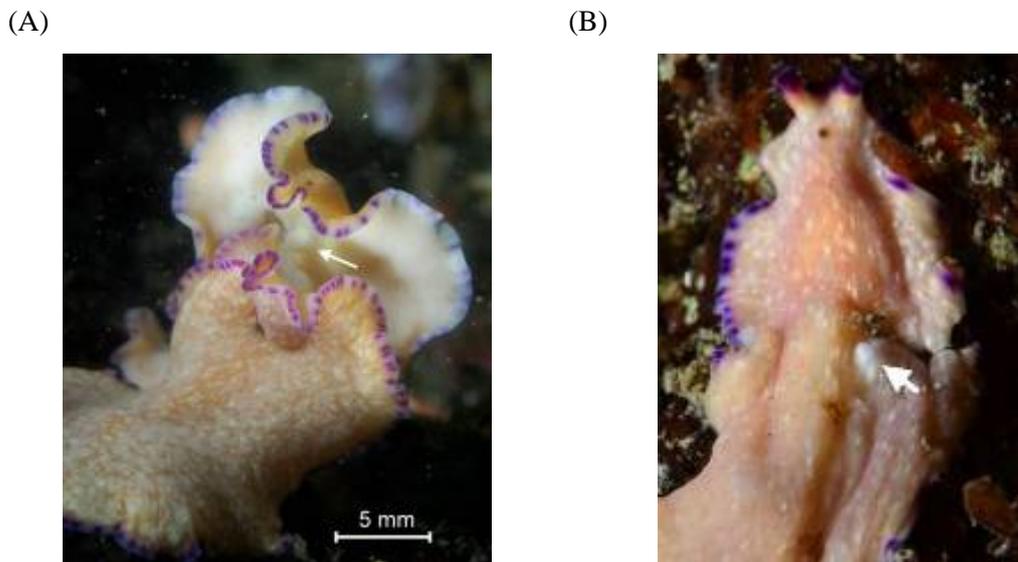


Fig. 5. The newly described species *Pseudoceros magangensis*. (A) Mating (Magang tide pool, April 14th 2013), with everted, outstretched penis indicated by an arrow. (B) Spermatophore (indicated by an arrow) resultant from dermal impregnation (Magang tide pool, June 2nd 2013.).

branched pharynx to slowly digest each zooid of the colonial ascidians. Without careful observation of the polyclad's extended pharynx, it is difficult to discern resting from feeding (Fig. 4B).

Based on twice-monthly observations from November, 2012 to December, 2013 in the Magang tide pool, *P. magangensis* recruitment starts from the beginning of Spring (February, 2013). The highest number of worms was quantified in summer (April to June), though a powerful tropical Typhoon (Soulik) struck the tide pool on July 13th, 2013, and no *P. magangensis* were sighted until November, 11th, 2014.

Reproductive behavior. Two mating sessions were recorded: April 2012 and April 2013. During copulation, two *P. magangensis* worms were observed to move towards and twist around each other in a ventral-to-ventral orientation, while simultaneously everting their stylets (Fig. 5A). They then attempted to inseminate each other. After successful copulation, one or more spermatophores could be seen on the dorsal side of each polyclad (Fig 5B). Between April and June 2012 and April and June 2013, individuals with spermatophores on their dorsal surfaces were commonly observed.

Remarks on color patterns. *Pseudoceros magangensis* is very easily confused with *P. indicus*, both of them have elongate, oval body shapes and mottled cream color patterns. *Pseudoceros magangensis* has a

cream background with a pink hue while the background color of *P. indicus* varies from opaque white to cream. Although both species have similar patterns of unevenly sized and spaced marginal spots around their bodies, *P. magangensis* has reddish purple marginal spots (Fig. 2A) while *P. indicus* has royal blue or dark purple spots (Fig. 7A). Depending on the lighting, the dark purple or reddish purple marginal spots on live animals may look similar.

