RAFFLES BULLETIN OF ZOOLOGY **73**: 348–384
Date of publication: 1 September 2025
DOI: 10.26107/RBZ-2025-0025
http://zoobank.org/urn:lsid:zoobank.org:pub:7AC418B0-6C58-43B4-B53A-02721AB14262

Flatworms of Singapore: molecular phylogeny of Polycladida (Platyhelminthes: Rhabditophora) and an annotated checklist of Cotylea species

Samuel Yong Jie **Diong**[1*], Rene S. L. **Ong**[2], Samantha J. W. **Tong**[3], Tashfia **Raquib**[2], Yin Cheong Aden **Ip**[1,4], Danwei **Huang**[1,2,3] & Jia Jin Marc **Chang**[1,2]

Abstract. The taxonomy of polyclads has been a subject of contention due to the inconsistent emphases on different morphological traits. While past molecular phylogenetic analyses have illuminated relationships amongst suborders and several families, uncertainties persist at the lower taxonomic levels. This study analysed 270 sequences of 28S rRNA from 135 species to gain insights into many of these relationships, particularly at lower taxonomic levels. The results recovered the monophyly of the suborders Acotylea and Cotylea, and of the cotylean families Pseudocerotidae and Prosthiostomidae. However, the family Euryleptidae and the genera *Pseudobiceros*, *Nymphozoon*, and *Thysanozoon* were shown to be non-monophyletic. Euryleptidae was split into two distinct clades while *Pseudobiceros*, *Nymphozoon*, and *Thysanozoon* have other genera nested within. The analysis recovered a clade with *Thysanozoon* and *Acanthozoon* that was sister to the clade containing *Pseudobiceros*, *Phrikoceros*, and *Nymphozoon*. The synonymies of *Tytthosoceros* and *Phrikoceros*, *Pseudobiceros hymanae*, and *Pseudobiceros splendidus*, as well as *Pseudoceros duplicinctus* and *Pseudoceros prudhoei* were rejected; our results supported their distinction and so they remain separate. New *Tytthosoceros* combinations were established from the phylogeny. The diagnosis of *Pseudoceros prudhoei* was also emended. Importantly, the study generated 26 novel cotylean sequences, further enriching our understanding of this diverse order.

Key words. Polycladida, phylogeny, Pseudocerotidae, Euryleptidae, Prosthiostomidae, Cotylea

INTRODUCTION

Polycladida is an order of free-living flatworms in the phylum Platyhelminthes. There are over 1,000 described living species within Polycladida, most of which are found in the marine environment (Tyler et al., 2022). Only two species of polyclads in the suborder Acotylea are known from non-marine environments, freshwater *Limnostylochus borneensis* and semi-terrestrial *Myoramyxa pardalota* (Ramos-Sánchez et al., 2019). These dorsoventrally flattened acoelomate worms display a wide array of colours and patterns on their dorsal surfaces. Polyclads found in the tropics and subtropics tend to display bright and conspicuous colourations, while those in higher latitudes tend to have duller colours (Bulnes, 2010).

The phylogeny of polyclads has been contested at nearly every taxonomic level apart from the suborders. The order Polycladida comprises two suborders, Acotylea and Cotylea (Lang, 1884), which are distinguished based on the absence and presence of ventral suckers, respectively. However, recent findings have suggested exceptions to this diagnostic criterion such as the families Cestoplanidae and Theamatidae (Bahia et al., 2017; Dittmann et al., 2019; Litvaitis et al., 2019).

A better understanding of the polyclad phylogeny based on DNA sequence data has led to taxonomic revisions and a more data-supported classification. Many recent studies used molecular markers to establish the interrelationships within Polycladida (Litvaitis et al., 2010; Rawlinson et al., 2011; Aguado et al., 2017; Bahia et al., 2017; Tsunashima et al., 2017; Litvaitis et al., 2019). The 28S ribosomal DNA (rDNA) is one of the most common phylogenetic genetic markers used for this purpose, although other molecular markers such as the 16S and 18S rDNA (Katayama et al., 1993; Aguado et al., 2017; Oya & Kajihara, 2020; Oya et al., 2020; Oya & Kajihara, 2021) have been used in various studies. Mitochondrial data, specifically the sequences of cytochrome c oxidase subunit I (COI) gene, have also been used for the analysis of phylogenetic relationships within Polycladida. However, the lack of universal primers for the mitochondrial

Accepted by: Lim Swee Cheng

[1]Department of Biological Sciences, National University of Singapore, 16 Science Drive 4, Singapore 117558, Singapore; Email: samdiong99@gmail.com (*corresponding author)

[2]Lee Kong Chian Natural History Museum, National University of Singapore, 2 Conservatory Drive, Singapore 117377, Singapore

[3]Tropical Marine Science Institute, National University of Singapore, S2S, 18 Kent Ridge Road, Singapore 119227, Singapore

[4]School of Marine and Environmental Affairs, University of Washington, Seattle, Washington 98105, United States

ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

marker in polyclads limited COI gene sequencing to only a handful of species, with only a few studies having sequenced and analysed the whole mitogenome (Kenny et al., 2019; Yonezawa et al., 2020). Another study by Goodheart et al. (2023) made use of transcriptomic data to reconstruct the polyclad phylogeny, providing another strong basis for understanding species relationships. With more genetic data becoming available, the analysis of multiple gene markers can provide better resolution of the phylogenetic relationships and more precise taxonomic classification within the order.

Studies on the polyclad molecular phylogeny have shown that previous morphological phylogenies were not able to adequately represent the relationships within the group and have challenged the classification of some families within these suborders. For example, Cestoplanidae and Theamatidae, which were traditionally placed in Acotylea due to possessing traditional acotylean traits, were found to be basal clades clustered within Cotylea in some analyses but could also be in Acotylea depending on the genetic markers used (Bahia et al., 2017; Dittmann et al., 2019; Litvaitis et al., 2019; Goodheart et al., 2023). Bahia et al. (2017) proposed that this incongruence challenged the traditional clusters of the suborders, but also noted that the monophyly of both Acotylea and Cotylea was still supported by all studies. Multiple studies have placed Theamatidae, previously placed under Acotylea, into a Cotylea clade containing the families Boniniidae and Amyellidae (Dittmann et al., 2019; Litvaitis et al., 2019). However, despite such strong support for this clade, there seem to be no phylogenetically relevant characteristics distinct to these families to elevate Boninioidea to superfamily level (Dittmann et al., 2019; Litvaitis et al., 2019).

Bahia et al. (2017) also suggested revisions to many traditional superfamilies within Polycladida as the molecular phylogeny showed that they are non-monophyletic. New diagnoses and classification of superfamilies were suggested and one such example is the superfamily of Pseudocerotoidea. Pseudocerotoidea was described by Faubel (1984) to include families with ruffled pharynx but was later found to be paraphyletic, which prompted the revision of the superfamily to include Euryleptidae and Pseudocerotidae, and the diagnosis to be an oval-shaped body, cerebral and tentacular eyespots, and developed marginal tentacles. These superfamilies were also supported by Dittmann et al. (2019), who also suggested revisions to the paraphyletic family of Euryleptidae. The proposed revision sought to retain the family name for the clade containing *Cycloporus japonicus*, *Cycloporus variegatus*, *Maritigrella*, and *Prostheceraeus*, while proposing a new family, Stylostomidae, which includes at least *Cycloporus gabriellae*, *Euryleptodes*, and *Stylostomum*. Despite these results by Dittmann et al. (2019) and Bahia et al. (2017), Litvaitis et al. (2019) and Dittmann et al. (2023) reconstructed a monophyletic *Cycloporus* clade in their study. This has led to conflicting results for the revision suggested by Dittmann et al. (2019), so further clarification is required.

Other studies have also attempted to resolve some of the relationships amongst some genera within Pseudocerotidae, such as *Acanthozoon*, *Phrikoceros*, *Pseudobiceros*, *Pseudoceros*, *Thysanozoon*, and *Yungia*, but have failed to come to a consensus (Tsunashima et al., 2017; Litvaitis et al., 2019; Goodheart et al., 2023). Pseudocerotidae can be distinguished from other cotylean families by morphological traits such as the type of pharynx, pseudotentacles, arrangement of eyespots, and number of gonopores. Most studies have shown that *Pseudoceros* is monophyletic and sister to the other clade containing *Acanthozoon*, *Phrikoceros*, *Pseudobiceros*, *Thysanozoon*, *Tytthosoceros*, and *Yungia*. However, the monophyly and relationship between these genera are still in contention, with Litvatis et al. (2019) supporting the monophyly of *Phrikoceros* and *Thysanozoon*, but Tsunashima et al. (2017), Bahia et al. (2017), and Dittmann et al. (2019) showing the opposite.

Many of these studies focused on the higher taxonomic levels, such as the superfamilies or families, and used representative genera to determine higher classifications, resulting in many uncertainties amongst the lower classification levels. There have been analyses focusing on the lower taxonomic levels of genus and species but the taxonomic coverage of species within the order remains limited. Therefore, more comprehensive sampling and analyses are needed to fully understand the evolutionary history and diversity of Polycladida.

Singapore hosts a tropical coastal environment where most known polyclad species are cotyleans, which are known to be more diverse and abundant in warmer regions (Bulnes, 2010; Bolaños et al., 2016). In recent years, there have been several published studies on the diversity of polyclads in Singapore waters. The most comprehensive biodiversity study of polyclads in Singapore by Ong & Tong (2018) places the number of described species at 38, with another 81 distinct but unknown species recorded. In 2020, another seven new cotylean records were documented (Ong et al., 2020). Of the estimated total of 126 species found in Singapore, 88 are from Cotylea and the remaining 38 are from Acotylea. There have been two studies by Tong & Ong (2020) and Chim et al. (2015) that examined the reproduction of polyclads, from unique mating behaviour to parental care and embryonic development. The molecular phylogenetic study of Singapore polyclads by Lim (2016) which used 24 locally collected samples and analysed a total of 48 species, was hitherto the first and only molecular phylogenetic study of local polyclads that provided insight into the lower taxonomic relationships within the cotylean families of Pseudocerotidae and Prosthiostomidae despite its limited scope. These previous studies collected numerous specimens for later DNA work, some of which are used in the present study to investigate the molecular phylogeny of polyclads in Singapore.

Here, we analyse the phylogeny of polyclads based on 182 specimens from Singapore representing 56 putative

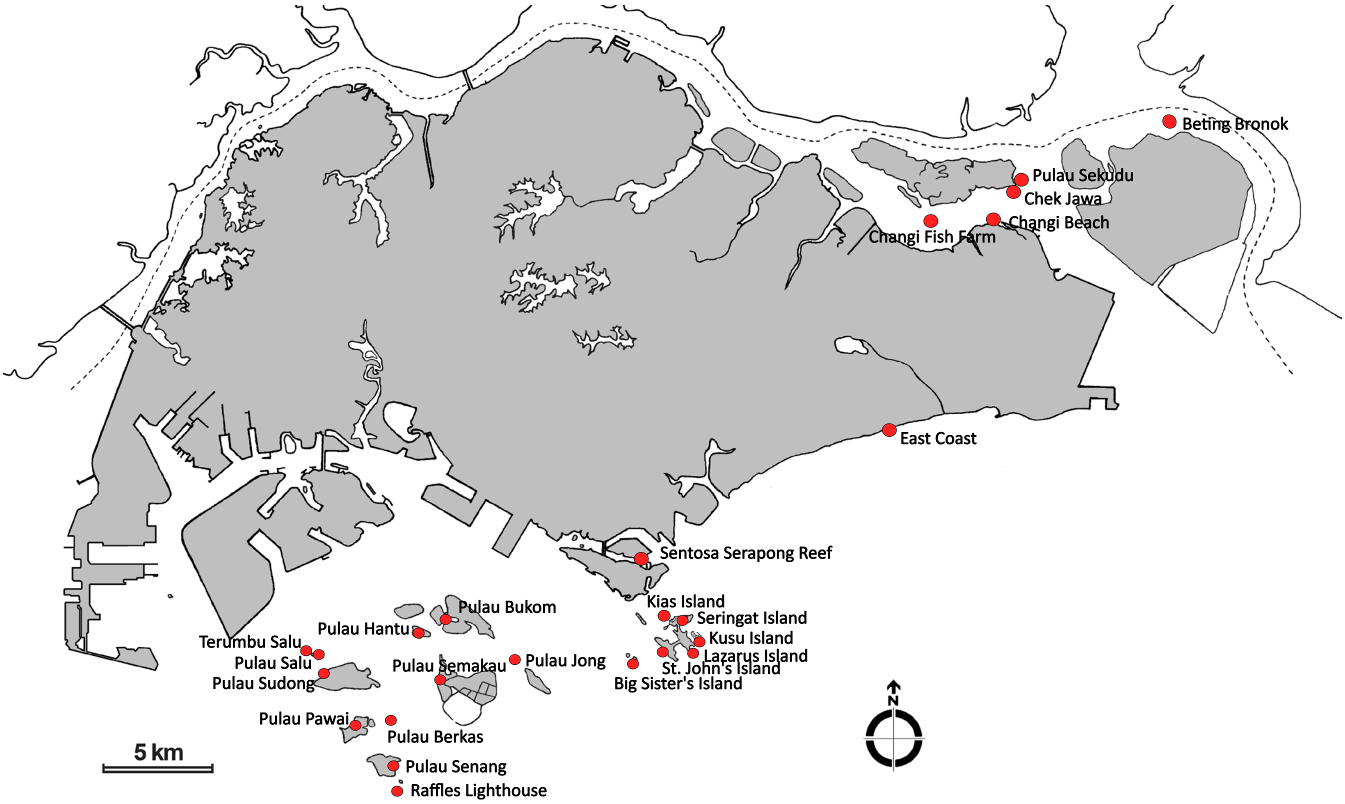

Fig. 1. A map of Singapore showing the sampling locations of polyclad specimens used in this study.

species across three cotylean families and three acotylean superfamilies. We sequence the 28S rRNA gene to reconstruct a species-rich phylogeny for comparison with past studies and current taxonomic classification. We expect that the comprehensive collection in this study, in conjunction with molecular phylogenetic analysis, will lead to better understanding of the flatworm phylogeny and the discovery of potential new species. Our results reveal 15 lineages that may constitute species new to science and further contribute 28S rRNA sequences from 26 polyclad species which have never been sequenced, inclusive of the 15 potential new species. More broadly, our study enhances the understanding of polyclad biodiversity in Singapore and provides support for an annotated checklist that is produced herein to document the diversity of this fascinating clade.

MATERIAL AND METHODS

Specimen collection and morphological identification. Polyclad specimens were either obtained from the Comprehensive Marine Biodiversity Survey (2011–2015), or collected by the authors at various intertidal, subtidal, and offshore dive locations in Singapore between 2014 and 2023 (Fig. 1). All specimens have been accessioned into the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum (LKCNHM), Singapore.

The flatworms were hand-collected with a soft paintbrush from various intertidal and subtidal habitats. Specimens were kept in individual containers for transportation. In the laboratory, they were placed in glass petri dishes, measured, and photographed using either a Nikon D800 digital SLR camera fitted with 60-mm macro lens and SU-800 flash system, or a Canon EOS 5D digital SLR camera with MP-E65 macro lens and 430EX II flash system. Flatworms were kept alive for a few hours for closer observation and documentation. For each polyclad specimen, a tissue subsample, approximately 2–5 mm in diameter, was cut from the edge for DNA extraction, after which they were preserved by being coaxed onto a piece of filter paper immersed in sea water, and then placed on a frozen block of 10% buffered formalin together with the filter paper. The specimens were left in the fixative for 24–48 hours before transferring to 70% ethanol. Tissue subsamples that were not immediately used for extraction were stored in 99% ethanol at -20ºC.

A total of 182 samples of polyclad flatworms were analysed in this study. Specimen identification to the lowest taxonomic level was conducted based on published morphological traits, referencing previous records in Singapore (Ong et al., 2015; Bolaños et al., 2016; Ong et al., 2018; Ong & Tong, 2018). Within Cotylea, the major characteristics used for identification to genus were the type of pharynx, cerebral eyespot arrangement, number of gonopores, and pseudotentacle characteristics. Colouration and pattern of the dorsal surface were used for identification to species level, based primarily on the colour and pattern system established by Newman & Cannon (1994, 1996a, 1996b, 1997, 1998). As histology was not conducted for the specimens, only some acotyleans with distinctive key morphology were identified to family level. The majority of the Acotylea specimens were only identified as acotylean, with a morphospecies number assigned, based on the general morphology of the ventral surface, arrangement of eyespots and tentacle characteristics (if any). Unidentified species are referred to by the nomenclature in Ong & Tong (2018) for consistency.

DNA extraction, amplification and sequencing. Genomic DNA from the subsampled tissues was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc.) according to manufacturer protocols. A partial fragment of the 28S rRNA (~1000 bp) gene was amplified through polymerase chain reaction (PCR) using 28S universal primers (LSU fw1: 5′-AGCGGAGGAAAAGAAACTA-3′; LSU rev2: 5′-ACGATCGATTTGCACGTCAG-3′) following Sonnenberg et al. (2007). Each 25 µl PCR reagent mixture contained 1.0 µl of gDNA extract, 12.5 µl of GoTaq Green Master Mix (Promega, Madison, Wisconsin), 1.0 µl of bovine serum albumin (BSA), 8.5 µl of water and 1.0 µl each of both forward and reverse primers. The mixtures then underwent a thermocycling profile of 94°C for 4 minutes, followed by 45 cycles of 20 seconds at 94°C, 20 seconds at 52.5°C, 90 seconds at 72°C, and 8 minutes final extension at 72°C. PCR success was confirmed through agarose gel electrophoresis on 1% agarose gels stained with GelRed (Biotium).

Successful 28S amplicons underwent purification, cycle sequencing with BigDye Terminator 5X Sequencing Buffer v3.1 and BigDye Terminator v3.1 (Applied Biosystems, Waltham), and Sanger sequencing on an ABI 3130XL DNA Analyzer (ThermoFisher Scientific) at Axil Scientific Pte Ltd. Sequencing reads were assembled and checked using Geneious Prime 2023.2 (https://www.geneious.com). A total of 138 specimens were successfully sequenced and used in the following phylogenetic analysis. The 28S rRNA sequences have been uploaded to NCBI GenBank (accession numbers PQ863115–PQ863252).

Phylogenetic analysis. The 28S rRNA sequences were consolidated and organised in a data matrix using Mesquite v3.81 (Maddison & Maddison, 2023) for alignment and the supplementation of GenBank sequences. Sequences were also searched against the online NCBI (National Center for Biotechnology Information) GenBank database with the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990; Camacho et al., 2023) on 7 December 2023 to determine preliminary identifications. The GenBank sequences representing identities with the highest scores with our unidentified specimens were recorded and added to the matrix. A total of 132 28S rRNA sequences (Supplementary Material), from BLAST sequences of identities with the highest scores and sequences referenced from Litvaitis et al. (2019), were obtained from GenBank for a total of 270 sequences. *Paromalostomum fusculum* (KP730517) and *Microstomum papillosum* (FJ715316) were added as outgroups as they were previously shown to be closely related to Polycladida (Karling, 1974; Katayama et al., 1996; Carranza et al., 1997; Litvaitis & Rohde, 1999; Baguñà & Riutort, 2004; Laumer & Giribet, 2014; Egger et al., 2015; Laumer et al., 2015;). All sequences were aligned with MAFFT v7.520 with G-INSI-I algorithm (Katoh et al., 2002; Katoh & Standley, 2013).

jModeltest v2.1.10 (Posada, 2008) was run to determine the best nucleotide substitution model for the 28S rRNA data. It returned a result of GTR+G+I which was the model used in the subsequent analyses. The maximum likelihood (ML) tree was reconstructed using RAxML-NG v1.2.1 (Kozlov et al., 2019), running 50 random starting trees. Node supports were assessed by bootstrapping the ML tree with 1,000 non-parametric replicates. Bayesian inference (BI) was performed using MrBayes v3.2.7a (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012), producing two runs of 12 million generations of four chains of Markov chain Monte Carlo (MCMC) with a sample frequency of 100 generations. Following assessments of run convergence based on the average standard deviation of split frequencies (<0.05) (Ronquist et al., 2012) and Tracer v1.7.1. (Rambaut et al., 2018), the first 20,001 sampled trees were discarded as burn-in. A total of 100,000 sampled trees remained that were summarised with majority-rule consensus.

RESULTS

A total of 270 sequences representing 135 species were included in the analysis, of which 59 were species collected in Singapore. These 59 species make up about 47% of the total species recorded in Singapore.

The phylogenetic tree presented in this study contains more cotylean species and is focused on the three families Euryleptidae, Prosthiostomidae, and Pseudocerotidae (Figs. 2, 3). Pseudocerotidae (Fig. 3) is the most common and abundant family found in Singapore waters and is the largest family represented in this study. Due to the lack of information on acotyleans (Fig. 4), there is an underrepresentation of local acotyleans, and most of the species within Acotylea used in the analysis were obtained from GenBank.

The results from analyses with maximum likelihood (ML) and Bayesian inference (BI) exhibited high congruence for both suborders. However, differences between the two analyses were observed, with greater discrepancies observed in the nodes of the Acotylea clade. Here, only the tree topology from the ML analysis is reported with the inclusion of branch support values from both analyses.

This study successfully recovered monophyletic suborders with high support values for the suborders Acotylea (maximum likelihood bootstrap support, BS=69, Bayesian posterior probability, PP=1) and Cotylea (BS=64, PP=1) (Fig. 2). Within Cotylea, Pericelidae is shown to be sister to the clade containing the other families found in Singapore (Fig. 2). The node splitting Prosthiostomidae from Euryleptidae and Pseudocerotidae shows moderate support by ML but is highly supported by BI (ML=54, PP=1) (Fig. 2).

In total, 31 species remained unidentified, with 16 species from Acotylea and 15 from Cotylea. Comparisons of existing records with the gross morphology, colouration, and pattern of the unidentified cotylean species did not

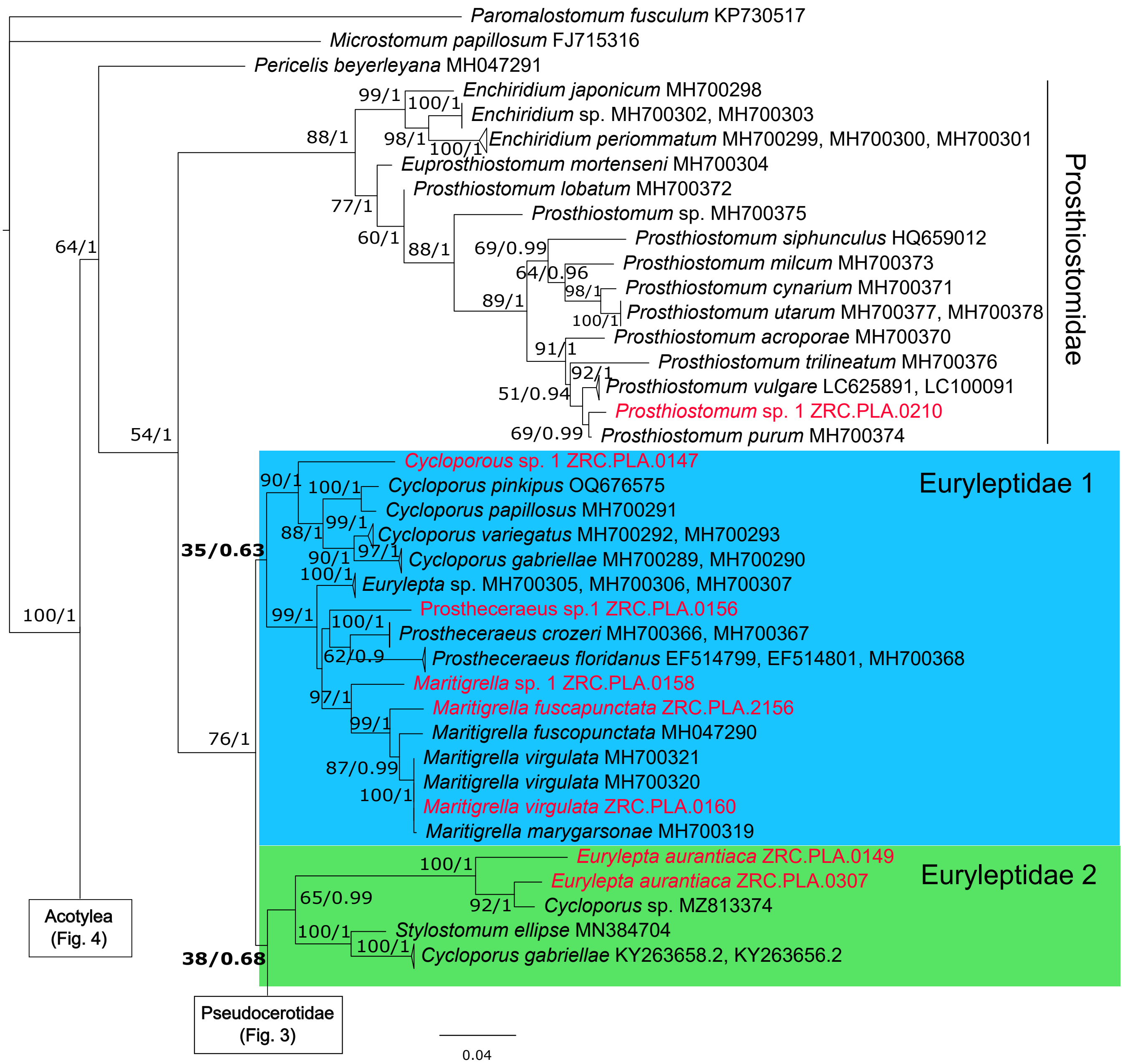

Fig. 2. Maximum likelihood phylogeny of Polycladida based on 28S rRNA gene sequences, displaying the relationships between the main groups within the order. Monophyletic species are represented by a single collapsed tip, with their identification numbers indicated. Taxa highlighted in red represent sequences generated in this study. The Pseudocerotidae and Acotylea branches of the tree are in separate figures, Fig. 3 and 4 respectively. Support values for each branch are given as maximum likelihood (ML) bootstrap support (≥50) and Bayesian posterior probability (≥0.85) in the format (ML/PP).

find any matches for these 15 cotylean species. Further investigations may show that they are new and do not yet have a formal description. The 16 Acotylea species were either undescribed or unidentified due to the lack of histological data. Furthermore, this study obtained novel 28S sequences for 26 Cotylea species, of which 11 are known: *Eurylepta aurantiaca*, *Nymphozoon bayeri*, *Phrikoceros baibaiye*, *Pseudobiceros bajae*, *Pseudobiceros fulgor*, *Pseudobiceros hymanae*, *Pseudoceros concinnus*, *Pseudoceros laingensis*, *Pseudoceros prudhoei*, *Pseudoceros rubrotentaculatus*, and *Pseudoceros scintillatus*.

Cotylea. A total of 97 species from 17 genera in Cotylea were analysed in this study, including 39 species from Singapore (Figs. 5–22). These species represent four families in Cotylea: Euryleptidae, Pericelidae, Prosthiostomidae, and Pseudocerotidae, which were part of three of the revised superfamilies recognised by Bahia et al. (2017), i.e., Euryleptidae and Pseudocerotidae within Pseudocerotoidea, and Prosthiostomidae and Pericelidae as the only family in Prosthiostomoidea and Periceloidea, respectively (Fig. 2).

Pseudocerotidae (BS=83, PP=1) and Prosthiostomidae (BS=88, PP=1) were recovered as clades with strong support values (Figs. 2, 3). Euryleptidae was non-monophyletic and divided into two distinct clades (Fig. 2). The first clade (henceforth referred to as Euryleptidae 1) contained the monophyletic genera *Cycloporus*, *Maritigrella*, and

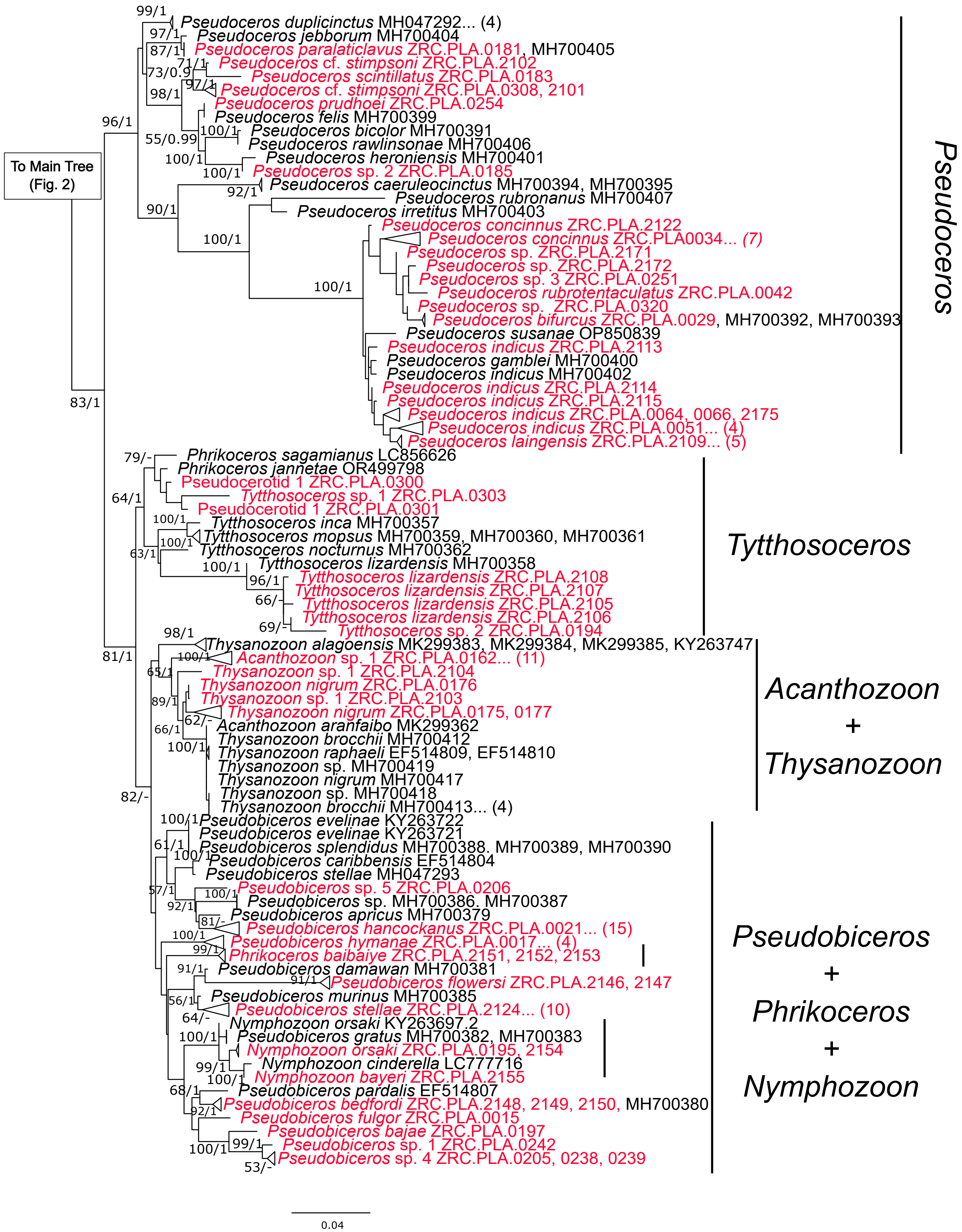

Fig. 3. Maximum likelihood phylogeny of family Pseudocerotidae. Monophyletic species are represented by a single collapsed tip, with their identification numbers indicated. Taxa highlighted in red represent sequences generated in this study. For species with numerous specimens, the total number of specimens is shown in brackets, and individual identification numbers are provided in the Supplementary Material. Support values for each branch are given as maximum likelihood (ML) bootstrap support (≥50) and Bayesian posterior probability (≥0.85) in the format (ML/PP). The bars indicate the genera within the family.

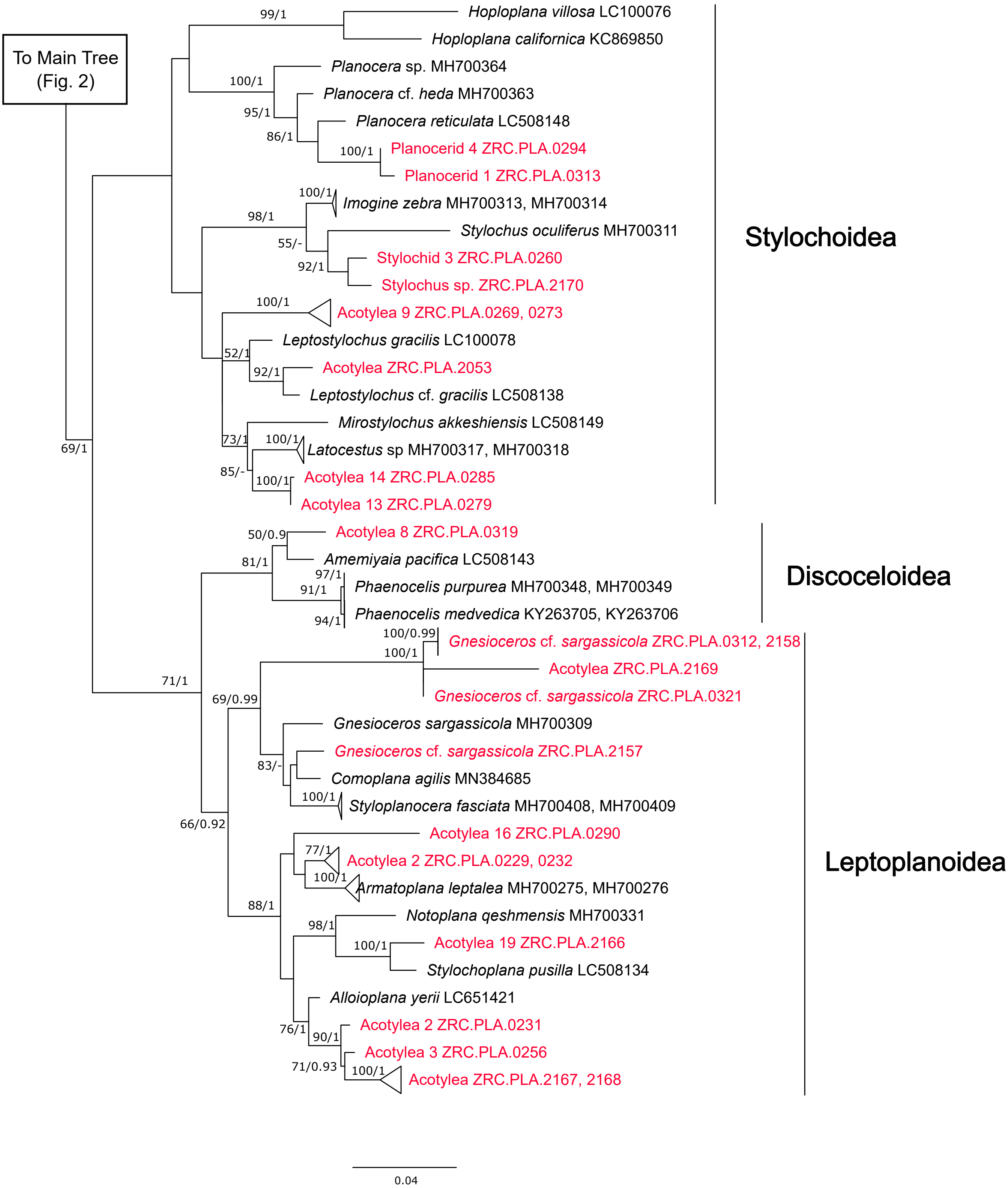

Fig. 4. Maximum likelihood phylogeny of suborder Acotylea. Monophyletic species are represented by a single collapsed tip, with their identification numbers indicated. Taxa highlighted in red represent sequences generated in this study. Support values for each branch are given as maximum likelihood (ML) bootstrap support (≥50) and Bayesian posterior probability (≥0.85) in the format (ML/PP). Numberings allocated to unknown species was based on Ong & Tong (2018), if any, for consistency. Species were distinguished by observable external morphological traits when specimen was collected.

Prostheceraeus. The second clade (henceforth referred to as Euryleptidae 2) encompassed the species *Eurylepta aurantiaca*, *Cycloporus* sp., and *Stylostomum ellipse*. Euryleptidae 2 was sister to Pseudocerotidae (BS=38, PP=0.68) while Euryleptidae 1 was sister to the clade containing Euryleptidae 2 and Pseudocerotidae (BS=35, PP=0.63).

The monophyly of most genera within Euryleptidae and Prosthiostomidae was supported in the analyses. They included *Cycloporus*, *Maritigrella*, and *Prostheceraeus* from Euryleptidae 1, as well as *Enchiridium* and *Prosthiostomum* from Prosthiostomidae. Only the genus *Eurylepta* was paraphyletic with species dispersed between Euryleptidae 1 and Euryleptidae 2.

The analysis included seven genera and 64 species from Pseudocerotidae (Fig. 3), with 32 of these species known from Singapore. The genera analysed were *Acanthozoon*, *Nymphozoon*, *Phrikoceros*, *Pseudobiceros*, *Pseudoceros*, *Thysanozoon*, and *Tytthosoceros*. Prosthiostomidae was represented by 15 species in the analysis, including one unidentified species from Singapore that was most closely related to *Prosthiostomum purum*. Within Pseudocerotidae, *Pseudoceros* was monophyletic and sister group to the rest of the family (BS=96, PP=1) (Fig. 3). *Thysanozoon* was found to be non-monophyletic, with *Thysanozoon alagoensis* in a separate lineage from the rest of the genus. *Tytthosoceros* formed a clade which was sister to a clade of two undescribed species, Pseudocerotid 1 (ZRC.PLA.0300, ZRC.PLA.0301) and *Tytthosoceros* sp. 1 (ZRC.PLA.0303), and *Phrikoceros jannetae* and *Phrikoceros sagamianus* (BS=63, PP=1). *Pseudobiceros* was not monophyletic (Fig. 3), with the genera *Nymphozoon* and *Phrikoceros* nested amongst its species. *Nymphozoon*, containing only three species, *Nymphozoon bayeri*, *Nymphozoon cinderella*, and *Nymphozoon orsaki*, was recovered as non-monophyletic with *Pseudobiceros gratus* nested within.

Acotylea. Many of the diagnostic traits of Acotylea groups are found internally and can only be reliably identified using histology. As histology was not performed on the specimens here, most of the acotyleans analysed in this study could not be accurately identified based on morphology (Fig. 4). They are loosely classified based on observable exterior morphological features such as the eyespot arrangement, presence/absence of nuchal (true) tentacles, as well as the arrangement of the pharynx and the reproductive organs. (Figs. 23–25). Different morphospecies numbers are considered to be distinct species. Morphospecies that are clearly separated phylogenetically are also considered distinct species.

There were 35 species of Acotylea analysed in this study, of which 17 species were from Singapore. These unidentified samples can be placed in their respective superfamilies with reference sequences available on GenBank. The 17 species of Acotylea present in Singapore were distributed across all three superfamilies, Discoceloidea, Leptoplanoidea, and Stylochoidea. One unidentified species, Acotylea 8 (ZRC.PLA.0319) was nested within Discoceloidea, and in the same clade as *Amemiyaia pacifica* (LC508143). For Leptoplanoidea, sequences obtained from Singapore were distributed throughout three clades in the superfamily (Fig. 4). *Gnesioceros* cf. *sargassicola* is the only acotylean species identified to species level albeit with some uncertainty. Due to the lack of available data to distinguish morphological differences between the specimens, they are treated as a single species despite one specimen (ZRC.PLA.2157) being placed in a different clade. Eight species were recovered in Stylochoidea and some within this superfamily were identified to family level: Planocerid 1, Planocerid 4, and Stylochid 3. All local Planoceridae species were in a single lineage, and the two Stylochidae species were nested in Stylochidae.

ANNOTATED CHECKLIST OF COTYLEA SPECIES

Provided here is an annotated compilation of Cotylea species that have been analysed based on the molecular phylogeny (Figs. 3, 4). Where applicable, accession numbers of examined specimens from the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum (LKCNHM), Singapore, are indicated with the collection sites as part of their distributions in Singapore. The numbering of unknown species used in this checklist follows that of Ong & Tong (2018) for consistency.

Place names: Pulau [= island], Terumbu [= submerged reef], Beting [= sandbar], Besar [= big], Sungei [= river or stream], Tengah [= middle], Laut [= sea ward].

Phylum Platyhelminthes Minot, 1876

Clade Rhabditophora Ehlers, 1986

Order Polycladida Lang, 1884

Suborder Cotylea Lang, 1884

Superfamily Euryleptoidea Stimpson, 1857

Family Euryleptidae Stimpson, 1857

***Cycloporus* sp. 1**
(Fig. 5A)

Cycloporus Newman & Cannon, 2002.
Cycloporus sp. 1 - Ong & Tong, 2018: 79.

Material examined. ZRC.PLA.0147 (Terumbu Berkas).

Distribution. Singapore.

Diagnosis. Dorsal background brownish cream with splotches of brown scattered around the submargin, and some along the median area. Short white or light-cream median line lightly bordered by brown pigments. Marginal band surrounding the entire body with interrupted, short darker brown lines

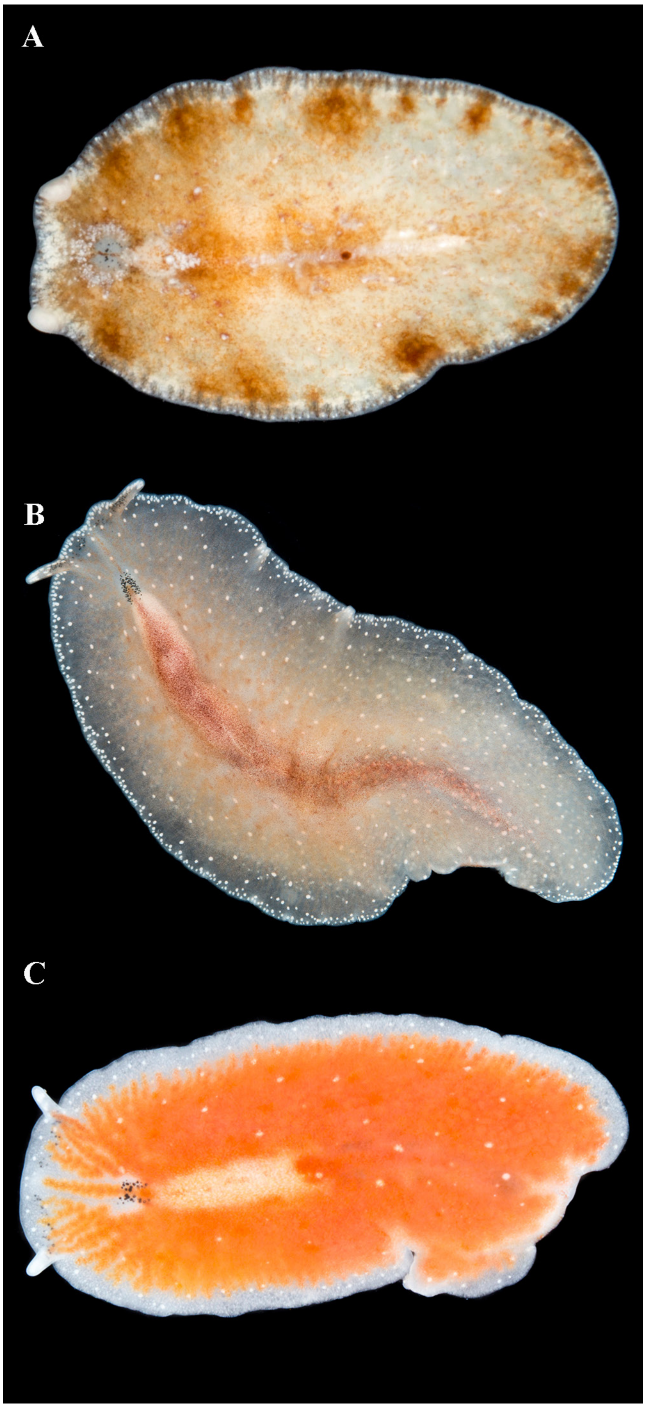

Fig. 5. Dorsal view of live flatworms. A, *Cycloporus* sp. 1 (ZRC.PLA.0147); B, C, *Eurylepta aurantiaca* (B, ZRC.PLA.0149; C, ZRC.PLA.0307).

with white dots and a clear white rim. Anterior margin and marginal tentacles lighter in colour, with a triangular area formed by numerous white spots in front of the cerebral eyespots. Numerous white dots circled around the cluster of cerebral eyespots. Small rounded, whitish marginal tentacles.

Remarks. Only one specimen of this small species was collected, so the degree of intraspecific variation is unknown. This species is distinct but sister to the rest of *Cycloporus* (Fig. 2).

***Eurylepta aurantiaca* Heath & McGregor, 1912**
(Fig. 5B, C)

Eurylepta aurantiaca Heath & McGregor, 1912: 481 (type locality: Monterey Bay, California, USA).
Eurylepta aurantiaca - Hyman, 1953: 370; Faubel, 1984: 220; Bolaños et al., 2003; Bahia et al., 2014: 520; Ong & Tong, 2018: 79.

Material examined. ZRC.PLA.0149 (Pulau Semakau), ZRC.PLA.0307 (Beting Bemban Besar).

Distribution. United States: California, Florida, Mexico, Columbia, Brazil, Singapore.

Diagnosis. Dorsal body colour ranges from light orange to peachy pink and slightly translucent nearer the margin with a darker medial line formed by numerous reddish-brown dots. White creamy spots are randomly scattered around the body. Marginal band consists of dense white spots surrounding the entire body, including the marginal tentacles. Pointed distinct, erect marginal tentacles extending from anterior margin.

Remarks. This is the only described species of *Eurylepta* in Singapore (Ong & Tong, 2018). Although it is classified as *Eurylepta*, this species and *Stylostomum ellipse* are phylogenetically distinct from the rest of Euryleptidae. This is the first molecular phylogenetic study to include this species. Previous studies have suggested that Euryleptidae is not monophyletic (Bahia et al., 2017; Dittmann et al., 2019). This study has also recovered two distinct clades of Euryleptidae. Our results show that *Eurylepta aurantiaca* is nested in the second clade of this family and this may provide additional support for the reclassification of the family (Fig. 2). However, it is recommended that more sampling should be done, especially with *Eurylepta aurantiaca* from other localities, to confirm this placement and for a clearer understanding of phylogenetic relationships more generally.

***Maritigrella fuscopunctata* (Prudhoe, 1978)**
(Fig. 6A, B)

Pseudoceros fuscopunctatus Prudhoe, 1978: 594 (type locality: Donsborough, Western Australia).
Eurylepta fuscopunctatus - Gosliner et al., 1996: 95.
Maritigrella fuscopunctata - Newman & Cannon, 2000: 195; Velasquez et al., 2018: 252; Ong & Tong, 2018: 81.

Material examined. ZRC.PLA.2156.

Distribution. Australia, Papua New Guinea, Indonesia, Israel, Singapore.

Diagnosis. Background whitish-cream with varying sizes of blackish spots arranged in a transverse row around the margin, including the marginal tentacles. Spots are surrounded by lighter blackish shade. Median area is orange or light orange-brown with whitish lines criss-crossing, loosely forming a pattern of honeycomb netting. Distinct broad marginal tentacle held upright with cerebral eyespots in two clusters (Fig. 6B).

Fig. 6. A, B, *Maritigrella fuscopunctata* (ZRC.PLA.2156); C, D, *Maritigrella virgulata* (ZRC.PLA.0160); E, F, *Maritigrella* sp. 1 (ZRC. PLA.0158). A, C, E, dorsal view of live flatworm; B, D, F, close-up of marginal tentacles.

Remarks. This species was originally described as a *Pseudoceros* by Prudhoe (1978). It was reassigned to Euryleptidae and genus *Maritigrella* by Newman & Cannon (2000) due to morphological traits such as the cylindrical pharynx, distinctive broad, ear-like, erect marginal tentacles and eyespots in a paired cluster. This study places this species within Euryleptidae and *Maritigrella* (Fig. 2). It is noted that the *Maritigrella fuscopunctata* sequenced here is not in the same clade as *Maritigrella fuscopunctata* of Velasquez et al. (2018).

Maritigrella virgulata Newman & Cannon, 2000
(Fig. 6C, D)

Maritigrella virgulata Newman & Cannon, 2000: 201 (type locality: Heron Island, southern Great Barrier Reef, Australia).
Maritigrella virgulata - Ng et al., 2011: 274; Ng, 2012: 105; Ong & Tong, 2018: 81.

Material examined. ZRC.PLA.0160.

Distribution. Australia, Singapore.

Diagnosis. Dorsal background whitish-cream with transverse black stripe extending from margin toward the median line. Black stripes are bordered by grey pigments and are of unequal lengths. Median line made up of irregular orange spots arranged in a longitudinal line starting from posterior to cerebral eyespots. Distinct broad marginal tentacle held upright with similar black stripes as the body, and cerebral eyespots in two clusters (Fig. 6D).

Remarks. This is a distinct species that is grouped with the rest of *Maritigrella,* which is nested within the main Euryleptidae clade. It is also in the same clade as the other *Maritigrella virgulata* and *Maritigrella marygarsonae* (Fig. 2).

Maritigrella sp. 1
(Fig. 6E, F)

Eurylepta sp. 6 - Ong & Tong, 2018: 81.

Material examined. ZRC.PLA.0158 (Pulau Sudong).

Distribution. Singapore.

Diagnosis. Dorsal background whitish-cream with numerous small blackish spots scattered around, with more concentrated at the median area. Larger dark brown or black spots bordered with lighter brown shade surrounds the entire body in the submarginal region. Distinct cream marginal band void of any spots surrounds entire body, stopping at the marginal tentacles. The anterior area to both sides of the cerebral eyespots are orange or light brownish-orange and does not contain the small brown spots like the rest of the body. Marginal tentacles filled with dark brown or black spots with cream tip, with such spots absent from the frontal-anterior portion (Fig. 6F). Cerebral eyespots in a pair of clusters.

Remarks. This species was previously identified as *Eurylepta* sp. 6 in Ong & Tong (2018). Based on the molecular analysis here, it is reidentified as *Maritigrella* sp. 1 as it is found to be sister to the rest of the identified *Maritigrella* species and falls into a single clade (Fig. 2). The diagnostic feature of *Maritigrella* that distinguishes it from the rest of the Euryleptidae is the lack of uterine vesicles (Newman & Cannon, 2000). As histology was not done on this specimen, this characteristic was not verified. Records of this species have been rare, and only one specimen has been collected. The patterns of *Maritigrella* are usually of lines, stripes and/or spots and dots, except for *Maritigrella stellata.* Importantly, *Maritigrella* spp. all have broad, erect, ear-like marginal tentacles (Fig. 6B, D, F) and eyespots in a pair of elongated clusters.

Prostheceraeus sp. 1
(Fig. 7)

Eurylepta sp. 4 - Ong & Tong, 2018: 79.

Material examined. ZRC.PLA.0156 (Pulau Berkas).