Due to the mottled cream patterns of *P. goslineri* and *P. concinnus*, the new species may also be easily mistaken as these two species. However, *P. magangensis* can be differentiated by the pink, and mottled cream background color, in contrast to the closely spaced purple and pink marginal irregular dots and spots, brick red dots concentrated midline of *P. goslineri* and the longitudinal, narrow, royal blue medium stripe of *P. concinnus*.

Remarks on anatomy. Three immature individuals (TMPL000018, TMPL000019, TMPL000020) was sectioned for reproductive system research. The male reproductive organs developed prior to the female reproductive system in all three specimens. In serial sections, it is also found that the male and female reproductive organs, other than the testes and ovaries, developed first (Fig.6).

In comparison to the original description of *P. indicus* (Newman & Schupp, 2002),

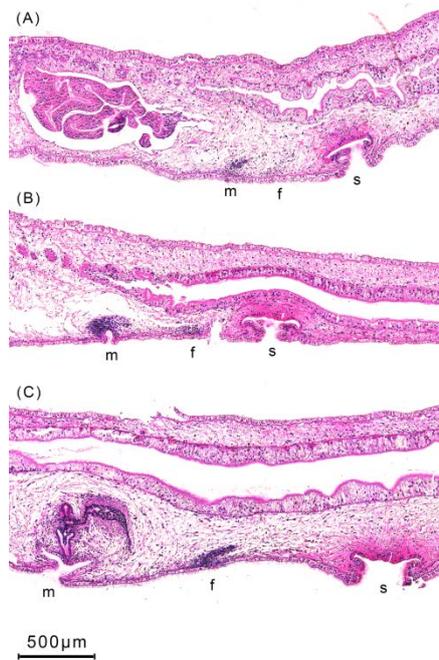


Fig. 6. Sagittal sections of three different sized immature *Pseudoceros magangensis* new sp. (A) TMPL000018, 15 mm length (B) TMPL000019, 21 mm length, and (C) TMPL000020, 35 mm length. The testes and ovaries are not found in specimens. f=female pore; m=male pore; s=sucker.

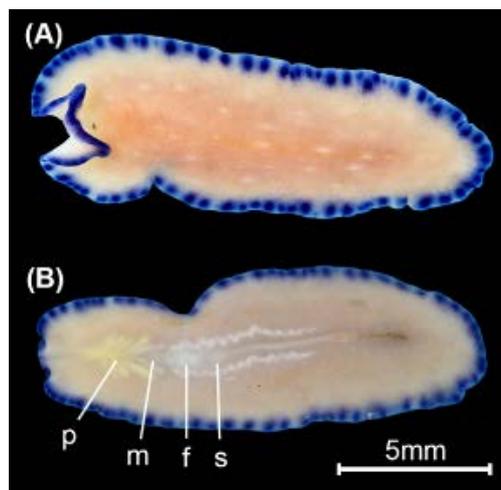


Fig. 7. *Pseudoceros indicus* (ZRC.PLA.0064). Anterior end to the left. (A) Dorsal view. (B) ventral view. f=female pore; m=male pore; p=pharynx; s= sucker.

the reproductive structures of *P. magangensis* are generally similar but larger than those of *P. indicus*. In particular, the stylet of *P. magangensis* is longer and sharper than those of *P. indicus*. According to the reconstruction of the reproductive structures (Fig. 3), the ejaculatory duct of *P. magangensis* is long and coiled, while that of *P. indicus* is short and coiled.

***Pseudoceros indicus* Newman & Schupp, 2002** 藍紋偽角扁蟲
(Fig. 7; Fig. 8B)

Pseudoceros undetermined sp. Stummer-Traunfels, 1933: 3565, Fig.16
Pseudoceros concinnus Hyman, 1954: 220; Prudhoe, 1989: 79, Fig. 21a, b, c
Pseudoceros indicus Newman & Schupp, 2002: 178, Fig. 1-5; Newman & Cannon, 2003, p.74; Newman et al., 2003: 197; Apte & Pitale, 2011: 109; Dixit & Raghunathan, 2013: 166; Sreeraj & Raghunathan, 2013: 39; Bolanos et al., 2016: 157

Examined materials

Examined materials and collecting sites from Singapore are listed in Table 2.

Description. Body elongate, oval, and leaf-like in shape. Background whitish to cream with well-defined and separated royal blue spots along its margin (Fig. 7A). Ventrally cream with a margin of blue

spots (Fig. 7B). Body with an average size of 15.6 x 6 mm when mature (n=5, standard deviation= 1.4 and 0.54 mm, respectively).

Reproductive organs. ZRC.PLA.0062, vas deferens unbranched. Seminal vesicles rounded and oblong (644 µm long). Ejaculatory duct short and coiled. Prostate gland round (237 µm wide). Stylet long and narrow (237 µm long x 34 µm wide); stylet length: width ratio 1:72 (Fig. 8B). Male antrum moderately deep and voluminous. Female antrum shallow.

Comparison of male reproductive organs of *P. magangensis* and *P. indicus*.

Male reproductive organs of *P. magangensis* and *P. indicus* look similar, but the stylets of *P. indicus* are shorter (Fig. 8A-B). Reconstructed histological serial sections of both species' reproductive structures also show that the ejaculatory duct of *P. indicus* is shorter and less coiled than the long, highly coiled one of *P. magangensis*.

***Pseudoceros goslineri* Newman & Cannon, 1994** 柯氏偽角扁蟲
(Fig. 9A-B)

Pseudoceros goslineri Newman & Cannon, 1994: 223, Fig. 16A-C; Gosliner et al., 1996: 106, Fig. 353. Newman & Cannon, 2003, p. 74

Material examined. TMPL000021, length 45 mm, width 14 mm, locality: Magang, New Taipei City, tidal pool, coll.

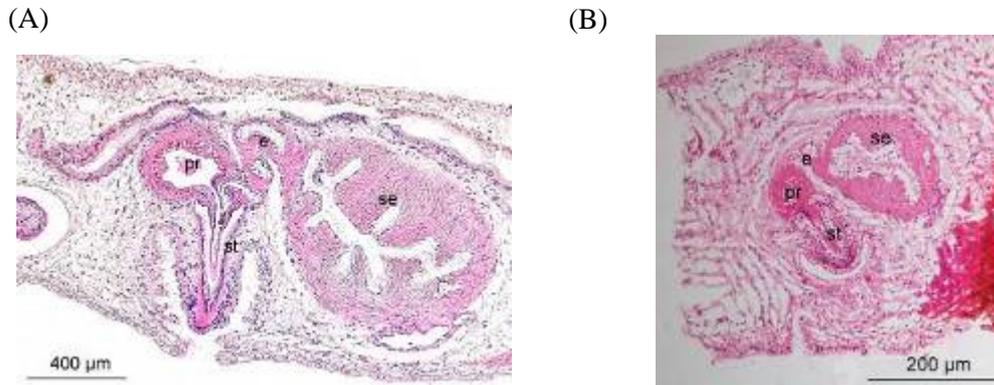


Fig. 8. Histological sections showing male reproductive organs of (A) *Pseudoceros magangensis* new sp. (TMPL000009) and (B) *Pseudoceros indicus* (ZRC.PLA.0062). e=ejaculatory duct. pr= prostate gland. se=seminal vesicle. st=stylet.