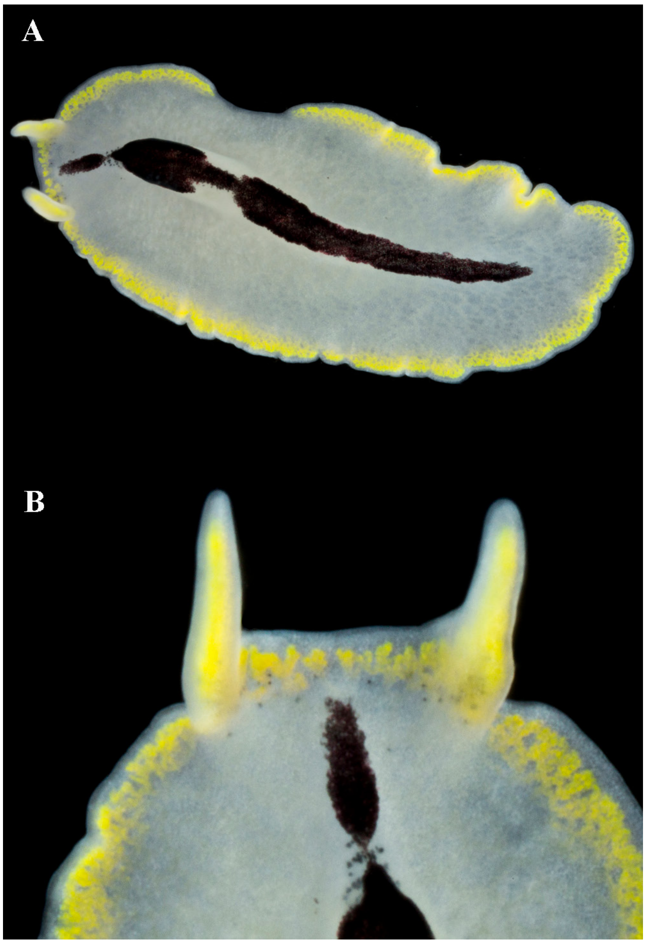

Fig. 7. *Prostheceraeus* sp. 1 (ZRC. PLA.0156). A, Dorsal view of live flatworm; B, Close-up of marginal tentacles.

Distribution. Singapore.

Diagnosis. Dorsal background whitish-grey with conspicuous dark brown or black median streak cutting through the cerebral eyespot, to the anterior end, and tapering off towards the posterior end. Yellow submargin, with a narrower whitish-grey outer marginal band surrounding entire body, including the marginal tentacles. Pointed, long marginal tentacles tapering at the top (Fig. 7B).

Remarks. This specimen was previously identified as *Eurylepta* sp. 4 in Ong & Tong (2018). However, it is shown to be in the same clade as the genus *Prostheceraeus* (Fig. 2). Both *Eurylepta* and *Prostheceraeus* are generally characterised by an elongate or oval body with slender, erected and pointed tentacles, tubular pharynx, and smooth dorsal body. This may easily cause confusion or misidentification. While the first three characteristics were observed in our specimen, histology is yet to be carried out to determine the characteristics of the reproductive system, where more differences might be noted. As *Prostheceraeus* is recovered as a monophyletic genus in this study as well as in Dittmann et al. (2019) and Litvaitis et al. (2019), this specimen is reidentified as *Prostheceraeus* based on the molecular analysis. Further sampling is required to establish its identity more precisely.

Fig. 8. *Prosthiostomum* sp. 1 (ZRC.PLA.0210). A, Dorsal view of live flatworm; B, Close-up of anterior area showing the cerebral and marginal eyespots arrangement.

Superfamily Prosthiostomoidea Lang, 1884

Family Prosthiostomidae Lang, 1884

***Prosthiostomum* sp. 1**
(Fig. 8)

Prosthiostomid 2 - Ong & Tong, 2018: 106.

Material examined. ZRC.PLA.0210 (Lazarus Island).

Distribution. Singapore.

Diagnosis. Body elongate with orange-brown dorsal background without spots. Pharynx long and tubular.

Remarks. This specimen was previously identified as Prosthiostomid 2 in Ong & Tong (2018). Molecular phylogenetic analysis places this species within the genus *Prosthiostomum* (Fig. 2).

Superfamily Pseudocerotoidea Lang, 1884

Family Pseudocerotidae Lang, 1884

***Acanthozoon* sp. 1**
(Fig. 9A)

Acanthozoon sp. - Lim et al., 1994: 71; Chua, 2007: 129; Ng et al., 2007; 89; Ng et al., 2011: 374; Ng, 2012: 105.
Acanthozoon sp. 1 - Ong & Tong, 2018: 82.

Material examined. ZRC.PLA.0162, ZRC.PLA.2048 (Serapong reef), ZRC.PLA.2049 (Serapong reef), ZRC.PLA.2159, ZRC.PLA.2160 (Lazarus Island), ZRC.PLA.2161 (Lazarus Island), ZRC.PLA.2162 (Lazarus Island), ZRC.PLA.2163 (Lazarus Island), ZRC.PLA.2164 (Lazarus Island), ZRC.PLA.2165 (Lazarus Island), ZRC.PLA.2173 (Lazarus Island).

Distribution. Singapore.

Diagnosis. Dorsal background black with numerous small yellow-tipped papillae and sparsely scattered white-based papillae with yellow tip. Some of these white-based papillae can be quite broad, giving the impression that white spots or patches are present. Broad white margin surrounds the entire body including pseudotentacles. Pseudotentacles single-fold, erect, with pointed tip.

Remarks. This species is very commonly seen in Singapore despite not having a formal description. This species is placed in a different clade from *Acanthozoon aranfaibo* while being distinct from *Thysanozoon*. The close phylogenetic relationship between the two genera supports the morphological similarity (presence of papillae on the dorsal body) between these genera (Fig. 3).

***Nymphozoon bayeri* Hyman, 1959**
(Fig. 9B)

Nymphozoon bayeri Hyman, 1959: 578 (type locality: Iwayama Bay, Palau, Micronesia).
Nymphozoon bayeri - Prudhoe, 1985: 30; Newman et al., 2003: 198; Bolaños et al., 2016: 135; Ong & Tong, 2018: 85.
Pseudobiceros sp. - Chua, 2007: 129.

Material examined. ZRC.PLA.2155.

Distribution. Micronesia, Singapore.

Diagnosis. Body mainly black and white with greyish dorsal background. Broad, black outer margin followed by an inner white band, surrounding the entire body including the pseudotentacles. Presence of a medial longitudinal black stripe bordered by a continuous white band that extends to the cerebral eyespots. Simple, single-fold, pointed pseudotentacles with white tips.

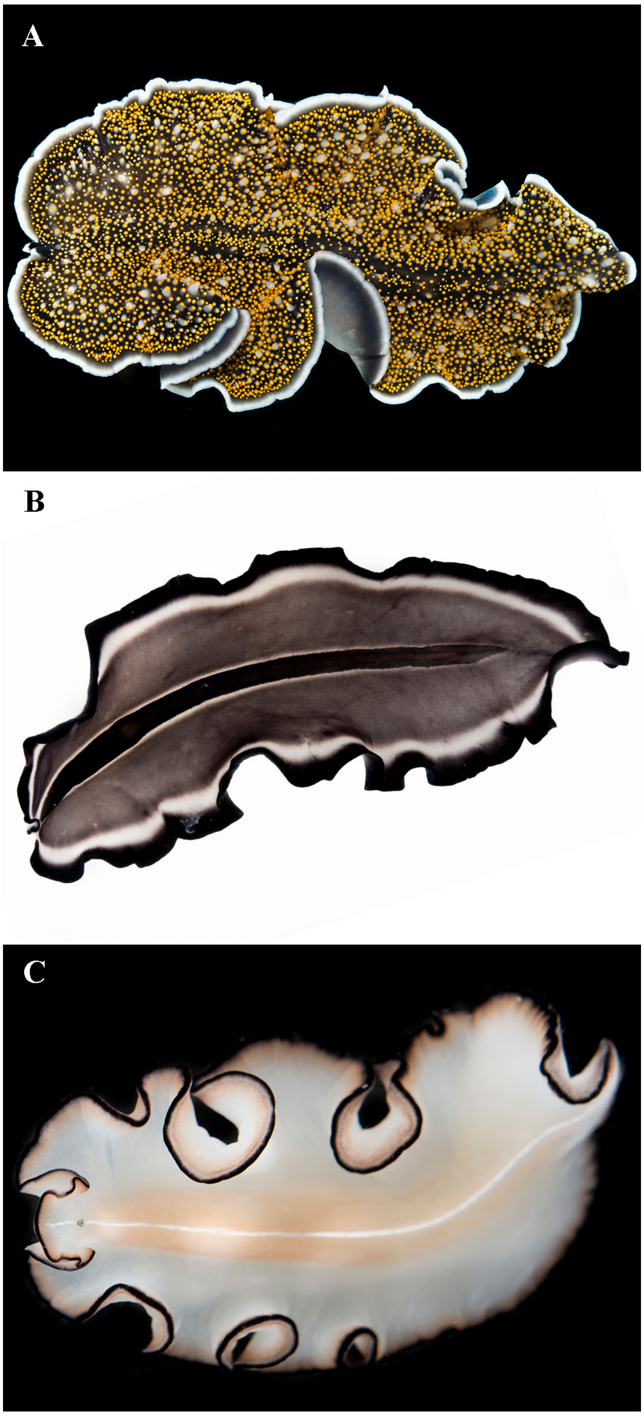

Fig. 9. Dorsal view of live flatworms. A, *Acanthozoon* sp. 1 (ZRC.PLA.2162); B, *Nymphozoon bayeri* (ZRC.PLA.0003); C, *Nymphozoon orsaki* (ZRC.PLA.0195).

Remarks. This is the type species of its genus, *Nymphozoon*. One of the defining characteristics of the genus is having multiple female gonopores, with the number increasing with age, arranged in a midventral longitudinal row and two male gonopores posterior to pharynx. Variations in this species are relatively low, with some having darker or lighter body colour and some greyish-brown rather than black. All *Nymphozoon* species are recovered in a single clade also containing *Pseudobiceros gratus*. This clade is nested within the genus *Pseudobiceros* (Fig. 3).

***Nymphozoon orsaki* (Hyman, 1959)**
(Fig. 9C)

Maiazoon orsaki Newman & Cannon, 1996b: 1427 (type locality: Dam Awan, Madang, Papua New Guinea).
Maiazoon orsaki - Gosliner et al., 1996: 99.
Maiazoon orsaki - Newman & Cannon, 2003: 71.
Flatworm - Leong et al., 2003: 110.
Nymphozoon orsaki - Bolaños et al., 2016: 136; Ong & Tong, 2018: 85.

Material examined. ZRC.PLA.0195, ZRC.PLA.2154 (Raffles Lighthouse).

Distribution. Papua New Guinea: Madang, Micronesia: Marshall Islands, Maldives: Male Atoll, Philippines, Indonesia, Thailand, Singapore.

Diagnosis. Dorsal body orange-cream or pinkish-cream with a thin white medial longitudinal line bordered by a darker brownish-orange smudge. Orange-brown inner marginal band followed by a narrow black rim. Squared pseudotentacles formed by deep lateral folds of the margin.

Remarks. This species was originally described as *Maiazoon orsaki* but has been reassigned to *Nymphozoon orsaki* by Bolaños et al. (2016) as *Maiazoon* was synonymised. The distinguishing feature of *Maiazoon*—three to five female gonopores and two sclerotised stylets—was also found in *Nymphozoon* which removed the need for the separation of genera. This genus contains three species, *Nymphozoon bayeri*, *Nymphozoon cinderella*, and *Nymphozoon orsaki*, which are recovered as part of a clade (Fig. 3) having *Pseudobiceros gratus* nested within. A *Nymphozoon orsaki* sequence (KY263697) was placed in a separate clade from the rest of the genus. There is no morphological information available to verify its identity for comparison with specimens used in this study.

***Phrikoceros baibaiye* Newman & Cannon, 1996**
(Figs. 10, 11A)

Phrikoceros baibaiye Newman & Cannon, 1996b: 1430 (type locality: Hastings Point, New South Wales, Australia).
Phrikoceros baibaiye - Newman & Cannon, 2003: 71; Ng et al., 2011: 314; Ng, 2012: 104; Bolaños et al., 2016: 138; Ong & Tong, 2018: 87.
Marine Flatworm - Chua, 2002: 67.
Pseudobiceros sp. - Tan & Yeo, 2003: 171.
cf. *Phrikoceros baibaiye* - Chua, 2007: 129.
Phrikoceros sp. - Ong et al., 2015: 182, fig. 7.

Material examined. ZRC.PLA.2151 (Seringat Kias), ZRC.PLA.2152 (Seringat Kias), ZRC.PLA.2153 (Seringat Kias).

Distribution. Australia: New South Wales, Singapore.

Diagnosis. Dorsal background ranging from bright orange, orange-red to orange-brown or rust coloured, with numerous or sparingly scattered white spots and or dots. White rim made up of dense white dots. A short line formed by white microdots behind the cerebral eyespots. Square-like, laterally-

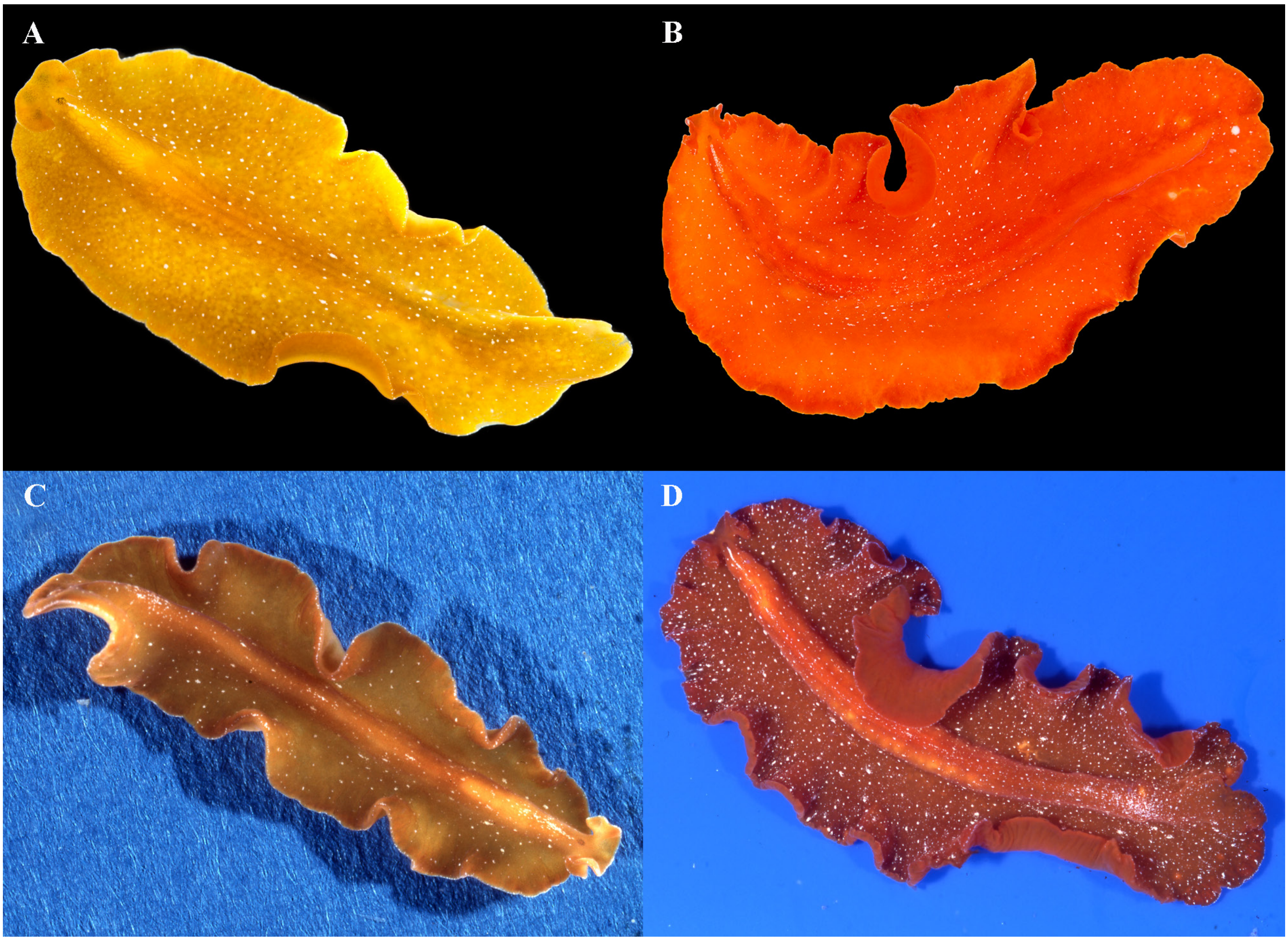

Fig. 10. Dorsal view of live *Phrikoceros baibaiye* (A, ZRC.PLA.2151; B, ZRC.PLA.0006). C, D, post-situ of *Phrikoceros baibaiye* from Newman & Cannon (1996b) (reproduced with permission from Queensland Museum).

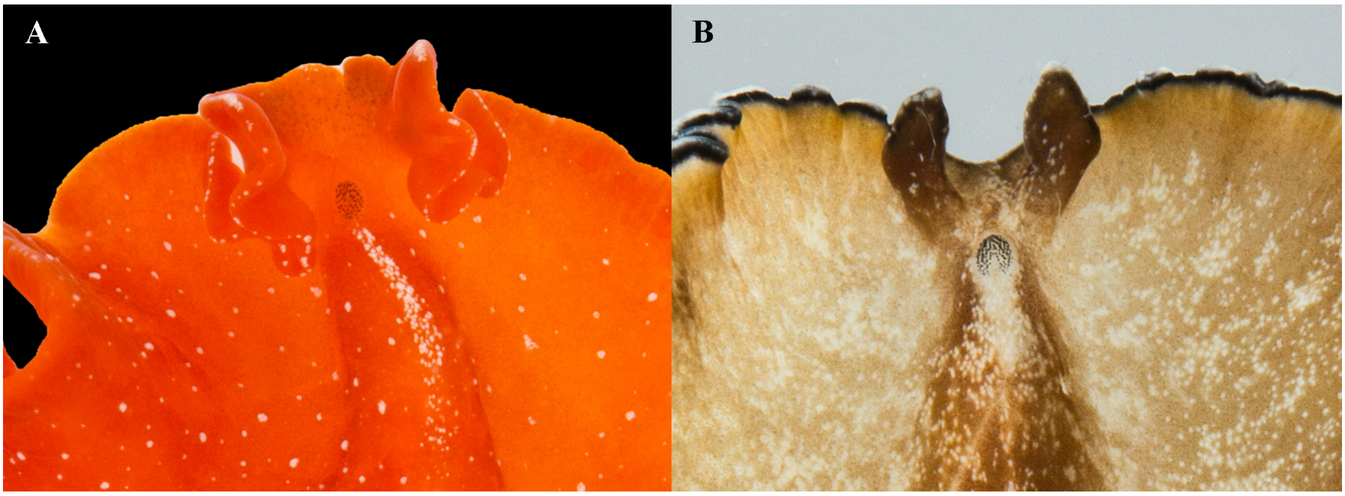

Fig. 11. A, Close-up of *Phrikoceros baibaiye* pseudotentacles (ZRC.PLA.0006); B, Close-up of *Tytthosoceros lizardensis* pseudotentacles (ZRC.PLA.2108).

ruffled pseudotentacles with similar rim made up of white dots (Fig. 11A).

Remarks. This is the type species of the genus. The specimens used in this study are a dull orange-brown (Fig. 10A), notably duller in colour in comparison to the usual bright orange or orange-red colour seen (Fig. 10B) in this species. It is also noted to be much smaller (only ~2 cm) than the typical size of the species (3–5 cm) sighted locally, despite being a sexually mature adult. In Newman & Cannon (1996b), similar colour variation was noted (Fig. 10C, D). *Phrikoceros baibaiye*, the only species in the genus in this analysis, although in a distinct clade, is nested within *Pseudobiceros* (Fig. 3).

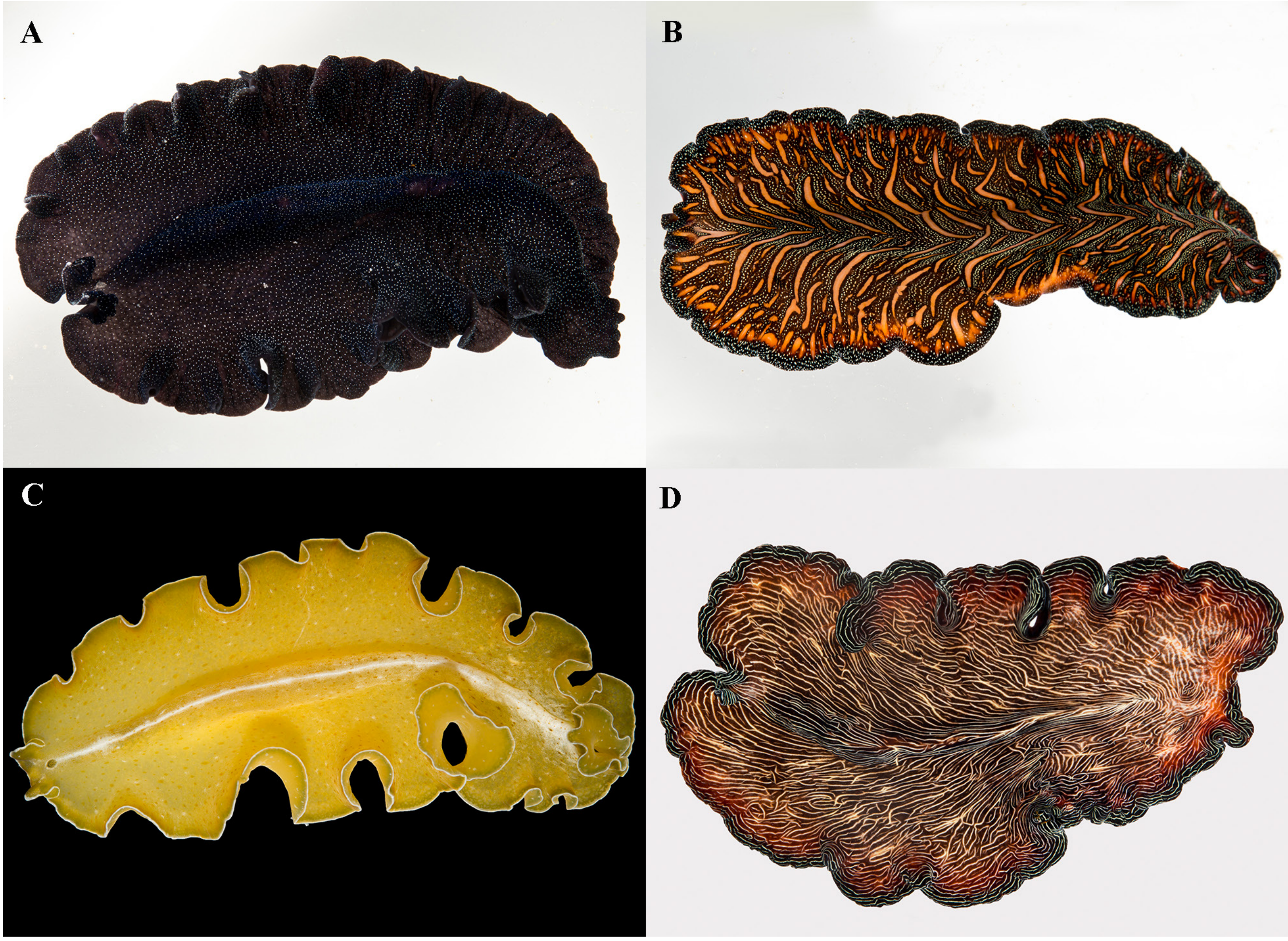

Fig. 12. Dorsal view of live flatworms. A, *Pseudobiceros bajae* (ZRC.PLA.0197); B, *Pseudobiceros bedfordi* (ZRC.PLA.0007); C, *Pseudobiceros flowersi* (ZRC.PLA.2146); D, *Pseudobiceros fulgor* (ZRC.PLA.0015).

Pseudobiceros bajae **(Hyman, 1953)**
(Fig. 12A)

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 & Schrödl, 2016: 109; Ong et al., 2018: 57, fig.4; Ong & Tong, 2018: 87

Material examined. ZRC.PLA.0197 (Terumbu Berkas).

Distribution. Mexico: Gulf of California, Singapore.

Diagnosis. Dorsal body black with small white dots scattered over the entire body. Ventral side of similar colour without any white dots. Laterally ruffled, square-like pseudotentacles with white tips.

Remarks. Similarities in colour and pattern between *Pseudobiceros bajae* and *Pseudobiceros stellae* have led to some confusion in their identification. They can be differentiated by the white dots on the dorsal surface: *Pseudobiceros bajae* has separate and evenly spaced white dots while the white dots on *Pseudobiceros stellae* are clustered. They have different types of pseudotentacles as well, with *Pseudobiceros bajae* having laterally ruffled, square pseudotentacles, while *Pseudobiceros stellae* has simple, ear-like, pointed pseudotentacles. This species is in a clade together with two undescribed species, *Pseudobiceros* sp. 1 and *Pseudobiceros* sp. 4 (Fig. 3).

Pseudobiceros bedfordi **(Laidlaw, 1903)**
(Fig. 12B)

Pseudoceros bedfordi Laidlaw, 1903: 314 (type locality: Singapore).
Pseudoceros bedfordi - Bock, 1913: 254; Kato, 1943: 87; Kato, 1944: 299; Marcus, 1950: 84; Dawydoff, 1952: 82; Hyman, 1954: 220; Hyman, 1959: 566; Prudhoe, 1989: 77.
Pseudoceros micronesianus - Hyman, 1955a: 78.
Pseudobiceros bedfordi - Faubel, 1984: 216; Newman & Cannon, 1994: 241; Newman & Cannon, 1997: 343; Newman & Cannon, 2003: 81; Newman et al., 2003: 197; Chua, 2007: 129; Ng et al., 2011: 374; Ng, 2012: 105; Sreeraj & Raghunathan, 2013: 39; Dixit & Raghunathan, 2013: 167; Bolaños et al., 2016: 138; Ong & Tong, 2018: 88.
Flatworm - Ng et al., 2007: 89.

Material examined. ZRC.PLA.2148 (Lazarus Island), ZRC.PLA.2149 (Terumbu Hantu), ZRC.PLA.2150 (Tanjung Hakim).

Distribution. Singapore, Micronesia: Ifaluk Atoll, Palau, Guam, Onotoa, Saipan, Marshall Islands, Philippines: Mindanao, Australia: Great Barrier Reef (Heron Island, Lizard Island), Western Australia (Coral Bay), Papua New Guinea: Madang, Laing Island, Indonesia: Sulawesi, Mozambique: Inhaca Island, India: Andaman and Nicobar Islands (Havelock Island, Campbell Bay).

Diagnosis. Dorsal body brownish to pinkish-brown with numerous transverse brownish to pinkish-orange streaks lineated with black. Transverse streaks are of varying lengths, some may form small arcs, and some contain a thin white line in the middle. Streaks start transverse nearer the edge and turn towards the longitudinal axis closer to the median. Broad black margin with white dots surrounds the whole body including pseudotentacles. Dorsal surface is covered with small yellow to white dots except in the interior of the streaks. Pseudotentacles simple, single-fold, and pointed.

Remarks. Despite colour variations, the transverse streaks are unique to this species. *Pseudobiceros bedfordi* is a distinct species and is most closely related to *Pseudobiceros pardalis* (Fig. 3).

Pseudobiceros flowersi Newman & Cannon, 1997
(Fig. 12C)

Pseudobiceros sp. 1 - Gosliner et al., 1996: 102.
Pseudobiceros flowersi Newman & Cannon, 1997: 347 (type locality: Lizard Island, Great Barrier Reef, Australia).
Pseudobiceros flowersi - Newman & Cannon, 2003: 81; Newman et al., 2003: 197; Ong et al., 2015: 182, fig. 4; Marquina et al., 2015: 364; Bolaños et al., 2016: 143; Ong & Tong, 2018: 88.

Material examined. ZRC.PLA.2146, ZRC.PLA.2147.

Distribution. Australia: Great Barrier Reef, Micronesia: Palau, Papua New Guinea, Philippines: Luzon, Singapore.

Diagnosis. Dorsal body varies from bright olive green to greenish-brown with speckles of white dots and spots, and/or brownish-green blotches. White, thin longitudinal median line starting at cerebral eyespot and ending anteriorly to the posterior margin bordered by dark pigment. Margin with three bands; inner wide, black; then olive green or brown, with a narrow, white rim. Pseudotentacles with white tips and a small white patch in between. Ventrally light green or brown with white dots similar to dorsal surface. Pseudotentacles square-like and laterally ruffled.

Remarks. There is some variation in the colouration of this species. The dorsal colouration has been recorded to range from light green to dark greenish-brown and some specimens lack the black inner margin. *Pseudobiceros flowersi* is subtended by a very long branch on the phylogeny and shows a clear separation from other species within the genus although it forms a sister group to *Pseudobiceros damawan* (Fig. 3).

Pseudobiceros fulgor Newman & Cannon, 1994
(Fig. 12D)

Pseudobiceros fulgor Newman & Cannon, 1994: 245 (type locality: Heron Islands, Great Barrier Reef, Australia).
Pseudobiceros fulgor - Gosliner et al., 1996: 101; Newman & Cannon, 1997: 348; Newman & Cannon, 2003: 81; Tan & Yeo, 2003: 172; Chua, 2007: 129; Bolaños et al., 2016: 144; Ong & Tong, 2018: 88.

Material examined. ZRC.PLA.0015.

Distribution. Australia: Great Barrier Reef (Heron Islands), Indonesia: Bali, Micronesia: Marshall Islands, Philippines: Anilao, Mauritius, Singapore.

Diagnosis. Dorsal body orange-brown or dark brown with numerous fine broken irregular white stripes. Black margin surrounds the whole body including pseudotentacles, with white streaks parallel to the rim. Random white or faint yellow blotches may be present along some of the white lines. Ventral surface light orange-brown with black marginal band. Pseudotentacles are simple, single-fold, pointed, and ear-like.

Remarks. Mild colour variation in overall body colour and pattern (the density of white lines) in this species seems to depend on the age of the specimen (pers. obs.). Bigger and mature specimens tend to be darker and have increased density of fine lines. *Pseudobiceros fulgor* is a distinct species in this study, and is sister group to a clade comprising *Pseudobiceros bajae*, *Pseudobiceros* sp. 1, and *Pseudobiceros* sp. 4 (Fig. 3).

Pseudobiceros hancockanus (Collingwood, 1876)
(Fig. 13A)

Proceros hancockanus Collingwood, 1876: 91 (type locality: Singapore).
Stylochopsis malayensis Collingwood, 1876: 94 (type locality: Pulo Barundum, Borneo).
Prostheceraeus hancockanus - Lang, 1884: 567.
Pseudoceros malayensis - Bock, 1913: 258, 259.
Pseudoceros hancockanus - Kaburaki, 1923: 639.
Flatworm - Ng et al., 2007: 89.
Pseudobiceros uniarborensis Newman & Cannon, 1994: 252 (type locality: Heron Islands, Great Barrier Reef, Australia).
Pseudobiceros uniarborensis - Newman & Cannon, 1997: 360; Newman et al., 2003: 197; Gosliner et al., 1996: 103; Apte & Pitale, 2011: 110; Ng, 2012: 142; Maghsoudlou & Rahimian, 2014: 332; Marquina et al., 2015: 367.
Pseudobiceros hancockanus - Newman & Cannon, 1994: 249 [Not *Pseudobiceros hancockanus* (Collingwood, 1876)]
Pseudobiceros hancockanus - Bolaños et al., 2016: 146; Ong & Tong, 2018: 90.

Material examined. ZRC.PLA.0021, ZRC.PLA.0027, ZRC.PLA.2050, ZRC.PLA.2051, ZRC.PLA.2052 (Sentosa), ZRC.PLA.2138, ZRC.PLA.2139, ZRC.PLA.2140, ZRC.PLA.2141 (Lazarus Island), ZRC.PLA.2142, ZRC.PLA.2143, ZRC.PLA.2144, ZRC.PLA.2145, ZRC.PLA.2146.

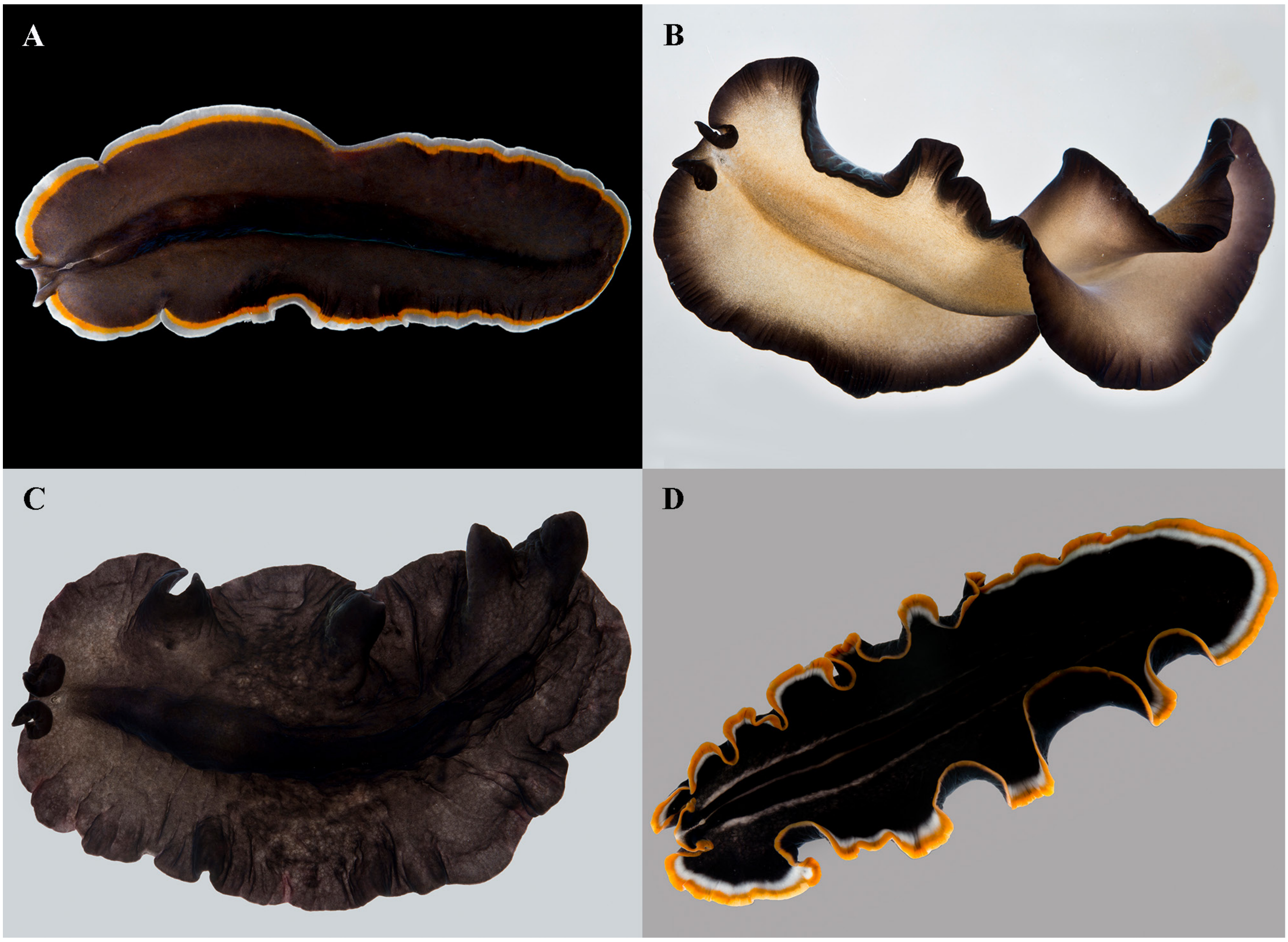

Fig. 13. Dorsal view of live flatworms. A, *Pseudobiceros hancockanus* (ZRC.PLA.2138); B, *Pseudobiceros* sp. 1 (ZRC.PLA.0242); C, *Pseudobiceros* sp. 4. (ZRC.PLA.0239); D, *Pseudobiceros* sp. 5 (ZRC.PLA.0206).

Distribution. Singapore, Borneo, Australia: Great Barrier Reef (Heron Island, One Tree Island, Lizard Island), Western Australia (Tantabiddi, Coral Bay), Papua New Guinea: Madang, Philippines, Micronesia: Guam, India: Lakshadweep (Kavratti Island), Iran: Gulf of Oman.

Diagnosis. Dorsal body dark brown to black with three marginal bands: the innermost a bright, distinct orange, followed by a middle transparent grey, and the outermost white rim. Ventral surface is brown with the same marginal bands. Black simple-folded pseudotentacles with white tips, bordered with only the white rim. A white, narrow line anterior and posterior to the cerebral eyespots.

Remarks. This species is one of the most common flatworms found in Singapore. There are some variations in the colouration of the marginal band where the middle grey band is indistinct, or appears whitish instead of grey. *Pseudobiceros hancockanus* is in a distinct clade of its own, sister to *Pseudobiceros apricus* (Fig. 3).

***Pseudobiceros hymanae* Newman & Cannon, 1997**
(Fig. 14)

Pseudoceros affinis - Hyman, 1960: 309 [Not *Pseudoceros affinis* (Collingwood, 1876)].

Pseudobiceros hymanae Newman & Cannon, 1997: 350 (type locality: Madang, Papua New Guinea).

Pseudobiceros hymanae - Sreeraj & Raghunathan, 2013: 39; Dixit & Raghunathan, 2013: 167; Bolaños et al., 2016: 149; Ong & Tong, 2018: 90.

Material examined. ZRC.PLA.0017, ZRC.PLA.2135, ZRC.PLA.2136, ZRC.PLA.2137.

Distribution. Papua New Guinea: Madang, Hawaii: Makapu‘u Point, Australia: Great Barrier Reef (Heron Island), Western Australia (Rottnest Island), India: Andaman and Nicobar Islands (South Button Island, Ritchie’s Archipelago), Singapore.

Diagnosis. Dorsal background velvety black with a broad orange marginal band and narrow black rim around the whole body. Square-like, laterally-ruffled, black pseudotentacles, with the same marginal bands laterally only (Fig. 14C). Ventral surface greyish-black with the same marginal bands.

Remarks. There are a few other species with similar colouration as *Pseudobiceros hymanae*, but most of them can be differentiated by the colouration and order of marginal bands. *Pseudobiceros splendidus* has identical colouration and pattern to *Pseudobiceros hymanae* except

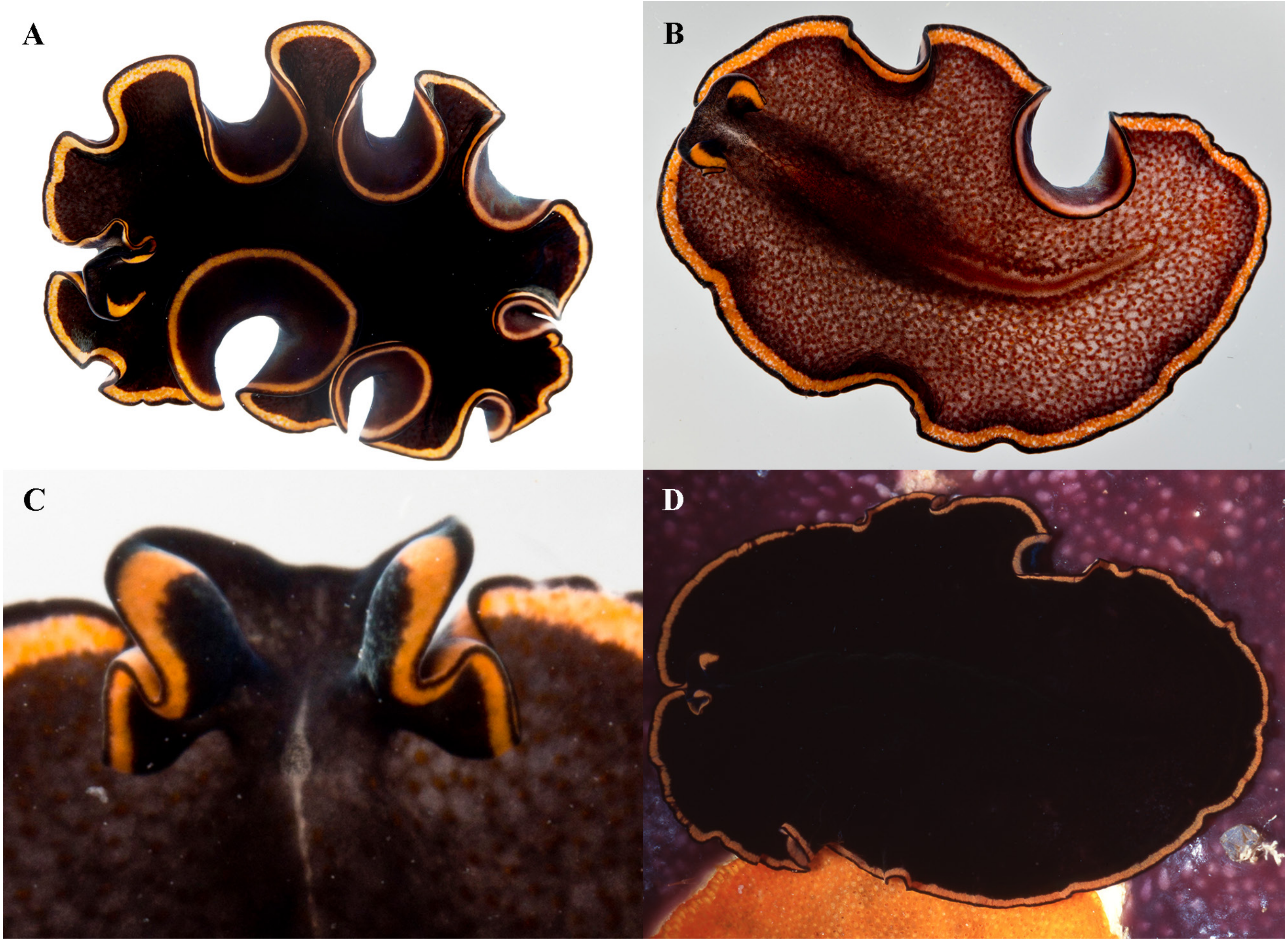

Fig. 14. Dorsal view of live *Pseudobiceros hymanae.* A, ZRC.PLA.2136; B, ZRC.PLA.0017; C, Close-up of pseudotentacles showing the marginal bands along the lateral ruffles; D, In-situ of *Pseudobiceros hymanae* from Newman & Cannon (1997) (reproduced with permission from Queensland Museum).

with microscopic white dots indistinguishable by the naked eye (Newman & Cannon, 1997). This has led to both these species being synonymised by Litvaitis et al. (2019). However, in this study, it is clear from the phylogenetic analysis that *Pseudobiceros hymanae* and *Pseudobiceros splendidus* are separate species despite being remarkably similar in colouration and pattern (Fig. 3).

Pseudobiceros stellae Newman & Cannon, 1994
(Fig. 15)

Pseudobiceros sp. - Poulter, 1987: pl. 2.I.2e.
Pseudobiceros stellae Newman & Cannon, 1994: 252 (type locality: Great Barrier Reef, Australia).
Pseudobiceros stellae - Newman & Cannon, 1997: 360; Newman & Cannon, 2003: 83; Apte & Pitale, 2011:110; Ng, 2012: 142; Marquina et al., 2015: 373; Sreeraj & Raghunathan, 2015: 87; Ong et al., 2015: 182, figs. 1–3; Bahia & Schrödl, 2016: 114; Ong et al., 2018: 58, fig. 5; Velasquez et al., 2018: 247; Ong & Tong, 2018: 90.

Material examined. ZRC.PLA.2124, ZRC.PLA.2125, ZRC.PLA.2126, ZRC.PLA.2127, ZRC.PLA.2128, ZRC.PLA.2129, ZRC.PLA.2130, ZRC.PLA.2131, ZRC.PLA.2132, ZRC.PLA.2133 (Seringat Kias).

Distribution. Australia: Great Barrier Reef, Coral Bay (Newman & Cannon, 2005), India, Papua New Guinea, Israel, Singapore.

Diagnosis. Dorsal background is blackish-brown or brown with white speckles and dots in clusters of different sizes (Fig. 15A–C). Interrupted narrow white spotted rim. Median area is darkened and raised. Simple, single-fold pseudotentacles with white tips.

Remarks. *Pseudobiceros bajae* and *Pseudobiceros stellae* are very similar in their colouration and pattern which can lead to confusion. Both species have black background with white dots over the dorsal surface. However, *Pseudobiceros bajae* has individually scattered white dots and square, ruffled pseudotentacles. *Pseudobiceros stellae* has been recovered in a clade that is sister to *Pseudobiceros murinus* (Fig. 3). It is noted that *Pseudobiceros stellae* of Velasquez et al. (2018) is not recovered in the same clade as the *Pseudobiceros stellae* in this study. The pattern of *Pseudobiceros stellae* described in Velasquez et al. (2018) differs from the original description. The specimen has white speckles randomly scattered across the body with large blotches of white and a median line formed by these speckles and blotches, instead of white dots arranged in 'flower-like' clusters. This

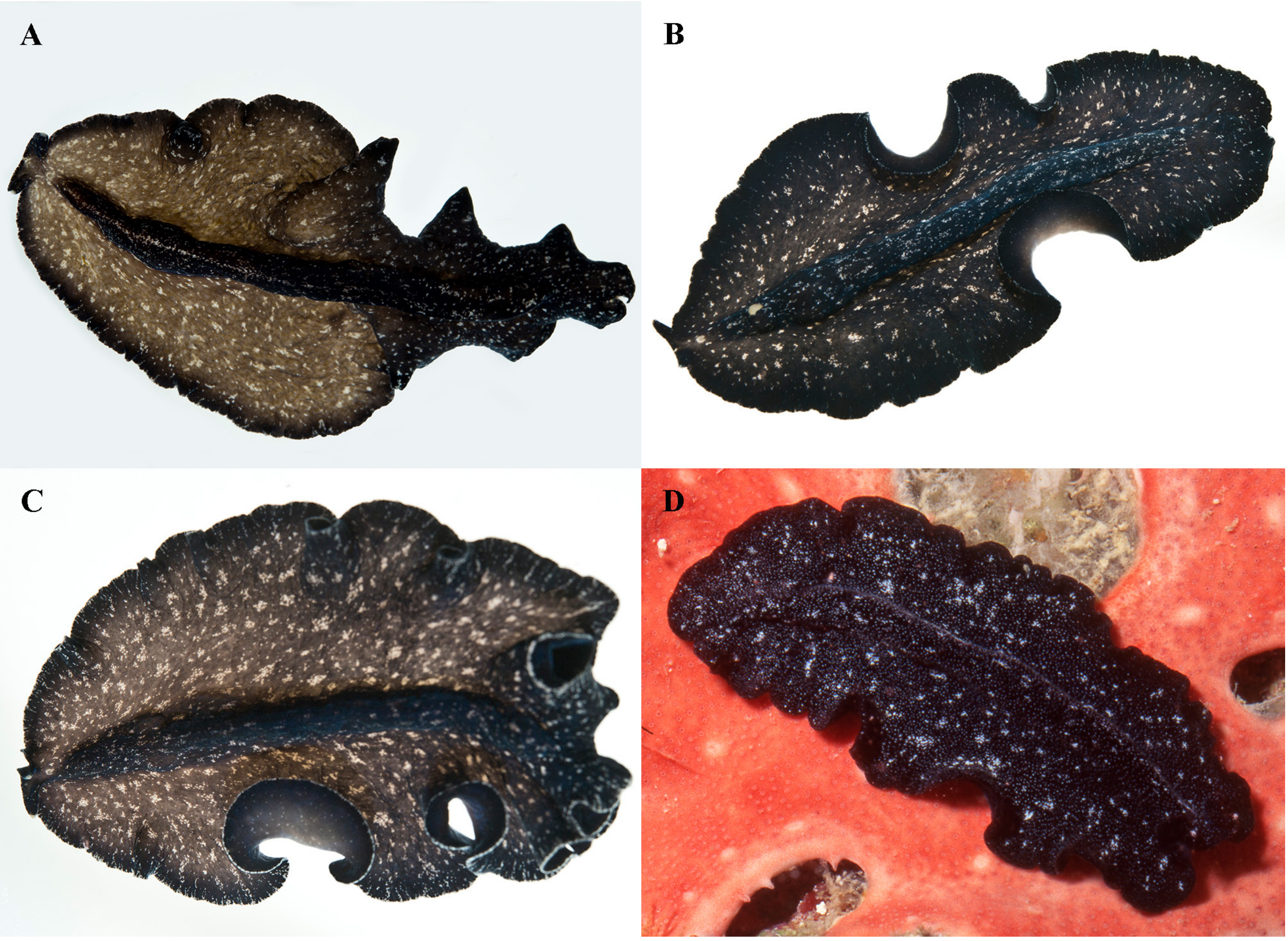

Fig. 15. Dorsal view of live *Pseudobiceros stellae*. A, with brownish body colouration (ZRC.PLA.2124); B, with blackish body colouration (ZRC.PLA.2132); C, with blackish-brown body colouration (ZRC.PLA.2130); D, blackish body colouration, in-situ photograph from Newman & Cannon (1994) (reproduced with permission from Queensland Museum).

difference was noted by the authors but it was still identified as *Pseudobiceros stellae*.

Pseudobiceros sp. 1
(Fig. 13B)

Pseudobiceros sp. 1 - Ong & Tong, 2018: 91.

Material examined. ZRC.PLA.0242.

Distribution. Singapore.

Diagnosis. Dorsal background brownish-cream to light brown. Broad, black margin surrounds the entire body, including the pseudotentacles, and fades lighter towards the interior. Square-like, laterally-ruffled pseudotentacles.

Remarks. The morphology of this species places it in *Pseudobiceros*; however, no existing species description fits the colour and pattern of this species. This is a distinct species on the phylogenetic tree and its closest relative is another undescribed species, *Pseudobiceros* sp. 4 (Fig. 3).

Pseudobiceros sp. 4
(Fig. 13C)

Pseudobiceros sp. 4 - Ong & Tong, 2018: 91.

Material examined. ZRC.PLA.0205, ZRC.PLA.0238 (Terumbu Salu), ZRC.PLA.0239.

Distribution. Singapore.

Diagnosis. Entire dorsal surface is black without any markings or margins. Square-like, laterally-ruffled pseudotentacles.

Remarks. The closest description to this species is *Pseudobiceros bajae* but it can be differentiated by the lack of white dots scattered over the body. Phylogenetic analysis also shows that this species is separate from *Pseudobiceros bajae*, although they, along with *Pseudobiceros* sp. 1, are closely related (Fig. 3).

Pseudobiceros sp. 5
(Fig. 13D)

Pseudobiceros sp. 5 - Ong & Tong, 2018: 91.

Material examined. ZRC.PLA.0206 (Terumbu Salu).

Distribution. Singapore.

Diagnosis. Dorsal body velvety black with two broad marginal bands, an inner white band and outer orange band, throughout its entire body, including the pseudotentacles. Square-like, laterally-ruffled pseudotentacles with a small whitish triangle in between. Cerebral eyespot in a clear area with a narrow white line running through from in between the pseudotentacles to fading off towards the posterior end of the body. Two white lines seemingly run parallel to this median line.