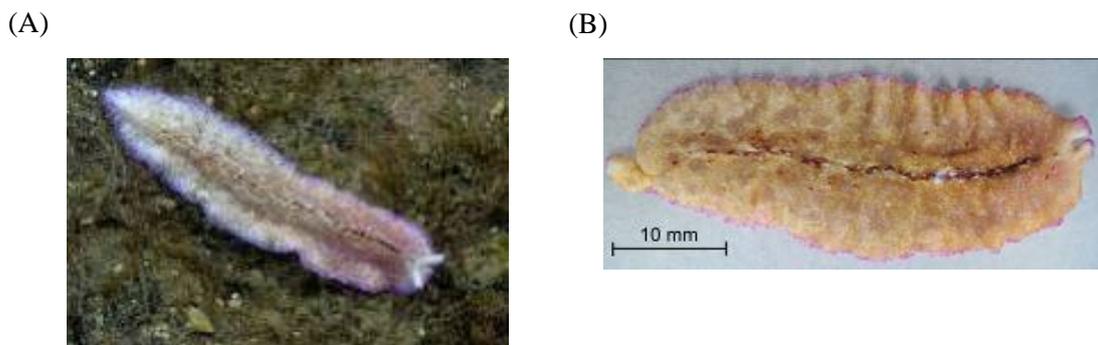


Fig. 9. *Pseudoceros goslineri* (A) *in situ* (Magang tide pool; November 27th 2011). (B) dorsal view of fixed specimen (TMPL000021). Anterior end to the right.

WB Jie, November 27th 2011; TMPL000022, length 41 mm, width 14 mm, locality: Magang, New Taipei City, tidal pool, coll. WB Jie, December 18th 2011; TMPL000023, serial histological sections, length 32 mm, width 10 mm, locality: Wanlitong, Pingtung Country, intertidal, coll. SC Kuo, February 1st 2013; TMPL000024, length 12 mm, width 8 mm, locality: Hejie, Pingtung Country, 5-10 m, coral reef, coll. WB Jie, February 1st 2013; TMPL000025, length 12 mm, width 8 mm, locality: Magang, New Taipei City, tidal pool, coll. WB Jie, November 1st 2014.

***Pseudoceros concinnus* (Collingwood, 1876)** 藍帶偽角扁蟲
(Fig. 10A-B)

Proceros concinnus Collingwood, 1876, p.90 – 91. Fig. 4

Pseudoceros concinnus (Collingwood, 1876) Kaburaki, 1923: 643; Hyman, 1954:221-223, Fig. 1; Newman & Cannon, 2003:73; Dixit & Raghunathan, 2013: 166; Sreeraj & Raghunathan, 2013: 4; Bolanos et al., 2016: 155

Material examined. TMPL000026, serial histological sections, length 39 mm, width 22 mm, locality: Magang, New Taipei City, tidal pool, coll. WB Jie, February 24th 2013. TMPL000027, length 38 mm, width 19 mm, locality: Wanlitong, Pingtung Country, intertidal, coll. WB Jie, April 4th 2015.

The differences between the color patterns and reproductive structures of these four similar-looking *Pseudoceros* species are listed and compared in Table 3.

(A)



(B)

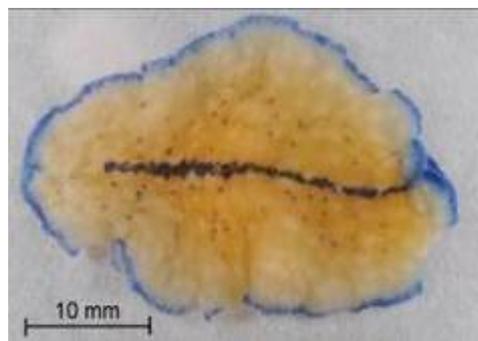


Fig. 10. *Pseudoceros concinnus* (A) *in situ* (Magang tide pool, February 24th 2013). (B) dorsal view of fixed specimen (TMPL000026). Anterior end to the right.