Remarks. There are other species with similar colour and pattern, and these species can be differentiated by the order and colour of their marginal bands. Two of such similar-looking species found in Singapore are *Pseudobiceros hancockanus*, where the order of marginal band from inner to outermost is orange, grey, and white, and *Pseudobiceros hymanae*, where the marginal bands are inner orange and outer black. Furthermore, none of these two species have the white longitudinal lines that easily distinguish them from *Pseudobiceros* sp. 5. Although this undescribed species is clearly distinct morphologically and phylogenetically, it is closely related to *Pseudobiceros hancockanus* and *Pseudobiceros apricus* (Fig. 3).

Pseudoceros bifurcus Prudhoe, 1989
(Fig. 16A)

Pseudoceros dimidiatus - George & George, 1979: 43, pl. 49 fig 7 [Not *Pseudoceros dimidiatus* Graff, 1893].
Pseudoceros bifurcus Prudhoe, 1989: 78 (type locality: M'Sanga Tsohole Reef, Benthedi, Mayotte, Comoro Island, Madagascar).
Pseudoceros bifurcus - Newman & Cannon, 1994: 216; Gosliner et al., 1996: 104; Newman & Cannon, 1998: 299; Newman & Cannon, 2003: 72; Ng, 2012: 105; Bolaños et al., 2016: 151; Ong & Tong, 2018: 93.

Material examined. ZRC.PLA.0029 (Lazarus Island).

Distribution. Comoro Islands, Madagascar, Australia: Great Barrier Reef (Heron Island, One Tree Island, Lizard Island), Indonesia: Sulawesi (Manado), Philippines, India: Andaman and Nicobar Islands (Little Andaman), Singapore.

Diagnosis. Dorsal body colouration ranges from evenly blue to bluish-lavender, or light cream-mauve to violet. Conspicuous median line of two colours, anterior bright orange and posterior interrupted or continuous white. Median line is bordered by a dark purple hue which may appear faded or absent in the anterior orange portion.

Remarks. This species' dorsal background colouration may vary slightly but the diagnostic characteristic of the species is the unique dual colouration of the median line. *Pseudoceros bifurcus* is recovered in a large clade of *Pseudoceros* including *Pseudoceros concinnus* and *Pseudoceros rubrotentaculatus* and is most closely related to *Pseudoceros* sp. (ZRC.PLA.0320) on the phylogeny (Fig. 3).

Pseudoceros concinnus (Collingwood, 1876)
(Fig. 16B)

Proceros concinnus Collingwood, 1876: 90 (type locality: Labuan, Pulo Daak, Borneo).
Proceros concinnus - Lang, 1884: 593.
Pseudoceros concinnus - Kaburaki, 1923: 64; Newman & Cannon, 1994: 208; Newman & Cannon, 2003: 73; Sreeraj & Raghunathan, 2011: 4; Dixit & Raghunathan, 2013: 166; Bolaños et al., 2016: 155; Ong & Tong, 2018: 93.
Pseudoceros sp. - Chou, 1988: 84; Chua, 2007: 15 & 129; Ng, 2012: 104.
Polyclad - Tan & Ng, 1988: 34; Lim et al., 1994: 71; Leong et al., 2003: 76; Ng et al., 2007: 90.

Material examined. ZRC.PLA.0034 (Pulau Pawai), ZRC.PLA.0035 (Pulau Salu), ZRC.PLA.0039, ZRC.PLA.2119, ZRC.PLA.2120, ZRC.PLA.2121, ZRC.PLA.2122, ZRC.PLA.2123.

Distribution. Malaysia: Borneo, Straits of Malacca, Philippines: Tawi Tawi Island, Papua New Guinea, India: Andaman and Nicobar Islands (Great Nicobar Island), Singapore.

Diagnosis. Dorsal background white to cream in colour with a double longitudinal blue medial line, that is often mistakenly seen as only one. A continuous blue marginal band along the entire body including the pseudotentacles.

Remarks. *Pseudoceros concinnus* is one of the most common flatworms found in Singapore. It shows minor intraspecific variation in its dorsal colour and pattern. Colour varies from white to cream with pink, orange, or yellow tint, likely due to intestinal content. Its phylogenetic position shows that it is closely associated with *Pseudoceros bifurcus*, *Pseudoceros rubrotentaculatus*, *Pseudoceros* sp. (ZRC.PLA.0320, 2171, 2172), and *Pseudoceros* sp. 3. *Pseudoceros concinnus* is recovered in two distinct lineages, but this may be due to the limitation of the 28S gene in resolving lower taxonomic groups as the pattern and colouration of the species are very similar in all specimens of this species (Fig. 3).

Pseudoceros indicus Newman & Schupp, 2002
(Fig. 16C)

Pseudoceros unidentified sp. - Stummer-Traunfels, 1933: 3565, fig. 16.
Pseudoceros concinnus - Hyman, 1954: 220; Prudhoe, 1989: 79. [Not *Pseudoceros concinnus* (Collingwood, 1876)]
Pseudoceros indicus Newman & Schupp, 2002: 178 (type locality: Dunwich, Stradbroke Island, Moreton Bay, Queensland, Australia).
Pseudoceros indicus - Newman & Cannon, 2003: 74; Newman et al., 2003: 197; Apte & Pitale, 2011: 109; Ng, 2012: 105; Dixit & Raghunathan, 2013: 166; Sreeraj & Raghunathan, 2013: 39; Bolaños et al., 2016: 157; Jie et al., 2016: 33; Ong & Tong, 2018: 94.
Flatworm - Tan & Yeo, 2003: 63.

Material examined. ZRC.PLA.0051 (Pulau Sekudu), ZRC.PLA.0064 (Pulau Pawai), ZRC.PLA.0066, ZRC.PLA.2113, ZRC.PLA.2114, ZRC.PLA.2115, ZRC.PLA.2116 (Chek

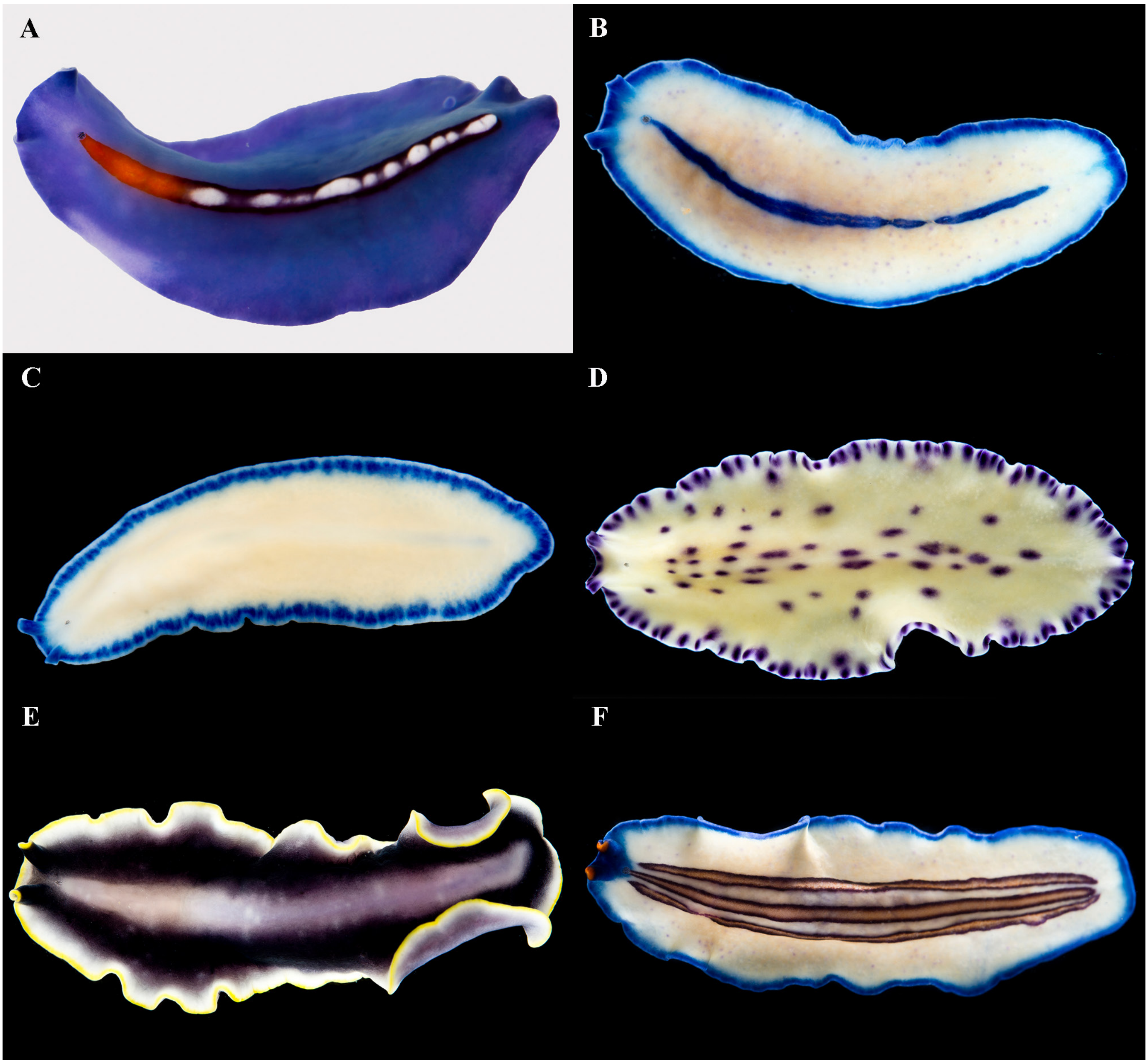

Fig. 16. Dorsal view of live flatworms. A, *Pseudoceros bifurcus* (ZRC.PLA.0029); B, *Pseudoceros concinnus* (ZRC.PLA.0035); C, *Pseudoceros indicus* (ZRC.PLA.0051); D, *Pseudoceros laingensis* (ZRC.PLA.2111); E, *Pseudoceros paralaticlavus* (ZRC.PLA.0181); F, *Pseudoceros rubrotentaculatus* (ZRC.PLA.0042).

Jawa), ZRC.PLA.2117 (Changi), ZRC.PLA.2118 (Changi), ZRC.PLA.2175.

Distribution. Australia: Queensland (Dunwich, Stradbroke Island, Moreton Bay), Philippines, New Guinea: Paidaido Island, Comoro Islands: Mayotte, India: Lakshadweep Island, Andaman and Nicobar Islands, Micronesia: Palau, Chuuk Lagoon, South Africa, Papua New Guinea: Madang, Singapore.

Diagnosis. Dorsal background cream or opaque white with well-defined, separate, royal blue or dark purple spots of varying sizes and spacing, forming a margin around the body, including the pseudotentacles.

Remarks. Similarity in colouration has led this species to be confused with *Pseudoceros concinnus*. However, the clear lack of blue median line provides a distinction between the two species. The phylogenetic analysis also shows that although these two species are closely related, they are placed in two separate clades. *Pseudoceros indicus* faces the same issue as *Pseudoceros concinnus* as it is also recovered in multiple lineages (Fig. 3).

Pseudoceros laingensis Newman & Cannon, 1998
(Fig. 16D)

Pseudoceros laingensis Newman & Cannon, 1998: 309 (type locality: Laing Island, Madang, Papua New Guinea).
Pseudoceros laingensis - Newman & Cannon, 2003: 75; Ng, 2012: 142; Bolaños et al., 2016: 159; Ong & Tong, 2018: 96.

Material examined. ZRC.PLA.2109, ZRC.PLA.2110, ZRC.PLA.2111, ZRC.PLA.2112, ZRC.PLA.2174.

Distribution. Papua New Guinea: Madang, Singapore.

Diagnosis. Dorsal background cream with randomly scattered purple spots. Dense purple spots of varying sizes forming a margin around the body, including the pseudotentacles.

Remarks. Variation in this species includes the body colour ranging from cream to cream-yellowish or pinkish, depending on presence of any intestinal food content, and the number of dorsal purple spots present. *Pseudoceros laingensis* is monophyletic and is closely related to *Pseudoceros indicus* (Fig. 3).

Pseudoceros paralaticlavus Newman & Cannon, 1994
(Fig. 16E)

Pseudoceros paralaticlavus Newman & Cannon, 1994: 232 (type locality: Heron Island, Australia).
Pseudoceros paralaticlavus - Gosliner et al., 1996: 107; Newman & Cannon, 1998: 311; Apte & Pitale, 2011: 110; Marquina et al., 2015: 358; Ong & Tong, 2018: 96.

Material examined. ZRC.PLA.0181 (Sister's Island).

Distribution. Australia: Heron Island, One Tree Island, Lizard Island, Moreton Bay; Papua New Guinea, India, Singapore.

Diagnosis. Dorsal body colour black, with a whitish longitudinal medial band. Medial band starts from behind the eyespots and stops near the posterior end of the body. There are two marginal bands, inner broad white and outer narrow yellow. Black pseudotentacles with only the outer narrow yellow margin.

Remarks. *Pseudoceros paralaticlavus* is in a distinct clade from other *Pseudoceros* species and is closely related to *Pseudoceros jebborum* (Fig. 3).

Pseudoceros prudhoei Newman & Cannon, 1994
(Fig. 17A–E)

Pseudoceros prudhoei Newman & Cannon, 1994: 235 (type locality: Heron Island, Australia).
Pseudoceros prudhoei - Newman & Cannon, 1998: 312; Newman & Cannon, 2003: 36, 76; Newman & Cannon, 2005; Apte & Pitale, 2011: 110; Dixit & Raghunathan, 2013: 167; Okuno & Naruse, 2013: 50; Marquina et al., 2015: 360.
Pseudoceros cf. *prudhoei* - Maghsoudlou & Rahimian, 2014: 333; Ong & Tong, 2018: 96.

Material examined. ZRC.PLA.0254 (Pulau Sudong).

Distribution. Australia: Great Barrier Reef (Heron Island); Papua New Guinea, India, Iran: Persian Gulf, Kenya: Shimoni, Israel, Singapore.

Diagnosis. Dorsal body colour is blackish to brownish-black, with two marginal bands, inner whitish to greyish-white and outer narrow bright yellow, surrounding the entire body, including the pseudotentacles.

Remarks. Descriptions of *Pseudoceros prudhoei* and *Pseudoceros duplicintus* are very similar and have led to much confusion. This study showed that the two species are placed in distinct clades and thus should remain separate (Fig. 3). Further analysis of this distinction is presented in the discussion section below.

Pseudoceros rubrotentaculatus Kaburaki, 1923
(Fig. 16F)

Pseudoceros rubrotentaculatus Kaburaki, 1923: 643 (type locality: Dumurug Point, Cataingan Bay, Masbate, The Philippines).
Pseudoceros rubrotentaculatus - Marcus, 1950: 88; Bolaños et al., 2016: 161; Ong & Tong, 2018: 97.
Pseudoceros sp. - Chua, 2007: 129; Auger, 2013: 165.
Pseudoceros tristriatus - Ng et al., 2011: 374; Ng, 2012: 105. [Not *Pseudoceros tristriatus* Hyman, 1959]

Material examined. ZRC.PLA.0042 (Pulau Jong).

Distribution. Philippines: Cataingan Bay, Singapore.

Diagnosis. Dorsal body creamy white with three non-connecting longitudinal light brown stripes bordered by darker brown or purplish brown. Bright blue marginal band surrounds the body including the area between the pseudotentacles and cerebral eyespots. Pseudotentacles with bright orange tips.

Remarks. The three stripes on the dorsal surface of *Pseudoceros rubrotentaculatus* may result in confusion with *Pseudoceros tristriatus*. However, the colouration between the two species is distinct with *Pseudoceros tristriatus* having a blue dorsal background while *Pseudoceros rubrotentaculatus* has a creamy-white dorsal background. This species is sister to a clade with two undescribed species: *Pseudoceros* sp. 3 and *Pseudoceros* sp. (ZRC.PLA.2172) (Fig. 3).

Pseudoceros scintillatus Newman & Cannon, 1994
(Fig. 18A)

Pseudoceros scintillatus Newman & Cannon, 1994: 237 (type locality: Heron Island, Australia).
Pseudoceros scintillatus - Ong & Tong, 2018: 97.

Material examined. ZRC.PLA.0183 (Pulau Jong).

Distribution. Australia: Heron Island, Singapore.

Diagnosis. Dorsal background black with varying numbers of large, irregularly sized yellow-green maculae encircled by a white rim. Maculae extend onto the wide distinct orange marginal band that runs around the entire body including the pseudotentacles.

Remarks. *Pseudoceros scintillatus* is in a clade together with *Pseudoceros* cf. *stimpsoni* (Fig. 3).

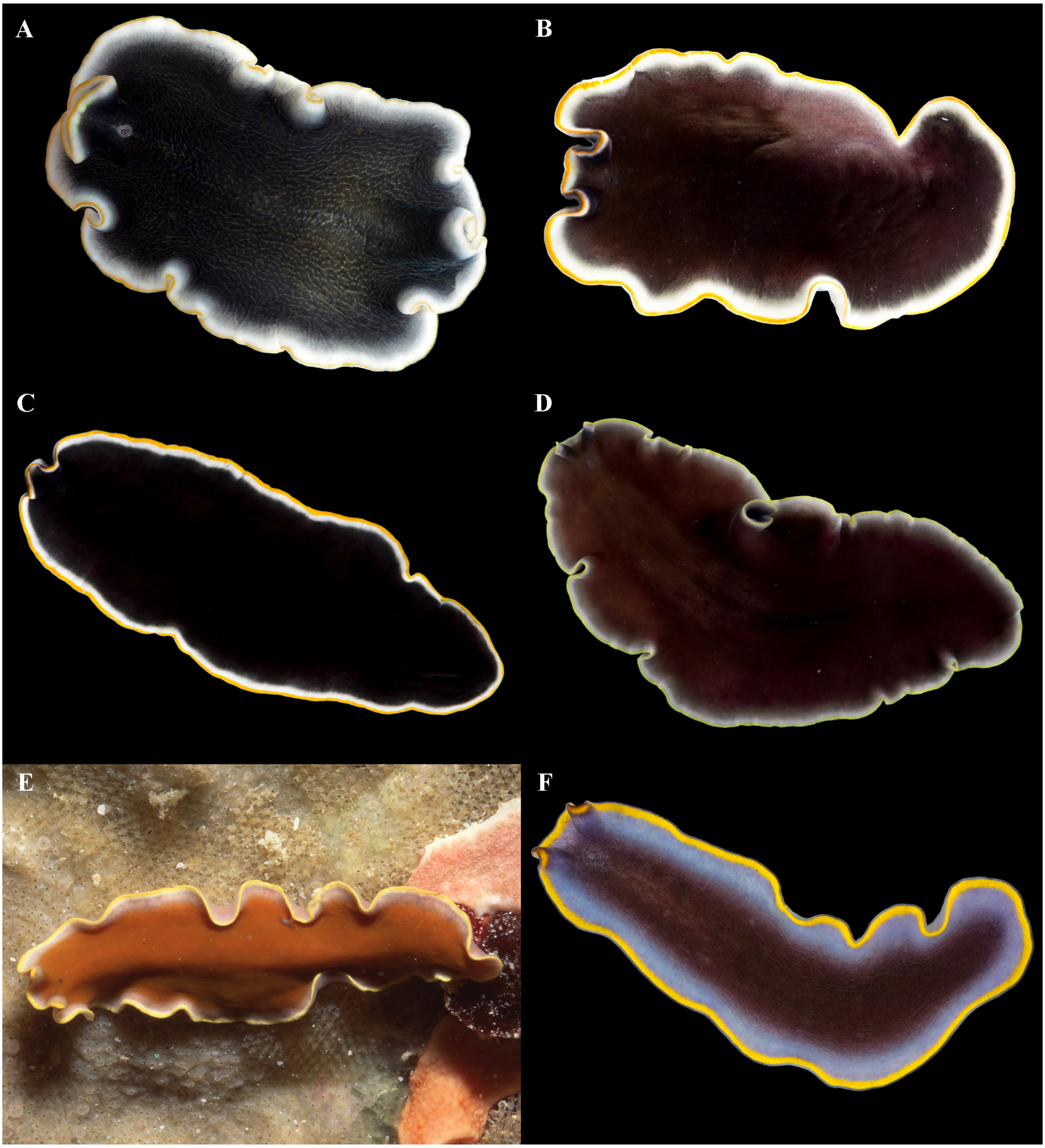

Fig. 17. A–E, Dorsal view of live *Pseudoceros prudhoei* (A, ZRC.PLA.0254; B, ZRC.PLA.0252; C, ZRC.PLA.0255; D, ZRC.PLA.0184); E, In-situ of *Pseudoceros prudhoei* from Newman & Cannon (1994) (reproduced with permission from Queensland Museum). F, Dorsal view of live *Pseudoceros duplicinctus* (ZRC.PLA.0178).

Pseudoceros cf. _stimpsoni_ Newman & Cannon, 1998
(Fig. 18B)

Pseudoceros stimpsoni Newman & Cannon, 1998: 315 (type locality: Madang, Papua New Guinea).
Pseudoceros stimpsoni - Marquina et al., 2015: 361.
Pseudoceros cf. *stimpsoni* - Ong & Tong, 2018: 98.

Material examined. ZRC.PLA.0308, ZRC.PLA.2101, ZRC.PLA.2102 (Changi Beach).

Distribution. Papua New Guinea, Australia: Lizard Island, Singapore.

Diagnosis. Dorsal colour pattern is mottled cream and light brown-grey background. Two interrupted submarginal bands, the inner black and outer orange. Pseudotentacles have a distinct orange submarginal band bordered with black.

Remarks. A difference in the marginal band is noted between the specimen examined in this study and the original

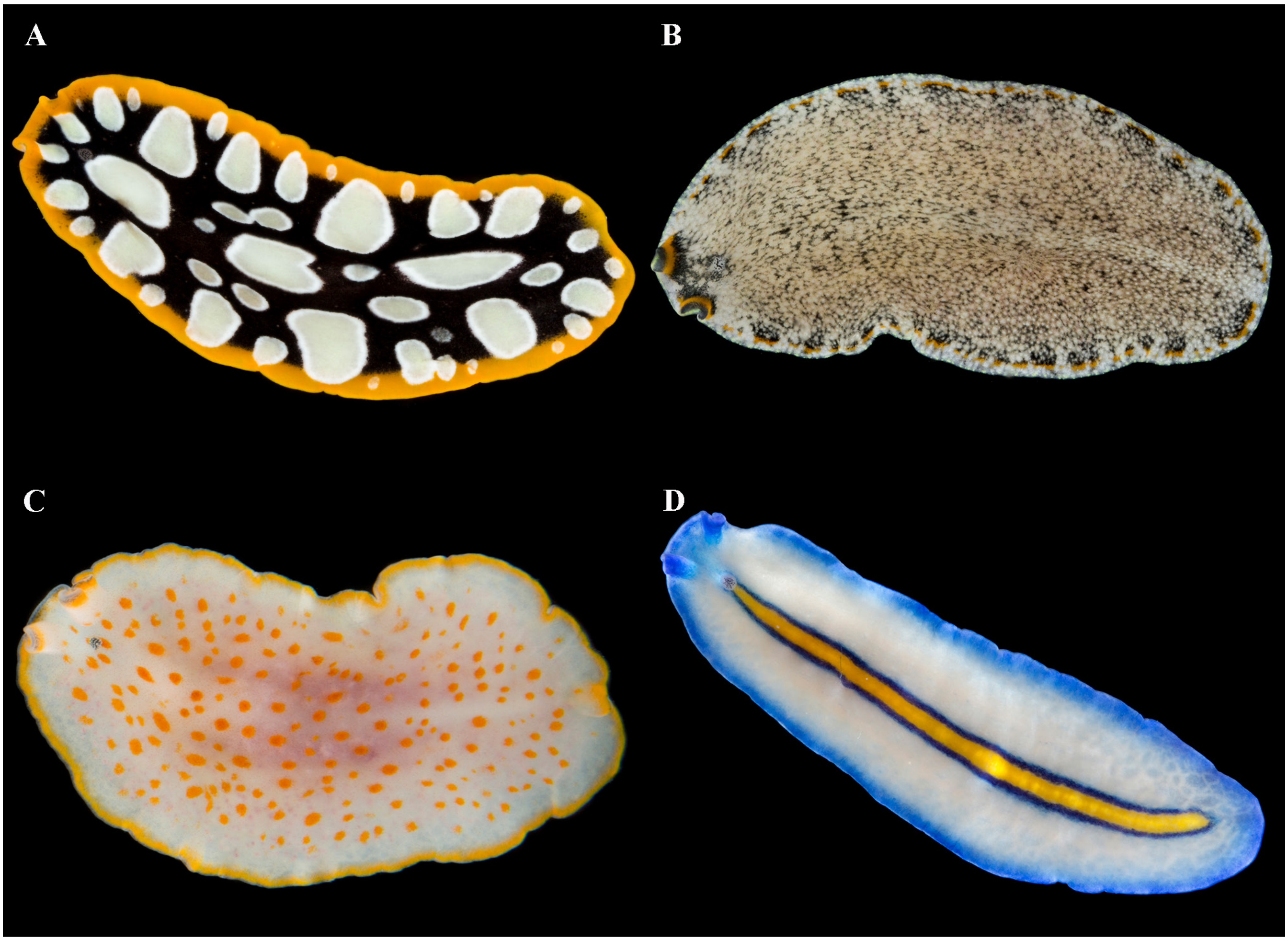

Fig. 18. Dorsal view of live flatworms. A, *Pseudoceros scintillatus* (ZRC.PLA.0183); B, *Pseudoceros* cf. *stimpsoni* (ZRC.PLA.0308); C, *Pseudoceros* sp. 2 (ZRC.PLA.0185); D, *Pseudoceros* sp. 3 (ZRC.PLA.0251).

description. Specifically, the specimen here does not have the submarginal orange band bordered with black at both sides. *Pseudoceros* cf. *stimpsoni* is not recovered in a monophyly and is in a clade together with *Pseudoceros scintillatus* despite having distinct colouration and pattern (Fig. 3).

***Pseudoceros* sp. 2**
(Fig. 18C)

Pseudoceros sp. 2 - Ong & Tong, 2018: 98.

Material examined. ZRC.PLA.0185 (Lazarus Island).

Distribution. Singapore.

Diagnosis. Dorsal body whitish-cream with yellowish-orange or orange spots of varying size all over the body. Orange spots absent nearing the margin. Similar yellowish-orange or orange marginal band surrounds the whole body including the pseudotentacles, with a narrow clear rim.

Remarks. This species is recovered in a clade with *Pseudoceros heroniensis* (Fig. 3). However, the description of *Pseudoceros heroniensis* does not fit *Pseudoceros* sp. 2 and is therefore considered a distinct species. *Pseudoceros heroniensis* was described by Newman & Cannon (1994) to have brown and white dots while *Pseudoceros* sp. 2 only possesses orange spots. The marginal bands are also different.

***Pseudoceros* sp. 3**
(Fig. 18D)

Pseudoceros sp. 3 - Ong & Tong, 2018: 98.

Material examined. ZRC.PLA.0251 (Pulau Pawai), ZRC.PLA.0320.

Distribution. Singapore.

Diagnosis. Dorsal body whitish-cream. Yellow median line bordered with dark blue that starts from after the cerebral eyespots and stops near the posterior end. Blue margin surrounds the whole body including pseudotentacles.

Remarks. This species closely resembles *Pseudoceros concinnus* or *Pseudoceros bifurcus* but can be differentiated by the median line. This species is also closely related to both *Pseudoceros concinnus* and *Pseudoceros bifurcus*, and is recovered in the same clade (Fig. 3).

***Thysanozoon nigrum* Girard, 1851**
(Fig. 19A)

Thysanozoon nigrum Girard, 1851: 137 (type locality: Coast of Florida, USA).
Thysanozoon brochii var. *nigrum* - Lang, 1884: 535.
Thysanozoon nigrum - Verrill, 1901: 41; Hyman, 1939: 15; Hyman, 1940: 484; Hyman, 1952: 196; Hyman, 1955b: 263; Hyman, 1955c: 137; Marcus & Marcus, 1968: 69; Ong et al., 2018: 56, fig. 3; Ong & Tong, 2018: 84; Nanda et al., 2024: 96.

Material examined. ZRC.PLA.0175, ZRC.PLA.0176, ZRC. PLA.0177 (Pulau Salu).

Distribution. United States: Coast of Florida, Sub-tropical western Atlantic (along the coast of Florida, Texas), Bermuda, Bahamas, Singapore, India: Gujarat coast.

Diagnosis. Dorsal surface black or dark grey covered with numerous black, elongated, and pointy papillae. White speckles or spots scattered over body. Narrow, white interrupted rim formed by small white dots randomly spaced around the entire body, including the pseudotentacles. Laterally ruffled, square-like pseudotentacles with white tips formed by white dots.

Remarks. Presence, quantity, and arrangement of white spots vary. The specimens of *Thysanozoon nigrum* used in this study are grouped together in a clade with *Thysanozoon* sp. 1 (ZRC.PLA.2103), while the sequences of the other *Thysanozoon* spp. in GenBank from Litvaitis et al. (2019) are placed in a different clade from others in the genus (Fig. 3).

***Thysanozoon* sp. 1**
(Fig. 19B)

Thysanozoon sp. 1 - Ong & Tong, 2018: 84.

Material examined. ZRC.PLA.2103 (Tanjung Hakim), ZRC. PLA.2104 (Lazarus Island).

Distribution. Singapore.

Diagnosis. Dorsal background reddish-brown to brown with numerous black blunt papillae with white dots and black tip. A black marginal band with white spots scattered randomly surrounds the whole body, including the pseudotentacles. Laterally ruffled, square-like pseudotentacles with white tip formed by small dots.

Remarks. Saturation of dorsal colour can change in an individual, as previously observed in two specimens kept in captivity which showed colours changing from reddish-brown to orange-brown, although papillae and margin colours remained the same (Ong & Tong, 2018). This suggests that the overall body colour of the flatworm may be affected by its intestinal content. Similar observations have also been made in other species, particularly with *Pseudoceros* spp. (Bolaños et al., 2016; Ong & Tong, 2018), but the changes in body colour contrast are much less pronounced. In all observations, the pattern and marginal bands, if any, are present and remain consistent.

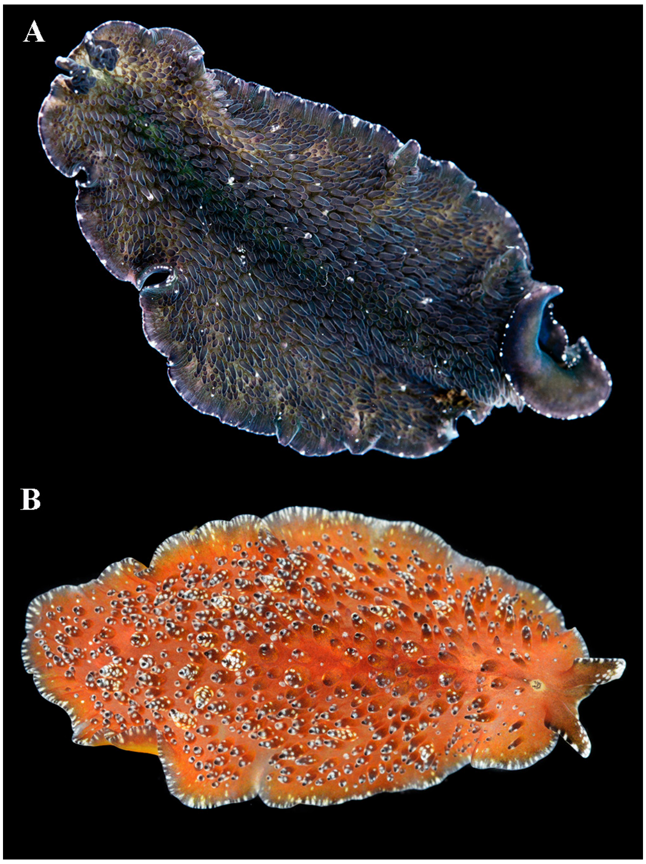

Fig. 19. Dorsal view of live flatworms. A, *Thysanozoon nigrum* (ZRC.PLA.0177); B, *Thysanozoon* sp. 1 (ZRC.PLA.2104).

***Tytthosoceros lizardensis* Newman & Cannon, 1996**
(Fig. 20)

Tytthosoceros lizardensis Newman & Cannon, 1996a: 485 (type locality: Heron Island, Australia).
Tytthosoceros lizardensis - Newman & Cannon, 2003: 86; Khalili et al., 2009: 41; Maghsoudlou & Rahimian, 2014: 336; Dixit et al., 2015: 3; Ong et al., 2015: 182, fig. 6; Bolaños et al., 2016: 163; Ong, 2016: 108; Ong & Tong, 2018: 102.

Material examined. ZRC.PLA.2105, ZRC.PLA.2106, ZRC. PLA.2107, ZRC.PLA.2108 (Seringat Kias).

Distribution. Australia: Great Barrier Reef (Heron Island); Iran: Qeshm Island; India: Andaman and Nicobar Islands (Great Nicobar Island, Campbell Bay); Singapore.

Diagnosis. Dorsal body varies from light brown to brown or olive green to greenish-brown covered with cream or white spots. The numerous white dots are either densely arranged on the medial area to form fine streaks leading laterally towards the margin or are aggregated into clusters resulting in a mottled pattern on the surface. Narrow black marginal band sometimes interrupted by white microdots and a white rim cover the entire body, including the pseudotentacles. Simple, single-folded, ear-like pointed pseudotentacles with white tip (Fig. 11B).

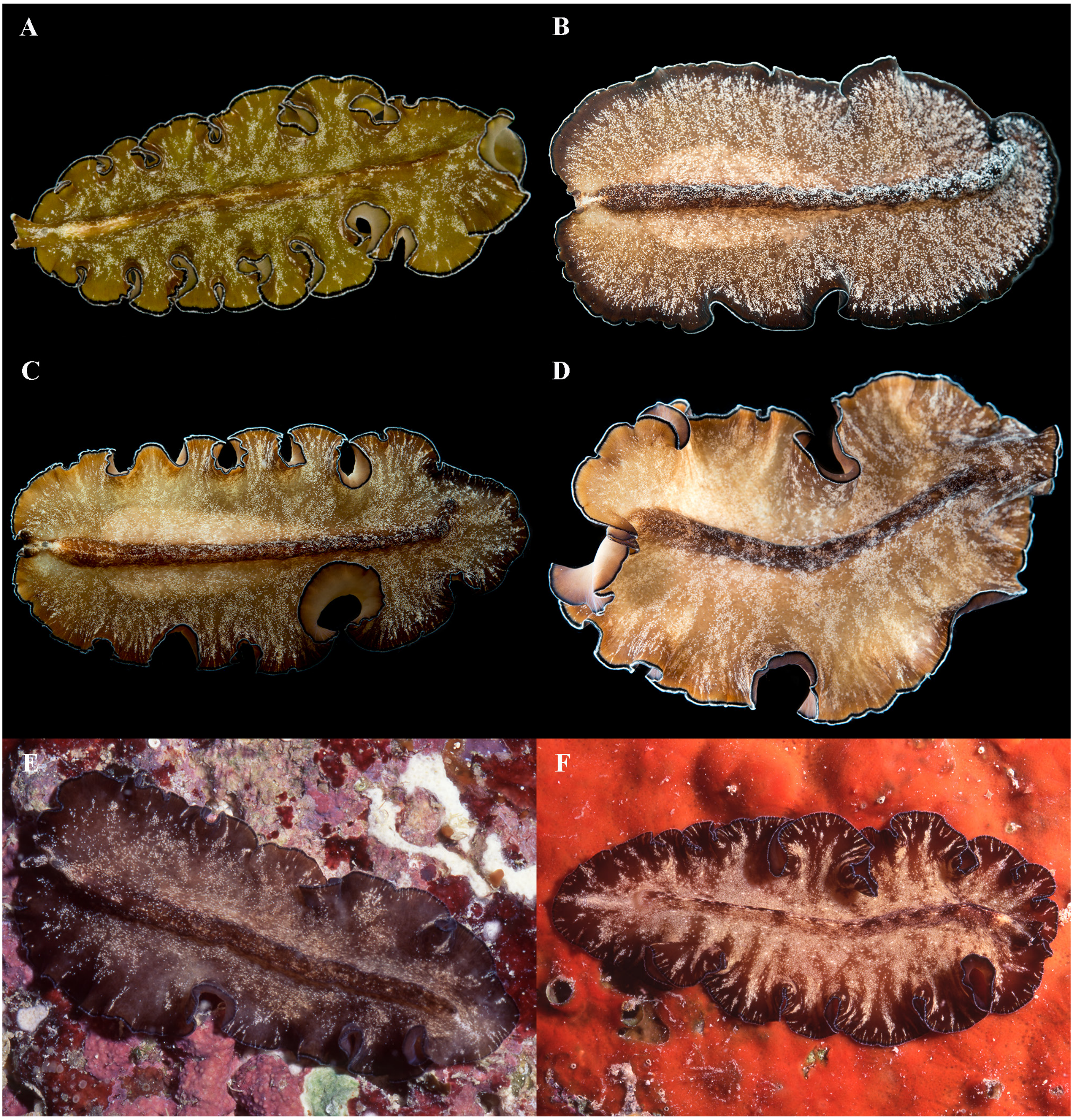

Fig. 20. A–F, Dorsal view of live *Tytthosoceros lizardensis*, showing the body colour and pattern variation (A, ZRC.PLA.2105; B, ZRC.PLA.2107; C, ZRC.PLA.2108; D, ZRC.PLA.2106); E, F, In-situ of *Tytthosoceros lizardensis* from Newman & Cannon (1996a) (reproduced with permission from Queensland Museum).

Remarks. Some variations in colour and pattern occur within the species. Mottled background forming transverse streaks and distinct marginal bands are the main identifying features to identify the species. *Tytthosoceros lizardensis* is not recovered as a clade as *Tytthosoceros* sp. 2 is nested within the species (Fig. 3). However, it is noted that the specimens of *Tytthosoceros lizardensis* used in this study fall into a distinct clade from the one available in GenBank (Lizard Island, Australia).

Tytthosoceros sp. 1
(Fig. 21A, B)

Tytthosoceros sp. 1 - Ong & Tong, 2018: 102.

Material examined. ZRC.PLA.0303 (St John's Island).

Distribution. Singapore.

Diagnosis. Dorsal body is a mottled yellowish-cream and brownish-black. Interrupted yellow submarginal band around the whole body, except for the pseudotentacles. Light cream

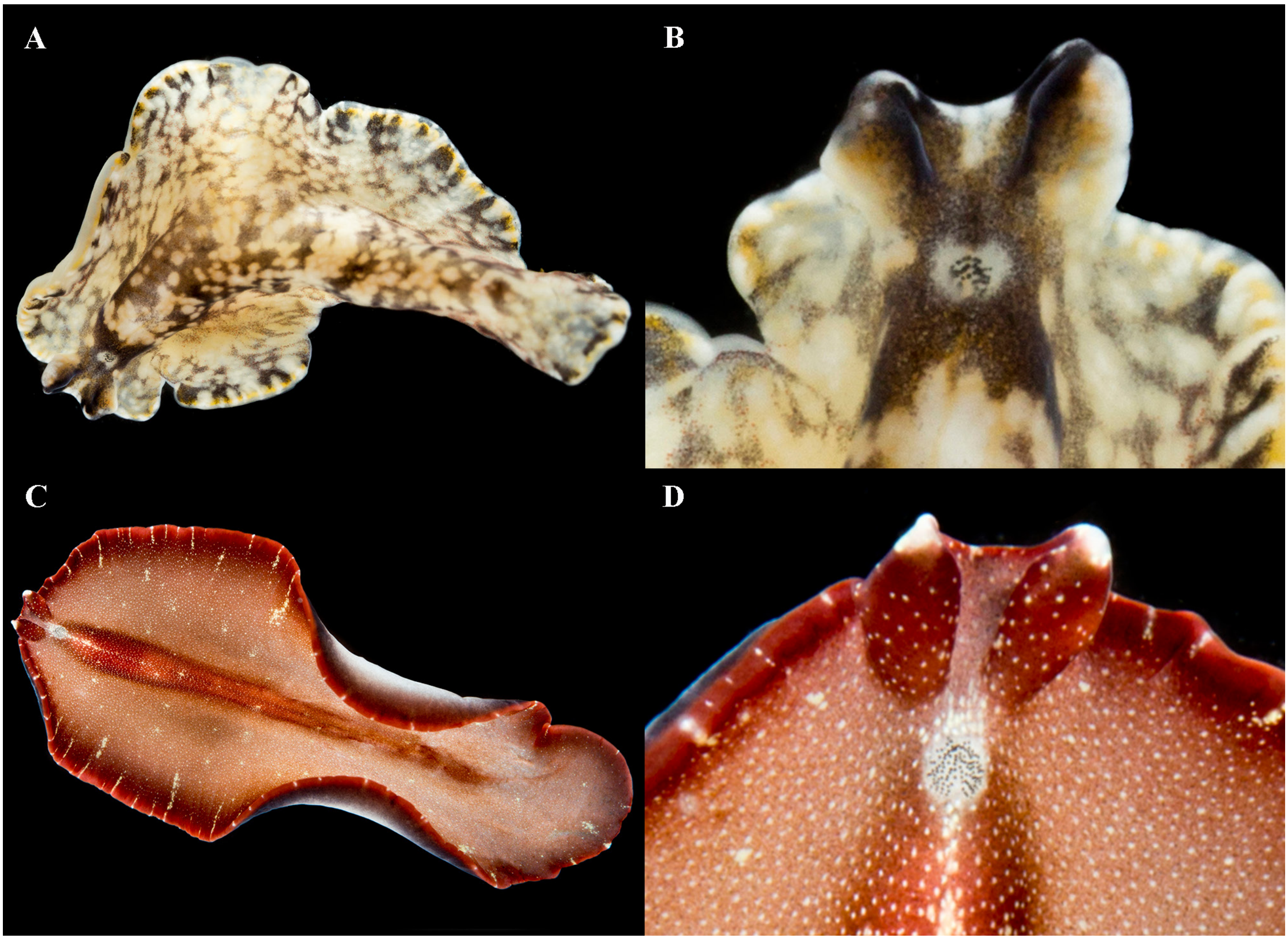

Fig. 21. A, B, *Tytthosoceros* sp. 1 (ZRC.PLA.0303); C, D, *Tytthosoceros* sp. 2 (ZRC.PLA.0194). A, C, dorsal view of live flatworm; B, D, close-up of pseudotentacles.

rim. Simple, single-folded, ear-like and pointed dark brown to black pseudotentacles with white tip (Fig. 21B).

Remarks. Only one specimen of this species has been found, and therefore variations in colour and pattern are unknown. This species is identified as *Tytthosoceros* and not *Phrikoceros* due to the presence of simple, single-folded, pointed pseudotentacles (Fig. 21B) instead of the square, laterally ruffled pseudotentacles found in *Phrikoceros*. However, this species does not fall in the same clade as the other species of *Tytthosoceros*, so the genus could be polyphyletic or it could be new to science (Fig. 3). More specimens will have to be examined to be conclusive.

Tytthosoceros sp. 2
(Fig. 21C, D)

Tytthosoceros sp. 2 - Ong & Tong, 2018: 104.

Material examined. ZRC.PLA.0194 (Pulau Hantu).

Distribution. Singapore.

Diagnosis. Dorsal body orange-cream to reddish-light brown with numerous white dots covering the whole body, except at the margin. Reddish-brown diffused median line starting from cerebral eyespots and ending a distance away from the posterior end. Red marginal band surrounding the whole body including pseudotentacles interrupted by cream or white lateral streaks formed by tiny dots. Simple, single-folded, ear-like and pointed pseudotentacles pointed with white tip (Fig. 21D).

Remarks. The morphology of this species fits the description of the genus *Tytthosoceros*, but not with any described *Tytthosoceros* species. This species is also placed in the same clade as *Tytthosoceros lizardensis* (Fig. 3).

Pseudocerotid sp. 1
(Fig. 22)

Pseudocerotid sp. 1 - Ong & Tong, 2018: 104.

Material examined. ZRC.PLA.0300, ZRC.PLA.0301 (Raffles Lighthouse).

Distribution. Singapore.

Diagnosis. Dorsal body is mottled whitish-grey and white or cream with reddish and blackish microdots. Raised median area that has more densely packed reddish and blackish microdots, giving the impression of a blackish-brown median

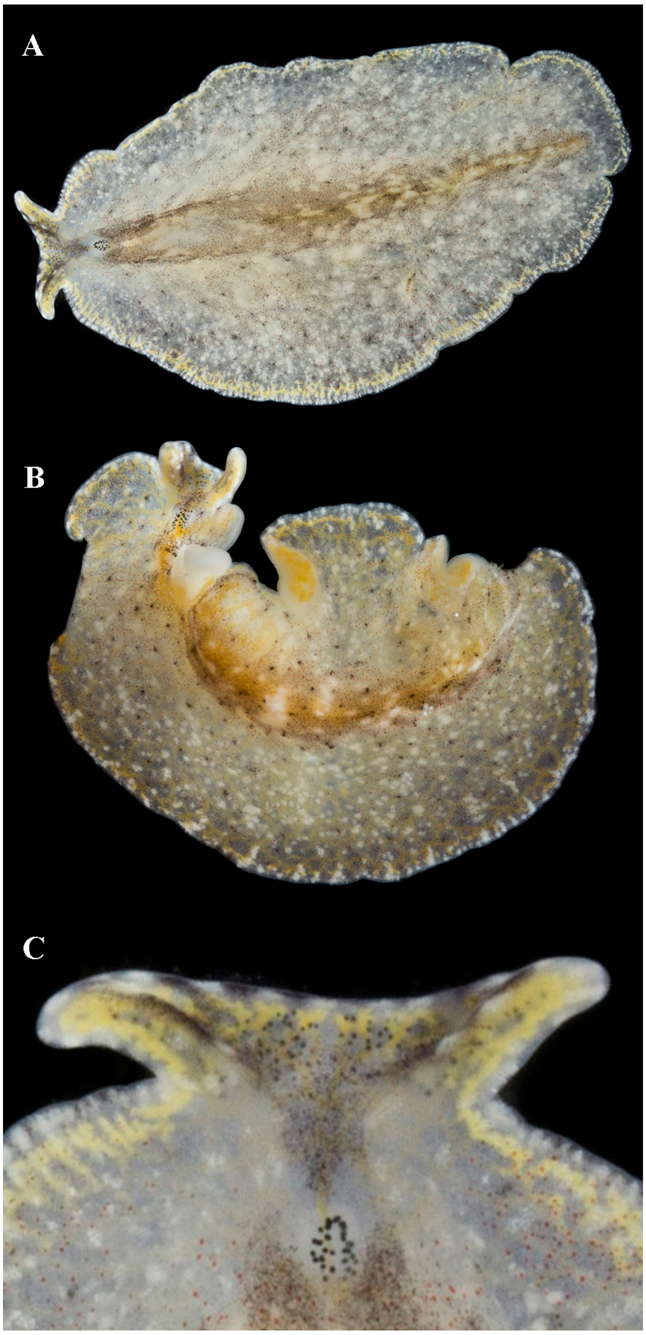

Fig. 22. A, B, Dorsal view of live Pseudocerotid 1 (A, ZRC.PLA.0300; B, ZRC.PLA.0301); C, Close-up of pseudotentacles.

line that extends from the cerebral eyespots to near the posterior end. White or cream blotches scattered within the median line. Narrow yellow submarginal band interrupted by dense white dots surrounds entire body, including the pseudotentacles. Broad, ear-like and erect marginal tentacles (Fig. 22C).

Remarks. This species contains a mixture of morphological characteristics of multiple genera and thus is not assigned to any one genus. Some of the traits are similar to *Tytthosoceros* such as the type of pharynx and single male gonopore. However, the morphology of the pseudotentacles (Fig. 22C) is not congruent with others in that genus or those in *Pseudobiceros* but suggests an affinity with Euryleptidae. This species forms a distinct clade with the other unknown species *Tytthosoceros* sp. 1 and together are sister group to the rest of *Tytthosoceros* (Fig. 3).

DISCUSSION

The split between Cotylea and Acotylea is strongly supported in this study (BS=100, PP=1), which is consistent with previous work (Bahia et al., 2017; Tsunashima et al., 2017; Dittmann et al., 2019; Litvaitis et al., 2019). The families that are usually contested, Cestoplanidae and Theamatidae, were not included in the present analysis (Fig. 2). Families within Cotylea, Pericelidae (BS=64, PP=1), Prosthiostomidae (BS=88, PP=1), and Pseudocerotidae (BS=83, PP=1) are strongly supported, although Euryleptidae is paraphyletic. Two of three superfamilies in Acotylea, Discoceloidea (BS=81, PP=1) and Leptoplanoidea (BS=66, PP=0.92), are monophyletic with strong support while the other superfamily Stylochoidea is recovered to be monophyletic but has weak support. Further discussions of the lower taxonomic levels are covered below.

Acotylea. The acotylean samples used in this study have not been examined with histology, thus the internal morphology cannot be determined. As the identification of acotylean species is mainly based on internal morphology, accurate identification of the samples based on external morphology is not possible. However, with reference sequences available on GenBank, the unidentified samples can be placed in their respective superfamilies (Fig. 4). While species identification is difficult to ascertain due to the lack of data, some observations can be made from the analysis.

Acotylea 19 is placed in the same clade as *Stylochoplana pusilla* with strong support values (100/1) (Fig. 4), which might suggest that they could be the same species. Acotylea 13 and 14 could also fall under Latocestidae based on its phylogenetic position. However, further sampling is required to increase the reliability of these claims as well as to determine the identity and relationships of the other unknown acotyleans.

One *Gnesioceros* cf. *sargassicola* specimen (ZRC.PLA.2157) is placed in a separate lineage from the rest of the identified species. This implies that they are likely a different species than the other *Gnesioceros* cf. *sargassicola* specimens despite being identified as the same species based on their similar external morphology. Moreover, none of the local *Gnesioceros* cf. *sargassicola* specimens fall into the same clade as the *Gnesioceros sargassicola* sequence obtained from GenBank (MH700309). More data, both molecular and histological, should be acquired in future studies to accurately identify the species.