Table 3. Comparison of color patterns and reproductive structures of four *Pseudoceros* species.

| | <i>P. magangensis</i> (new sp.) | <i>P. indicus</i> | <i>P. goslineri</i> | <i>P. concinnus</i> |
|----------------------------|--|---|--|--|
| Color group | Spots, Dots, & Mottling (Group 4)* | Spots, Dots, & Mottling (Group 4)* | Spots, Dots, & Mottling (Group 4)* | Spots, Dots, & Mottling (Group 3)** |
| Background | Mottling cream, pink centrally | White to cream | Mottling cream | Mottling cream |
| Longitudinal stripe | No | No | No | Blue, narrow, medium stripe |
| Spots/dots | No | No | Orange, pink, and brick red dots | None or few |
| Marginal spots | Reddish purple, uneven in size and spacing between spots | Royal blue or dark purple, uneven in size and spacing between spots | Purple, pink, irregular, closely spaced spots and dots | Royal blue |
| Reproductive organs | | | | |
| Reproductive Organ | Single male and female pore | Single male and female pore | Single male and female pore | Single male and female pore |
| Male antrum | Deep | Deep | Deep | Deep |
| Seminal vesicle | Rounded oblong | Rounded oblong | Elongated | Elongated |
| Ejaculatory duct | Long, coiled | Short, coiled | Short, coiled | Coiled |
| Prostate gland | Round | Round | Round | Round |
| Stylet | Long, narrow (1:6.75 [#] *) | Long, narrow (1:7.2 [#] *) | Short | Long, narrow |
| Female antrum | Deep | Shallow | Deep | Deep |

* Color pattern groups for recognizing *Pseudoceros* spp. proposed by Newman & Canon (2002).

** Group pattern changed by Bolanos et al.(2016).

[#] Stylet length:width ratio.

Discussion

In this study, we have described a new species of *Pseudoceros*, *P. magangensis*, which has a similar color pattern to that of the congeneric *P. indicus*, *P. goslineri* and *P. concinnus*. Indeed, *P. magangensis* was likely misidentified as *P. indicus* in the past when only photographs were assessed. Hence, Newman and Schupp (2002) emphasized the importance of examining living specimens to accurately record their color patterns. In terms of anatomical structure, *P. magangensis* can also be distinguished from *P. indicus* in that the former has a longer and sharper stylet, as well as longer and more coiled ejaculatory ducts.

Pseudoceros indicus is widely distributed throughout the Indo-Pacific and can be found in eastern Australia and Micronesia (Newman & Schupp, 2002). We found that it is very abundant in Singapore but rare in Taiwan. Thus far, *P. magangensis* has only been sighted in Taiwan. The habitats of this two species are also different. *P. indicus* was reported to be abundant 1) around mangrove roots in Chuuk, Micronesia, 2) under rocks covered with live and dead oysters in Moreton Bay, Queensland, and 3) in mud flats of Queensland, though rare amidst coral rubble in Lizard Island Lagoon (northern Great Barrier Reef), Ningaloo Reef, Australia and Madang, Papua New Guinea (Newman & Schupp, 2002). In contrast, *P. magangensis* has only been

found in shallow rocky seashore area, especially of Northeast coast of Taiwan.

P. magangensis was observed to feed on colonial ascidians (Fig. 4), and its population fluctuated over the observation period. This fluctuation is similar to the population growth pattern of stylochid flatworms, oyster parasites also known as “oyster leeches” (Shu & Lin, 1980). The stylochid flatworm population grows when the density of oysters peaks during summer, and its population drops dramatically after the oysters have been harvested or before winter comes. We found that the mating season of *P. magangensis* in the Magang tide pool is from April to July. However, spawning was not observed. The Magang tide pool population of *P. mangangensis* was wiped out by Typhoon Soulik on July 13th 2013. Although the colonial ascidians were still abundant in the pool, polyclad recruitment was not observed again until November 1st 2014. The fluctuation in the number of *P. mangangensis* worms in the Magang tide pool suggests that this species’ density is highly influenced by seasonal and local climate shifts.

Mature specimens have both well developed male and female organs, and in general, mature flatworms can be easily distinguish from juveniles; the former have swollen vas deferens that are easily seen from ventral side. This characteristic will be useful for collecting mature flatworms as voucher specimens.

Acknowledgements

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