Non-monophyly of Euryleptidae. This study supports the paraphyly of Euryleptidae, but our results indicate a different division of the family compared to other studies (Fig. 2). The results from Bahia et al. (2017) and Dittmann et al. (2019) suggested that the genera *Eurylepta*, *Stylostomum*, and species *Cycloporus gabriellae* were recovered as the

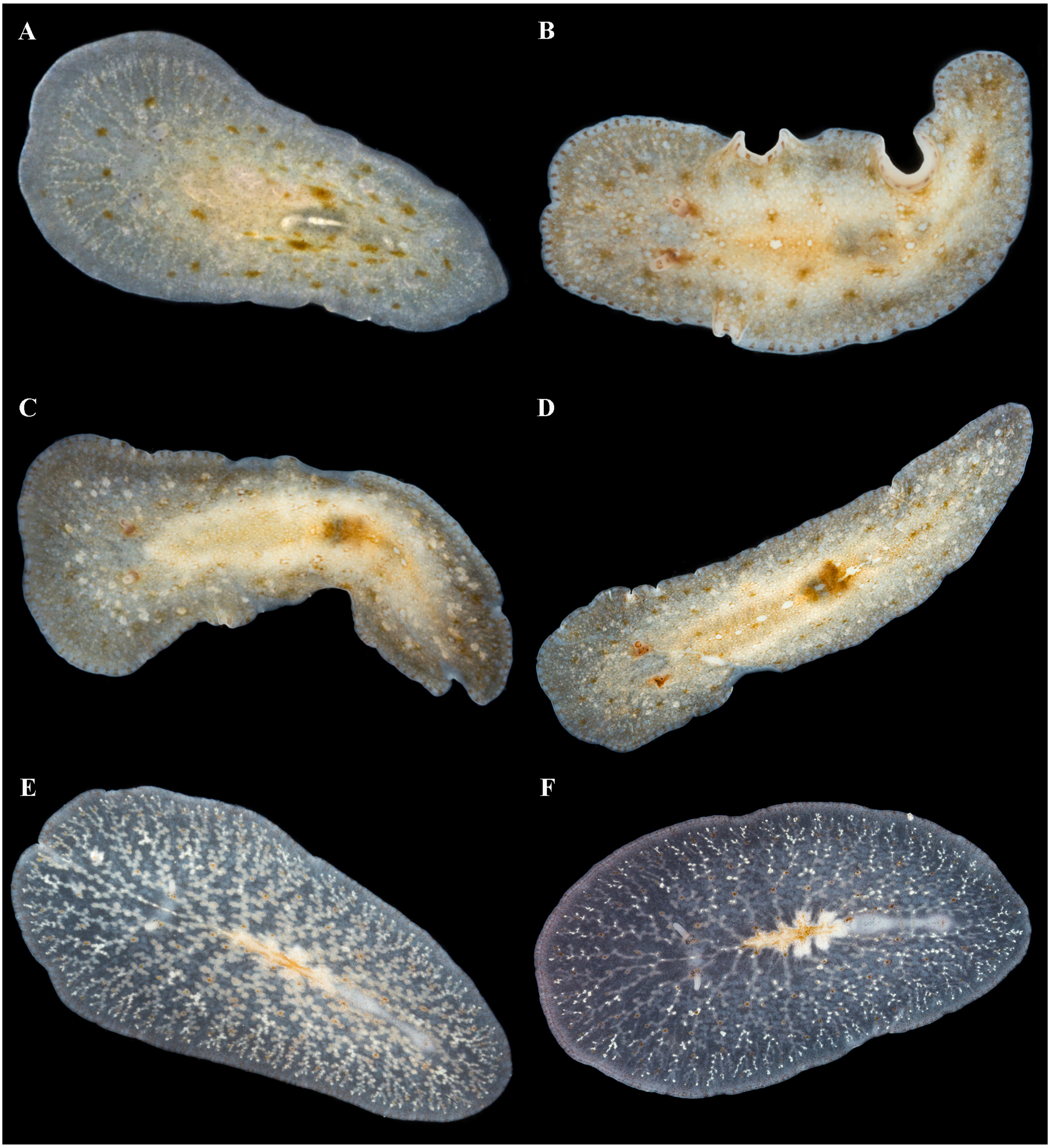

Fig. 23. Dorsal view of live flatworms. A–D, *Gnesioceros* cf. *sargassicola* (A, ZRC.PLA.2157; B, ZRC.PLA.2158; C, ZRC.PLA.0312; D, ZRC.PLA.2157); E, Planocerid 1 (ZRC.PLA.0313); F, Planocerid 4 (ZRC.PLA.0294).

sister clade of Pseudocerotidae, while the other genera and the remaining species of *Cycloporus* form another clade. In contrast, this study recovers a strongly supported *Cycloporus* clade (BS=90, PP=1), with the exception of some *Cycloporus* sequences, similar to the studies by Litvaitis et al. (2019) and Dittmann et al. (2023). A comprehensive study by Dittmann et al. (2023) concluded that *Cycloporus* is monophyletic and some sequences on GenBank are likely misidentified due to the morphological similarities within the family. It is also to be noted that in their study, *Cycloporus* sp. (MZ813374) was also in a clade with other *Eurylepta* species as is the case in this study as well, thus supporting the misidentification of a possible *Eurylepta* species. Furthermore, *Cycloporus gabriellae* (KY263656 and KY263658) are also in separate clades from the rest of the *Cycloporus*, further supporting the assertion of Dittmann et al. (2023) that they were wrongly identified. Regardless, the paraphyly of Euryleptidae remains, with one clade containing *Eurylepta* and *Stylostomum*, and the other containing the monophyletic genera of *Cycloporus*, *Maritigrella*, and *Prostheceraeus* (Fig. 2).

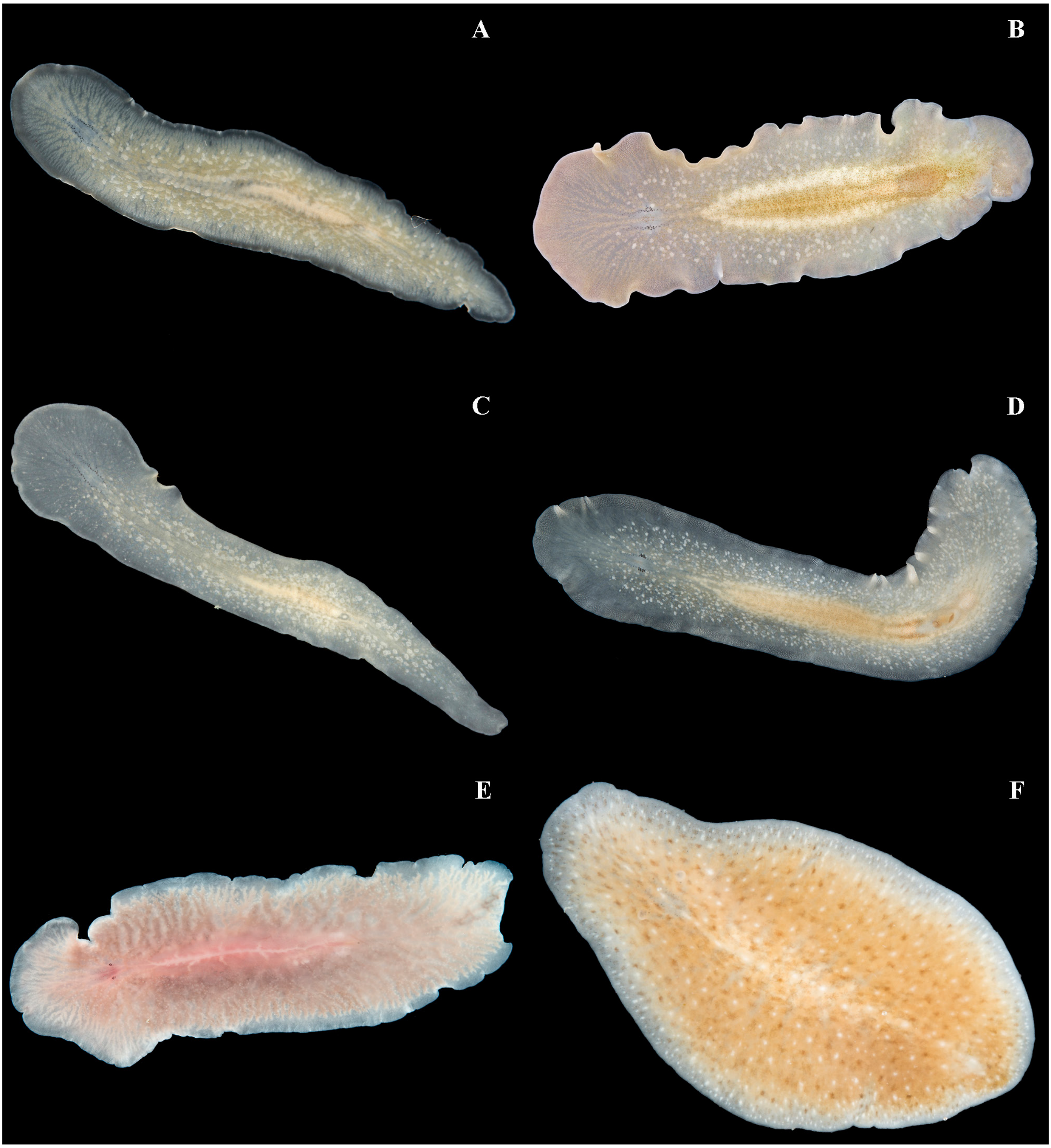

Fig. 24. Dorsal view of live flatworms. A–C, Acotylea 2 (A, ZRC.PLA.0299; B, ZRC.PLA.0231; C, ZRC.PLA.0232); D, Acotylea 3 (ZRC.PLA.0256); E, Acotylea 8 (ZRC.PLA.0319); F, Stylochid 3 (ZRC.PLA.0260).

The analysis further emphasises the uncertainty of the placements of the two Euryleptidae clades. Euryleptidae 1, the clade with *Cycloporus*, *Maritigrella*, and *Prostheceraeus,* is sister to the larger clade containing Pseudocerotidae and Euryleptidae 2. Euryleptidae 2, containing *Stylostomum* and *Eurylepta*, is sister to Pseudocerotidae (Fig. 2). The support values for both clades are very low (Fig. 2), showing that there are insufficient data to determine the placement of these clades. This ambiguity is also observed in the 18S rRNA analysis by Dittmann et al. (2019). Further studies with more genetic markers and species are needed to better understand the relationships between these taxa, and closer morphological inspection may reveal diagnostic characters for each of them.

***Acanthozoon* and *Thysanozoon* within Pseudocerotidae.** This study has provided evidence to support the placements of *Acanthozoon* and *Thysanozoon* as a clade within Pseudocerotidae. This relationship has often been assumed, given that these are the only genera within Pseudocerotidae that possess the morphological trait of having dorsal papillae, and are distinguished by *Acanthozoon* having only one male

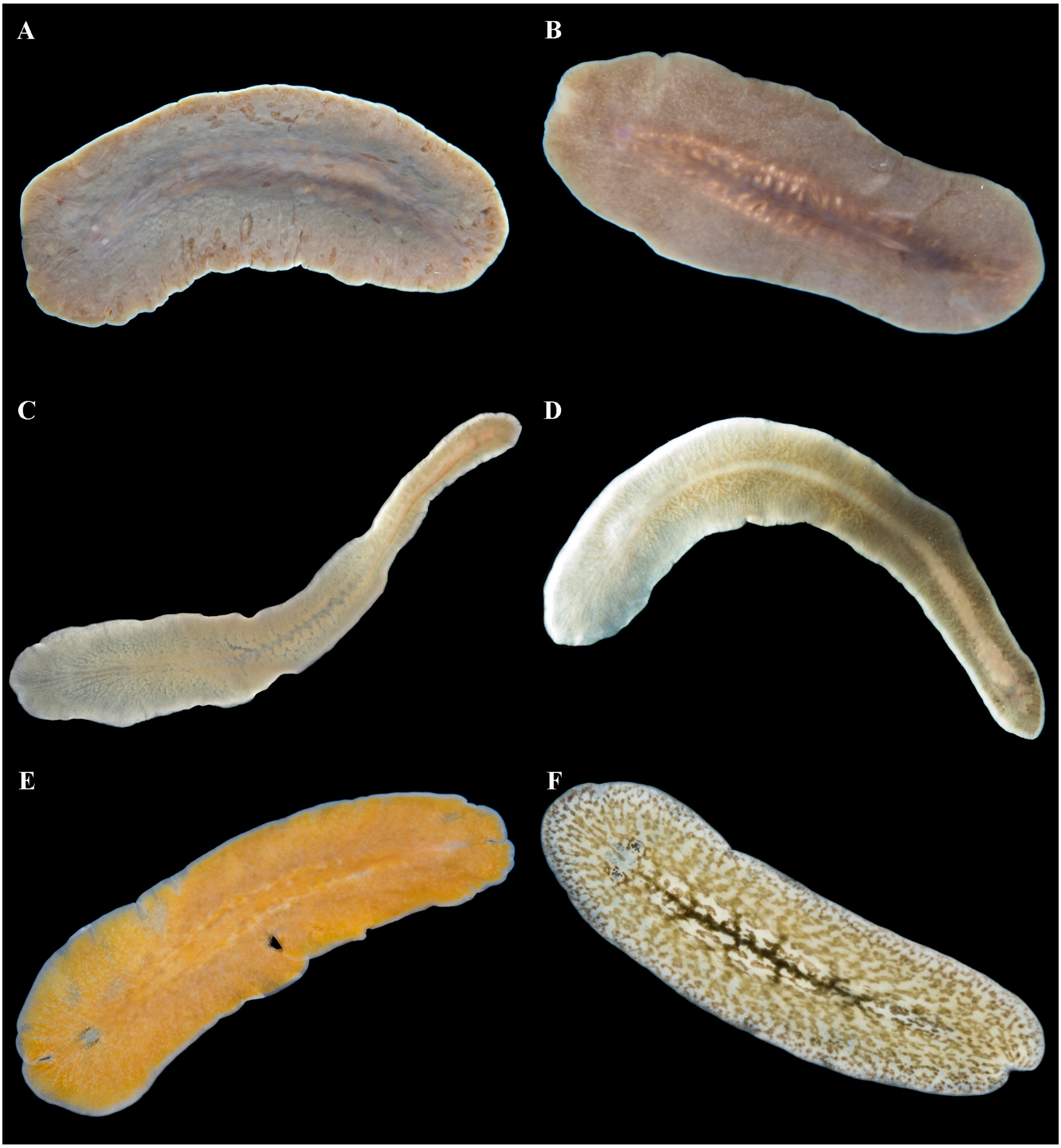

Fig. 25. Dorsal view of live flatworms. A, B, Acotylea 9 (A, ZRC.PLA.0269; B, ZRC.PLA.0273); C, Acotylea 13 ZRC.PLA.0279); D, Acotylea 14 (ZRC.PLA.0285); E, Acotylea 16 (ZRC.PLA.0290); F, Acotylea 19 (ZRC.PLA.2166).

gonopore and *Thysanozoon* with two male gonopores. Our study provides empirical support for this relationship with other genera within the family. These findings indicate that the clade containing both *Acanthozoon* and *Thysanozoon* is a sister group to the clade containing the paraphyletic *Pseudobiceros*.

Molecular phylogenetic analysis of 12 samples of *Acanthozoon* sp. 1 has provided strong support for its classification as a distinct taxon. The only other *Acanthozoon* species analysed, *Acanthozoon aranfaibo* (MK299362), is nested amongst other *Thysanozoon* species. Unfortunately, there are no other *Acanthozoon* species available for comparison. Our analysis recovers a paraphyletic *Thysanozoon* with *Acanthozoon* nested within the genus. *Thysanozoon alagoensis* is the only species that does not clade together with the other species of its genus. However, many of the nodes do not have very strong support values, indicating uncertainty surrounding the relationships within these genera. Future analysis with more data may help elucidate this relationship.

***Phrikoceros* and *Tytthosoceros*.** *Phrikoceros* and *Tytthosoceros* differ from the other genera within Pseudocerotidae by having the *Pseudoceros* trait of single male and female reproductive systems while possessing *Pseudobiceros* traits of pseudotentacle shape and simple ruffled pharynx. The two genera are further differentiated by the shape of their pseudotentacles with *Phrikoceros* having square, laterally ruffled pseudotentacles (Fig. 11A) while *Tytthosoceros* has simple-folded, pointed, ear-like pseudotentacles (Fig. 11B).

Litvaitis et al. (2019) synonymised *Tytthosoceros* with *Phrikoceros* as the two genera were monophyletic in their analysis. However, it is to be noted that in their synonymisation of the two genera, only a single *Phrikoceros mopsus* sample was used to represent the genus while also not being the type species of the genus. In a recent study by Bahia (2022), the misclassification of *Phrikoceros mopsus* in Quiroga et al. (2004) was noted, and the morphological differences between the two genera highlighted. When Quiroga et al. (2004) established the new combination *Phrikoceros mopsus* from *Pseudoceros mopsus*, the pseudotentacles' differences between the two genera were not noted or compared. The reclassification was based on marginal ruffling, simple pharyngeal folds and the arrangement of the eyespots. Bahia (2022) showed that *Phrikoceros mopsus* used in Litvaitis et al. (2019) has pointed, ear-like pseudotentacles and should have been placed in *Tytthosoceros* instead. Although no *Phrikoceros* species were used in Bahia's (2022) phylogenetic analysis, the results showed that all the *Tytthosoceros* spp., including *Tytthosoceros mopsus*, new combination, were recovered in a clade. Bahia (2022) thereby resurrected the synonymised genus *Tytthosoceros*.

A similar finding was reported in Gammoudi et al. (2025) and Tsuyuki et al. (2025), where the newly described species *Phrikoceros jannetae* and the reclassified *Phrikoceros sagamianus* were both assigned to *Phrikoceros*. This misplacement is not surprising, as both species possess simple-folded, pointed, ear-like pseudotentacles—features characteristic of *Tytthosoceros*, not *Phrikoceros*.

The phylogenetic distinction between the two genera, with *Phrikoceros baibaiye* and *Tytthosoceros lizardensis* included as type species of their respective genera, is demonstrated in this study. Results clearly show that they are distinct, with *Phrikoceros* nested within *Pseudobiceros* while *Tytthosoceros* is in a separate clade, supporting Bahia (2022) in re-establishing *Tytthosoceros*, as well as re-classifying *Phrikoceros mopsus* as *Tytthosoceros mopsus*. The present study rejects the synonymy of *Tytthosoceros* and *Phrikoceros,* and propose the reclassification of *Phrikoceros jannetae* as *Tytthosoceros jannetae*, new combination and *Phrikoceros sagamianus* as *Tytthosoceros sagamianus*, new combination.

The confusion between *Phrikoceros* and *Tytthosoceros* originated from the initial misidentification of *Phrikoceros mopsus*, which was mistakenly classified as a *Phrikoceros*. As a result, no true *Phrikoceros* species were available for comparison, and none of the species described by Newman & Cannon (1996b) had been sampled until this study. Although Gammoudi et al. (2025) attempted to observe pseudotentacles in live specimens, they reported being unable to identify the pseudotentacle type described by Newman & Cannon (1996b) and Bahia (2022) for *Phrikoceros*. They attributed this to the plasticity of the pseudotentacles. However, since the species described by Gammoudi et al. (2025) is a *Tytthosoceros*, the characteristic square, laterally ruffled pseudotentacles of *Phrikoceros* would not be present regardless of the flatworm's movement or condition.

Polyphyly of *Pseudobiceros*. In this study, the genus *Pseudobiceros* has been shown to be polyphyletic, being split into two distinct clades. One clade consists solely of *Pseudobiceros* species, specifically *Pseudobiceros apricus*, *Pseudobiceros caribbensis*, *Pseudobiceros evelinae*, *Pseudobiceros hancockanus*, *Pseudobiceros splendidus*, *Pseudobiceros stellae*, and *Pseudobiceros* sp. 5 (Fig. 3). The other clade contains the remaining *Pseudobiceros* species, the three species of the genus *Nymphozoon*, and *Phrikoceros baibaiye.*

Our examination of the gross morphology of specimens reveals that, while the key diagnosis of *Pseudobiceros* involves the presence of two male gonopores, there are no distinguishing characteristics between the two *Pseudobiceros* clades. There are two distinctive pseudotentacle types among the *Pseudobiceros* species: i) simple-folded, ear-like and pointed pseudotentacles and ii) square, laterally ruffled pseudotentacles, but these characteristics can be found throughout both clades. Despite the three genera, *Phrikoceros*, *Pseudobiceros*, and *Nymphozoon*, being closely related, there are differences in their morphology such as the shape of pseudotentacles and number of male and female gonopores. An interesting difference between the two clades is that the clade containing only *Pseudobiceros* species includes more species that are found in the Caribbean as compared to the other clade, suggesting that geographic locality could explain the phylogenetic differentiation within the genus.

Other studies have also found the genus to be non-monophyletic. The phylogeny reconstructed by Bahia et al. (2017) and Dittmann et al. (2019) also showed that *Nymphozoon orsaki*, previously classified as *Maiazoon orsaki*, is nested within *Pseudobiceros*, rendering the genus paraphyletic. More comprehensive sampling of *Pseudobiceros* spp., particularly from different geographical locations and across the two clades of *Pseudobiceros*, is required to better understand the genus.

***Pseudobiceros splendidus* and *Pseudobiceros hymanae*.** Our results show that *Pseudobiceros splendidus* and *Pseudobiceros hymanae* are phylogenetically distinct, with the species separated into the two clades of *Pseudobiceros* (Fig. 3). *Pseudobiceros splendidus*, first described by Lang in 1884, was compared to *Pseudobiceros hymanae* (Newman & Cannon, 1997) because of their very similar body colour and marginal bands. Newman & Cannon (1997) emphasised the presence of microscopic white dots, a characteristic originally noted by Lang (1884) for *Pseudobiceros splendidus*, as a distinguishing feature that led them to describe *Pseudobiceros hymanae* (Fig. 14D) as a new species. The geographical distributions of these species are also distinct, with *Pseudobiceros hymanae* found in the Indo-Pacific region and *Pseudobiceros splendidus* in the Mediterranean and Atlantic (Newman & Cannon, 1997). *Pseudobiceros evelinae* (Marcus, 1950) was not included in the comparison by Newman & Cannon (1997) as the original description indicated the body to be red, which sets this species apart distinctively.

However, Litvaitis et al. (2019) challenged this distinction. They synonymised these two species, along with *Pseudobiceros evelinae* and *Pseudobiceros periculosus* (Newman & Cannon, 1994) together based on general body colouration and similar marginal bands, even though *Pseudobiceros hymanae* was not included in their phylogenetic study. They proposed that the diet and nutritional state of the polyclad could influence the presence of microscopic white dots, and these can be considered intraspecific variations. Conversely here, observations of live flatworms kept in captivity for durations ranging from a few days to weeks, noted that while dietary factors can make a difference in the overall colouration of the body, the difference is in the way of lighter or darker shades or overall tonal colour, while the body colour generally remains the same (Tong & Ong, 2020; R. S. L. Ong, pers. obs.). Importantly, no changes were noted or seemed to affect the patterns and the marginal bands. Therefore, the hypothesis that diet and nutritional state of the polyclads can affect or change the pattern and/or marginal bands is currently unsupported.

Furthermore, there are subtle differences in the marginal bands between these similar species, relating to the continuation or absence of the same margins along the pseudotentacles as well as the width of these marginal bands that have not been addressed. In view of the phylogenetic separation between *Pseudobiceros splendidus* and *Pseudobiceros hymanae* presented in this study, the latter should be considered a separate, distinct species.

***Pseudoceros duplicinctus* and *Pseudoceros prudhoei*.** Newman & Cannon (1994) described *Pseudoceros prudhoei* as "brown covered in brown microdots: two marginal bands; inner band wide, blue or light purple; outer band narrow, bright yellow", while the photographs of the live species do not show the inner band as blue or light purple; instead, it is whitish/greyish-white. Examples available in Newman & Cannon (1994, 2003, 2005) showed a white inner margin instead of a blue or light purple band. The description of the marginal bands provided more closely matches *Pseudoceros duplicinctus* (Fig. 17F), yet available images showed otherwise. We propose that the description of *Pseudoceros prudhoei* (Fig. 17E) should be amended according to the photographs to avoid further confusion with *Pseudoceros duplicinctus.* Velasquez et al. (2018) synonymised these two species, likely due to the confusion in the descriptions. While they recognised three morphotypes of *Pseudoceros prudhoei*, it was noted that there were no specimens resembling *Pseudoceros duplicintus* sensu Prudhoe, 1989. It is noted that younger (juvenile) *Pseudoceros prudhoei* seems to be more solid black dorsally (Fig. 17A) while the mature, biggest specimen appears to be blackish-brown (Fig. 17D).

In Ong & Tong (2018), the discrepancies and confusion between these two species were also highlighted and explained. This study shows that *Pseudoceros prudhoei* in this study is distinct from *Pseudoceros duplicinctus* in Velasquez et al. (2018). These two species should thus remain separate until additional specimens are examined and molecular analyses, including comparisons of any potential morphotypes, are conducted. Furthermore, the description of *Pseudoceros prudhoei* should be revised to, "velvety orange-brown or blackish body with an inner marginal band of white followed by a narrow yellow rim", to more accurately reflect the observed colouration and pattern.

Putative new genera and species. This study has identified several potential new genera and species within Cotylea. There is a potential new genus within Pseudocerotidae based on two undescribed species, *Tytthosoceros* sp. 1 and Pseudocerotid 1, and two known species of *Tytthosoceros jannetae*, new combination, and *Tytthosoceros sagamianus*, new combination (Fig. 3). These species form a clade that is both distinct and sister to *Tytthosoceros*. Further examination of morphological traits of these species should be done to determine if there are traits that are unique and distinct to this clade.

Several other undescribed species within Cotylea (15 in total) exhibiting distinctive patterns and colouration have been identified and listed in the annotated checklist above. Presently, comparisons of these species with recorded taxa hitherto indicate that there are no good matches morphologically. Results from our phylogenetic analysis have also shown that these species are distinct from known polyclad species that have been sequenced before. Further investigation of existing literature and analyses of more collections could provide further evidence on whether these specimens represent new species awaiting formal description.

CONCLUSIONS

The present study has performed a comprehensive analysis of 270 sequences from 135 species within Polycladida. The results provide additional insights into the relationships of the lower taxonomic levels of Polycladida and have led to a better understanding of the diversity of Singapore flatworms with phylogenetic support. In particular, the monophyletic suborders of Cotylea and Acotylea, the acotylean superfamilies of Leptoplanoidea, Stylochoidea, and Discoceloidea, and the cotylean families of Pseudocerotidae and Prosthiostomidae have been recovered. However, some groups within Polycladida, such as Euryleptidae and the genera *Pseudobiceros*, *Thysanozoon*, and *Nymphozoon*, are not monophyletic. The phylogenetic analysis supports the separation of the genera *Tytthosoceros* and *Phrikoceros*, as well as the reestablishment of *Pseudobiceros hymanae* and

Pseudobiceros splendidus, and *Pseudoceros prudhoei* and *Pseudoceros duplicinctus,* as separate species. Furthermore, this study provides phylogenetic support for the subsequent description of 15 potentially new species within Cotylea and contributes 28S sequences from 138 Polycladida specimens.

Within Cotylea, congeners are often differentiated by their body colour, patterns and pseudotentacles rather than their reproductive morphology. The reproductive anatomy has been found to be too similar within the same genus to be useful in species identification, thus leading to the current system of identification of species based on colour and pattern (Newman & Cannon, 1994, 1996a, b, 1997, 1998, 2000). It is, therefore, important to observe and document the morphological characters of live flatworms to ensure accurate depictions of the characteristics as these traits are often unobservable following preservation. The integration of live morphological observation and molecular analysis has led to more reliable identification, and will furthermore create a more stable and consistent phylogenetic framework for understanding the relationships between polyclads. As demonstrated in Dittmann et al. (2019), taxon sampling, choice of marker genes, alignment method, and the phylogenetic analysis parameters can greatly affect and influence the outcomes of the phylogenetic analysis. We suggest that the inclusion of mitochondrial gene and genomic data can provide even better phylogenetic resolution at lower taxonomic levels to shed light on the lesser-known relationships (Aguado et al., 2017; Tsunashima et al., 2017), potentially resolving the taxonomic issues highlighted in Dittmann et al. (2019).

While this study has contributed observations and molecular data on a large number of polyclad species in an understudied locality—59 of the estimated 126 species recorded to date in Singapore—this only makes up about 47% of the known species diversity. There is clearly a need for more targeted sampling of the rarer polyclads and cryptobenthic species for inclusion in future phylogenetic analyses.

ACKNOWLEDGEMENTS

We are grateful for the generous assistance and support rendered by Mal Bryant (Queensland Museum). This compilation is made possible by the Comprehensive Marine Biodiversity Survey (CMBS) jointly organised by National Parks Board and National University of Singapore (2010–2015). We are also grateful to the following sponsors for their generous contributions in support of the Comprehensive Marine Biodiversity Survey II: Dalio Philanthropies, GSK-EDB Trust Fund, HSBC, ExxonMobil Asia Pacific, and Shell Eastern Trading (Pte) Ltd.

LITERATURE CITED

Aguado MT, Noreña C, Alcaraz L, Marquina D, Brusa F, Damborenea C, Almon B, Bleidorn C & Grande C (2017) Phylogeny of Polycladida (Platyhelminthes) based on mtDNA data. Organisms Diversity & Evolution, 17(4): 767–778. DOI: https://doi.org/10.1007/s13127-017-0344-4

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology, 215(3): 403–410. DOI: https://doi.org/10.1016/S0022-2836(05)80360-2

Apte D & Pitale R (2011) New records of polyclad flatworms (Platyhelminthes: Turbellaria) from coral reefs of Lakshadweep Island, India. Journal of the Bombay Natural History Society, 108: 109–113.

Auger T (2013) Living in a garden: the greening of Singapore. National Parks Board, Singapore, 200 pp.

Baguñà J & Riutort M (2004) Molecular phylogeny of the Platyhelminthes. Canadian Journal of Zoology, 82(2): 168–193.

Bahia J (2022) Twofold complication: *Phrikoceros* or *Tytthosoceros* (Platyhelminthes: Polycladida) in South America. Poster Presentation, 5th International Congress on Invertebrate Morphology, University of Vienna, August 2022. DOI: https://doi.org/10.13140/RG.2.2.24821.24805

Bahia J & Schrödl M (2016) *Pseudobiceros wirtzi* sp. nov. (Polycladida: Cotylea) from Senegal with revision of valid species of the genus. Zootaxa, 4097(1): 101–117.

Bahia J, Padula V & Schrödl M (2017) Polycladida phylogeny and evolution: Integrating evidence from 28S rDNA and morphology. Organisms Diversity & Evolution, 17(3): 653–678. DOI: https://doi.org/10.1007/s13127-017-0327-5

Bahia J, Padula V, Lavrado HP & Quiroga S (2014) Taxonomy of Cotylea (Platyhelminthes: Polycladida) from Cabo Frio, southeastern Brazil, with the description of a new species. Zootaxa, 3873(5): 495–525.

Bock S (1913) Studien über Polycladen. Zoologiska bidrag från Uppsala, 2: 29–344.

Bolaños DM, Gan BQ & Ong RSL (2016) First records of pseudocerotid flatworms (Platyhelminthes: Polycladida: Cotylea) from Singapore: A taxonomic report with remarks on colour variation. Raffles Bulletin of Zoology, Supplement 34: 130–169.

Bolaños MD, Quiroga SY, Ardila NE & Litvaitis MK (2003) An initial survey of the polyclad fauna of the Caribbean coast of Colombia. Integrative and Comparative Biology, 43(6): 945.

Bulnes V (2010) Polycladida-Marine Flatworms. In: Häussermann V & Försterra G (eds.) Marine Benthic Fauna of Chilean Patagonia. Nature in Focus, Chile, pp. 356–368.

Camacho C, Boratyn GM, Joukov V, Vera Alvarez R & Madden TL (2023) ElasticBLAST: accelerating sequence search via cloud computing. BMC Bioinformatics, 24(1): 117. DOI: https://doi.org/10.1186/s12859-023-05245-9

Carranza S, Baguñà J & Riutort M (1997) Are the Platyhelminthes a monophyletic primitive group? An assessment using 18S rDNA sequences. Molecular Biology and Evolution, 14(5): 485–497.

Chim CK, Ong RSL & Gan BQ (2015) Penis fencing, spawning, parental care and embryonic development in the Cotylean flatworm *Pseudoceros indicus* (Platyhelminthes: Polycladida: Pseudocerotidae) from Singapore. Raffles Bulletin of Zoology, Supplement 31: 60–671.

Chou LM (1988) A Guide to the Coral Reef Life of Singapore. Singapore Science Centre, Singapore, 128 pp.

Chua EK (2002) Chek Jawa: Discovering Singapore's biodiversity. Simply Green, Singapore, 115 pp.

Chua EK (2007) Singapore's Splendour: Life on the Edge. Simply Green, Singapore, 184 pp.

Collingwood C (1876) On thirty-one species of marine planarians, collected partly by the late Dr Kelaart, F.L.S. at Trincomalee, and partly by Dr Collingwood, F.L.S. in the eastern seas. Transactions of the Linnean Society of London, Series 2, Zoology, 1: 83–98.

Dawydoff C (1952) Contribution a l'etude des invertebres de la faune marine benthique de l'Indochine. Bulletin Biologique de la France et de la Belgique, Supplement 37: 1–158.

Dittmann IL, Dibiasi W, Noreña C & Egger B (2019) Description of the snail-eating flatworm in marine aquaria, *Pericelis tectivorum* sp. nov. (Polycladida, Platyhelminthes). Zootaxa, 4565(3): 383–397.

Dittmann IL, Grosbusch AL, Bertemes P & Egger B (2023) A new species of *Cycloporus* from the Adriatic Sea, with an updated phylogeny of the families Euryleptidae and Stylostomidae (Polycladida, Platyhelminthes). Zootaxa, 5319(2): 235–248. DOI: https://doi.org/10.11646/zootaxa.5319.2.5

Dixit S & Raghunathan C (2013) Polyclads of Andaman and Nicobar Islands. Journal of the Andaman Science Association, 18: 165–169.

Dixit S, Sivaperuman C & Raghunathan C (2015) Three new records of polyclad flatworms from India. Marine Biodiversity Records, 8: e29. DOI: https://doi.org/10.1017/S1755267214001043

Egger B, Lapraz F, Tomiczek B, Müller S, Dessimoz C, Girstmair J & Telford MJ (2015) A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms. Current Biology, 25(10): 1347–1353.

Ehlers U (1986) Comments on a phylogenetic system of the Platyhelminthes. Hydrobiologia, 132: 1–12.

Faubel A (1984) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut, 80: 189–259.

Gammoudi M, Dittmann IL, Girstmair J, Tomancak P, Egger B & Bulnes VN (2025) A new *Phrikoceros* species (Polycladida: Cotylea) from Ghar El Melh lagoon (Tunisia) with some remarks on the morphology of the genera *Phrikoceros* and *Thytthosoceros*. Biologia, 80: 101–115. DOI: https://doi.org/10.1007/s11756-024-01818-y

George JD & George JJ (1979) Marine Life: An Illustrated Encyclopedia of Invertebrates in the Sea. Rigby Ltd, Sydney, 288 pp.

Girard C (1851) Descriptions of a new Planaria and a new Nereries from the coast of Florida. Proceedings of the Boston Society of Natural History 1851 to 1854, 4: 137–367

Goodheart JA, Collins AG, Cummings MP, Egger B & Rawlinson KA (2023) A phylogenomic approach to resolving interrelationships of polyclad flatworms, with implications for life-history evolution. Royal Society Open Science, 10(3): 220939.

Gosliner TM, Behrens DW & Williams GC (1996) Coral Reef Animals of the Indo-Pacific. Sea Challengers, Monterey, California, 288 pp.

Heath H & McGregor EA (1912) New polyclads from Monterrey Bay, California. Proceedings of the Academy of Natural Sciences of Philadelphia, 64: 455–488.

Huelsenbeck JP & Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8): 754–755.

Hyman LH (1939) Acoel and Polyclad Turbellaria from Bermuda and the Sargassum. Bulletin of the Bingham Oceanographic Collection, 7(1): 1–26.

Hyman LH (1940) The polyclad flatworms of the Atlantic Coast of the United States and Canada. Proceedings of the United States National Museum, 89: 449–495.

Hyman LH (1952) Further notes on the Turbellarian fauna of the Atlantic Coast of the United States. Biological Bulletin, 103: 195–200.

Hyman LH (1953) The polyclad flatworms of the Pacific Coast of the North America. Bulletin of the American Museum of Natural History, 100(2): 265–392.

Hyman LH (1954) The polyclad genus *Pseudoceros*, with special reference to the Indo-Pacific region. Pacific Science, 8: 219–225.

Hyman LH (1955a) Some polyclads from Polynesia and Micronesia. Proceedings of the United States National Museum, 105: 65–82.

Hyman LH (1955b) A further study of the polyclad flatworms of the West Indian Region. Bulletin of Marine Science of the Gulf and Caribbean, 5: 259–268.

Hyman LH (1955c) Some polyclad flatworms from the West Indies and Florida. Proceedings of the United States National Museum, 104(3341): 115–150.

Hyman LH (1959) A further study of Micronesian polyclad flatworms. Proceedings of the United States National Museum, 108: 543–597.

Hyman LH (1960) Second report on Hawaiian polyclads. Pacific Science, 14: 308–309.

Jie WB, Gan BQ, Chen VY & Kuo SC (2016) *Pseudoceros magangensis*: a new species of pseudocerotid flatworm (Platyhelminthes: Polycladida) from Taiwan. Platax, 13: 33–50.

Kaburaki T (1923) The polyclad turbellarians from the Philippine Islands. Bulletin of the United States National Museum, 100: 635–649.

Karling TG (1974) On the anatomy and affinities of the turbellarian orders. In: Riser NW & Morse MP (eds.) Biology of the Turbellaria. McGraw-Hill, New York, pp. 1–16.

Katayama T, Nishioka M & Yamamoto M (1996) Phylogenetic relationships among turbellarian orders inferred from 18S rDNA sequences. Zoological Science, 13(5): 747–756.

Katayama T, Yamamoto M, Wada H & Satoh N (1993) Phylogenetic position of acoel turbellarians inferred from partial 18S rDNA sequences. Zoological Science, 10(3): 529–536.

Kato K (1943) Polyclads from Palao. Bulletin of the Biogeography Society of Japan, 13: 79–90.

Kato K (1944) Polycladida from Japan. The Journal of the Sigenkagaku Kenkyusko, 1: 257–318.

Katoh K, Misawa K, Kuma KI & Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30(14): 3059–3066.

Katoh K & Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30(4): 772–780.

Kenny NJ, Noreña C, Damborenea C & Grande C (2019) Probing recalcitrant problems in polyclad evolution and systematics with novel mitochondrial genome resources. Genomics, 111(3): 343–355.

Khalili Z, Rahimian H & Pazooki J (2009) First record of the family Pseudocerotidae (Platyhelminthes, Polycladida, Cotylea) from the Persian Gulf, Iran. ZooKeys, 31: 39–51.

Kozlov AM, Darriba D, Flouri T, Morel B & Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics, 35(21): 4453–4455. DOI: https://doi.org/10.1093/bioinformatics/btz305

Laidlaw FF (1903) On a collection of Turbellaria Polycladida from the Straits of Malacca. Proceedings of the Zoological Society of London, 1: 301–318.

Lang A (1884) Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. In: Fauna und Flora des Golfes von Neapel, No. 11. Verlag von Wilhellm Engelmann, Leipzig, XI + 688 pp. Separate atlas with 39 plates.

Laumer CE & Giribet G (2014) Inclusive taxon sampling suggests a single, stepwise origin of ectolecithality in Platyhelminthes. Biological Journal of the Linnean Society, 111(3): 570–588.

Laumer CE, Hejnol A & Giribet G (2015) Nuclear genomic signals of the ‘microturbellarian’ roots of platyhelminth evolutionary innovation. eLife, 4: e05503.

Leong KP, Lee K, Wong D, Gouw-Iwata L, Low KYJ & Chua SC (2003) Singapore Waters: Unveiling Our Seas. Nature Society (Singapore), Singapore, 145 pp.

Lim SSL, Ng PKL, Tan LWH & Wee YC (1994) Rhythm of the Sea: The Life and Times of Labrador Beach. Division of

Biology, Nanyang Technology University and Department of Zoology, National University of Singapore, Singapore, 160 pp.
Lim SSQ (2016) Molecular phylogeny of polyclad flatworms in Singapore. Unpublished BSc thesis. Department of Biological Sciences, National University of Singapore, 24 pp.
Litvaitis MK & Rohde K (1999) A molecular test of platyhelminth phylogeny: inferences from partial 28S rDNA sequences. Invertebrate Biology, 118: 42–56.
Litvaitis MK, Bolaños DM & Quiroga SY (2010) When names are wrong and colours deceive: unravelling the *Pseudoceros bicolor* species complex (Turbellaria: Polycladida). Journal of Natural History, 44(13–14): 829–845.
Litvaitis MK, Bolaños DM & Quiroga SY (2019) Systematic congruence in Polycladida (Platyhelminthes, Rhabditophora): are DNA and morphology telling the same story? Zoological Journal of the Linnean Society, 186(4): 865–891.
Maddison WP & Maddison DR (2023) Mesquite: a modular system for evolutionary analysis. Version 3.81. http://www.mesquiteproject.org [Accessed 7 December 2023]
Maghsoudlou A & Rahimian H (2014) Contribution to the knowledge of cotylean flatworms (Turbellaria, Polycladida) from Iranian coasts: Introducing a new species, with remarks on new records. Zootaxa, 3860: 325–342
Marcus E (1950) Turbellaria brasileiros. Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Zoologia, 15(8): 69−190.
Marcus E & Marcus E (1968) Polycladida from Curaçao and faunistically related regions. Studies on the Fauna of Curaçao and other Caribbean Islands, 101: 1−133.
Marquina D, Aguado MT & Norena C (2015) New records of Cotylea (Polycladida, Platyhelminthes) from Lizard Island, Great Barrier Reef, Australia, with remarks on the distribution of the *Pseudoceros* Lang, 1884 and *Pseudobiceros* Faubel, 1984 species of the Indo-Pacific Marine Region. Zootaxa, 4019: 354–377. DOI: https://doi.org/10.11646/zootaxa.4019.1.14
Minot CS (1876) On the classification of the lower worms. Proceedings of the Boston Society for Natural History, 19: 17-25.
Nanda SS, Kumar A & Vachhrajani K (2024) First record of *Thysanozoon nigrum* Girard, 1851 and *Tytthosoceros nocturnus* Newman and Cannon, 1996 (Platyhelminthes: Polycladida: Pseudocerotidae) from the Gujarat coast, India. Journal of the Marine Biological Association of India, 66(2): 95.
Newman LJ & Cannon LRG (1994) *Pseudoceros* and *Pseudobiceros* (Platyhelminthes, Polycladida, Pseudocerotidae) from Eastern Australia and Papua New Guinea. Memoirs of the Queensland Museum, 37(1): 205–266.
Newman LJ & Cannon LRG (1996a) *Bulaceros*, new genus, and *Tytthosoceros*, new genus (Platyhelminthes: Polycladida) from the Great Barrier Reef, Australia and Papua New Guinea. Raffles Bulletin of Zoology, 44(2): 479–492.
Newman LJ & Cannon LRG (1996b) New genera of pseudocerotid flatworms (Platyhelminthes; Polycladida) from Australian and Papua New Guinean coral reefs. Journal of Natural History, 30(10): 1425–1441.
Newman LJ & Cannon LRG (1997) Nine new species of *Pseudobiceros* (Platyhelminthes: Polycladida) from the Indo-Pacific. Raffles Bulletin of Zoology, 45(2): 341–368.
Newman LJ & Cannon LRG (1998) *Pseudoceros* (Platyhelminthes: Polycladida) from the Indo-Pacific with twelve new species from Australia and Papua New Guinea. Raffles Bulletin of Zoology, 46(2): 293–323.
Newman LJ & Cannon LRG (2000) A new genus of euryleptid flatworm (Platyhelminthes, Polycladida) from the Indo-Pacific. Journal of Natural History, 34(2): 191–205.
Newman LJ & Cannon LRG (2002) The genus *Cycloporus* (Platyhelminthes: Polycladida) from Australian waters. Raffles Bulletin of Zoology, 50(2): 287–299.
Newman LJ & Cannon LRG (2003) Marine Flatworms: The World of Polyclads. CSIRO Publishing, Melbourne, 112 pp.
Newman LJ & Cannon LRG (2005) Fabulous Flatworms: A Guide to Marine Polyclads. ABRS and CSIRO Publishing, Canberra and Melbourne, Australia. CD-ROM interactive guide.
Newman LJ, Paulay G & Ritson-Williams R (2003) Checklist of polyclad flatworms (Platyhelminthes) from Micronesian coral reefs. Micronesica, 35–36: 189–199.
Newman LJ & Schupp P (2002) A new species of pseudocerotid flatworm (Platyhelminthes, Polycladida) from the Indo-Pacific. Micronesica, 34(2): 177–184.
Ng MFC (2012) Habitats in Harmony: The Story of Semakau Landfill. Second Edition. National Environment Agency, Singapore, 158 pp.
Ng PKL, Corlett R & Tan HTW (2011) Singapore Biodiversity: An Encyclopedia of the Natural Environment and Sustainable Development. The Raffles Museum of Biodiversity Research, 560 pp.
Ng PKL, Lim SSL, Wang LK & Tan LWH (2007) Private Lives: An Exposé of Singapore's Shores. Editions Didier Millet and Raffles Museum of Biodiversity Research, Singapore, 212 pp.
Okuno J & Naruse T (2013) First records of five flatworms belonging to the genus Pseudoceros Lang (Turbellaria: Polycladida) from Japanese waters. Biological Magazine Okinawa, 51: 57–66.
Ong RSL (2016) Aggregation of *Tytthosoceros lizardensis* flatworms at Beting Bronok. Singapore Biodiversity Records, 2016: 108–109.
Ong RSL, Bolaños DM & Tong SJW (2018) New records of marine flatworms (Platyhelminthes: Polycladida: Cotylea) from Singapore. Nature in Singapore, 11: 53–62.
Ong RSL, Loh KS, Ng BL, Tan TS & Toh CH (2020) Seven new records of marine polyclads for Singapore. Singapore Biodiversity Records, 2020: 43–44.
Ong RSL, Tong S & Tay TS (2015) Marine flatworms at Seringat Kias. Singapore Biodiversity Records, 2015: 182–184.
Ong RSL & Tong SJW (2018) A preliminary checklist and photographic catalogue of polyclad flatworms recorded from Singapore. Nature in Singapore, 11: 77–125.
Oya Y & Kajihara H (2020) Molecular phylogenetic analysis of Acotylea (Platyhelminthes: Polycladida). Zoological Science, 37(3): 271–279.
Oya Y & Kajihara H (2021) Description and phylogenetic relationships of a new genus of Planoceridae (Polycladida, Acotylea) from Shimoda, Japan. Journal of the Marine Biological Association of the United Kingdom, 101(1): 81–88.
Oya Y, Tsuyuki A & Kajihara H (2020) A new species of *Zygantroides* (Platyhelminthes: Polycladida) from Amakusa, Japan. Species Diversity, 25(2): 189–196.
Posada D (2008) jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution, 25(7): 1253–1256. DOI: https://doi.org/10.1093/molbev/msn083
Poulter J (1987) Reef and shore fauna of Hawaii. Section 2: Platyhelminthes through Phoronida. Boston Museum Special Publication, 64: 29–58.
Prudhoe S (1978) Some polyclad turbellarians new to the fauna of the Australian coasts. Records of the Australian Museum, 31: 586–604.
Prudhoe S (1985) A Monograph on Polyclad Turbellaria. Oxford University Press, London, 259 pp.
Prudhoe S (1989) Polyclad turbellarians recorded from African waters. Bulletin of the British Museum (Natural History) Zoology, 55: 47–96.

Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology, 67(5): 901–904. DOI: https://doi.org/10.1093/sysbio/syy032

Ramos-Sánchez M, Bahia J & Bastida-Zavala JR (2019) New genus, new species and new records of marine Acotylean flatworms (Platyhelminthes: Polycladida: Acotylea) from Oaxaca, southern Mexican Pacific. Zootaxa, 4700(1): 30–58. DOI: https://doi.org/10.11646/zootaxa.4700.1.2

Rawlinson KA, Gillis JA, Billings RE & Borneman EH (2011) Taxonomy and life history of the *Acropora*-eating flatworm *Amakusaplana acroporae* nov. sp. (Polycladida: Prosthiostomidae). Coral Reefs, 30: 693–705.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA & Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539–542. DOI: https://doi.org/10.1093/sysbio/sys029

Sonnenberg R, Nolte AW & Tautz D (2007) An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. Frontiers in Zoology, 4: 6. DOI: https://doi.org/10.1186/1742-9994-4-6

Sreeraj CR & Raghunathan C (2011) New records of pseudocerotid polyclads from Andaman and Nicobar Islands, India. Marine Biodiversity Records, 4: 1–5.

Sreeraj CR & Raghunathan C (2013) Pseudocerotid polyclads (Platyhelminthes, Turbellaria, Polycladida) from Andaman and Nicobar Islands, India. Proceedings of the International Academy of Ecology and Environmental Sciences, 3(1): 36.

Sreeraj CR & Raghunathan C (2015) A report on the coral reef dwelling polyclads of Nicobar Islands, India. Proceedings of the International Academy of Ecology and Environmental Sciences, 5(2): 83–88.

Stimpson W (1857) Prodromus descriptionis animalium evertebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem a Republica Federata missa, Johanne Rodgers Duce, observavit et descripsit. Pars I, Turbellaria Dendrocoela. Proceedings of the Academy of Natural Sciences of Philadelphia, 9: 19–31.

Stummer-Traunfels R von (1933) Polycladida. In: Bronn HG (ed.) Klassen und Ordnungen des Thier-Reichs. Band IV, Abteilung 1c, Lief. 179, pp. 3485–3596, 176 text-figs., 1 pl.

Tan LWH & Ng PKL (1988) A Guide to Seashore Life. Singapore Science Centre, Singapore, 160 pp.

Tan R & Yeo A (2003) Chek Jawa Guidebook. Simply Green, Singapore, 219 pp.

Tong SJW & Ong RS (2020) Mating behavior, spawning, parental care, and embryonic development of some marine pseudocerotid flatworms (Platyhelminthes: Rhabditophora: Polycladida) in Singapore. Invertebrate Biology, 139(2): e12293.

Tsunashima T, Hagiya M, Yamada R, Koito T, Tsuyuki N, Izawa S, Kosoba K, Itoi S & Sugita H (2017) A molecular framework for the taxonomy and systematics of Japanese marine turbellarian flatworms (Platyhelminthes, Polycladida). Aquatic Biology, 26: 159–167.

Tsuyuki A, Kohtsuka H & Okuno J (2025) Description of a new species of *Prostheceraeus* Schmarda, 1859 and redescription of a poorly known species, *Phrikoceros sagamianus* (Kato, 1937) comb. n., with inference of their phylogenetic positions within Pseudocerotoidea (Platyhelminthes: Polycladida: Cotylea). Journal of Natural History, 59(5–8): 303–330.

Tyler S, Schilling S, Hooge M & Bush LF (comp.) (2006–2022) Turbellarian taxonomic database. Version 2.07. https://turbellaria.umaine.edu (Accessed 25 January 2024).

Velasquez X, Bolaños DM & Benayahu Y (2018) New records of cotylean flatworms (Platyhelminthes: Polycladida: Rhabditophora) from coastal habitats of Israel. Zootaxa, 4438(2): 237–260.

Verrill AE (1901) Additions to the fauna of the Bermudas from the Yale expedition of 1901, with notes on other species. Transactions of the Connecticut Academy of Arts and Sciences, 11: 15–62.

Yonezawa R, Itoi S, Igarashi Y, Yoshitake K, Oyama H, Kinoshita S, Suo R, Yokobori S, Sugita H & Asakawa S (2020) Characterization and phylogenetic position of two sympatric sister species of toxic flatworms *Planocera multitentaculata* and *Planocera reticulata* (Platyhelminthes: Acotylea). Mitochondrial DNA Part B, 5(3): 2352–2354. DOI: https://doi.org/10.1080/23802359.2020.1730255

SUPPLEMENTARY MATERIAL

The list of specimens and GenBank sequences used in this study are available online at https://doi.org/10.5281/zenodo.16880689

Supplementary Table 1. List of Singapore specimens examined in this study, with information on Zoological Reference Collection (ZRC) catalogue numbers and localities.

Supplementary Table 2. List of GenBank sequences used in this study, with information on accession numbers and localities.