New species and new records of *Melayonchis* slugs (Gastropoda: Euthyneura: Pulmonata: Onchidiidae)

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Abstract. In the context of a complete revision of the family Onchidiidae, two new species of *Melayonchis* Dayrat & Goulding *in* Dayrat et al., 2017, are described from western Peninsular Malaysia, in the Strait of Malacca: *Melayonchis eberlyi* Dayrat & Goulding, new species, and *Melayonchis tillieri* Dayrat, new species. The genus *Melayonchis* includes a total of six species, the four species originally described in 2017 and the two new species described here. The delineation of all six species is supported by comparative anatomy as well as mitochondrial (COI and 16S) and nuclear (ITS2 and 28S) DNA sequences. Both new species are identifiable in the field using external traits. New records are provided for *Melayonchis eloisae* Dayrat *in* Dayrat et al., 2017 in eastern Sumatra, and for *Melayonchis siongkiati* Dayrat & Goulding *in* Dayrat et al., 2017, in the Northern Territory, Australia. The isolated presence of *Melayonchis* in northern Australia suggests that the geographical distribution of *Melayonchis* is more extended than what was originally discovered (Andaman Sea, Strait of Malacca, and South China Sea). The diagnosis of the genus *Melayonchis* and the anatomical differences between *Melayonchis* species are updated. A noteworthy trait is the presence in *M. tillieri* of up to 420 radular lateral teeth in each half row (for a total of 840 lateral teeth per row), making it the onchidiid species with the highest number of teeth. Finally, differences between the intestinal types are clarified, most especially the types II and III, both found in *Melayonchis*.

Key words. biodiversity, integrative taxonomy, mangrove, South-East Asia

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INTRODUCTION

The pulmonates, or air-breathing gastropods, include the highly-diverse land snails and slugs, or Stylommatophora, and a series of taxa basal to the land snails and slugs (Dayrat et al., 2011; White et al., 2011). Some of those basal pulmonates are freshwater (Hygrophila) or terrestrial (Veronicellidae) but most of them, as the onchidiid slugs, are marine: they develop in the seawater and live in the intertidal, even though, being air-breathing organisms, they die if they are immersed in seawater for more than just a few hours. Naturally, as always, there are exceptions, and a few onchidiid species are adapted to a terrestrial life in high-elevation rainforests (Dayrat, 2010). So, from an evolutionary perspective, onchidiid slugs are fascinating, like the other taxa of basal pulmonates (Dayrat et al., 2011; White et al., 2011). However, because they mostly diversified in mangroves of the tropical Indo-West Pacific, and most particularly in South-East Asia, their diversity has remained largely overlooked (Dayrat, 2009). Also, onchidiid slugs are notoriously difficult to identify, even at the genus level, to the point where, until recently, most specimens in museum collections were merely identified as "Onchidiidae," "*Onchidium* Buchannan, 1800" (if collected from the tropical Indo-West Pacific), or "*Onchidella* J.E. Gray, 1850" (if collected outside the tropical Indo-West Pacific).

In the past few years, in an effort to improve our knowledge of onchidiid taxonomy, each genus is being methodically revised following an integrative approach (Dayrat et al., 2016, 2017, 2018, 2019a, b; Dayrat & Goulding, 2017; Goulding et al., 2018a, b, c): thousands of fresh specimens have been collected worldwide, especially in South-East Asia where onchidiids are by far the most abundant and diverse; species are delineated using anatomy and DNA sequences; the application of all existing names is addressed; new species and genera are described as needed; the colour of live animals is documented to help species identification in the field.

The genus *Melayonchis* Dayrat & Goulding *in* Dayrat et al., 2017 was originally described as a distinct clade including four species: *M. aileenae* Dayrat & Goulding *in* Dayrat et al., 2017, *M. annae* Dayrat *in* Dayrat et al., 2017, *M. eloisae*

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Dayrat *in* Dayrat et al., 2017 (the type species), and *M. siongkiati* Dayrat & Goulding *in* Dayrat et al., 2017. No external trait distinguishes *Melayonchis* slugs from all other onchidiid slugs. However, all but one species are easily identifiable in the field because of distinctive external traits. *Melayonchis annae* is the only species that could potentially be confused. Also, each species is characterised by a unique internal anatomy.

Here, two new species, *Melayonchis eberlyi* Dayrat & Goulding, new species, and *Melayonchis tillieri* Dayrat, new species, are described from the Matang mangrove, near Kuala Sepatang, in western Peninsular Malaysia. Both new species are rare but are externally and internally distinct from other known species. The four species described in 2017 were delineated using comparative anatomy and mitochondrial (COI and 16S) DNA sequences. Here, molecular data are provided for both mitochondrial (COI and 16S) as well as nuclear (ITS2 and 28S) DNA sequences, which all yield identical relationships.

New records are provided for *M. eloisae* from eastern Sumatra and for *M. siongkiati* from the Northern Territory, Australia. The presence of *M. siongkiati* in the Northern Territory is surprising because it is far away from the rest of the distribution of the genus (the Andaman Sea, the Strait of Malacca, and the South China Sea). All *Melayonchis* species are exclusively restricted to mangrove forests where live animals are found on tree trunks and roots as well as logs.

Importantly, the present study is also an opportunity to clarify the distinction between the types of intestinal loops. Four intestinal types (types I to IV) were recognised by Plate (1893) to which Labbé (1934) added a type V. However, intestinal loops vary far more than what was originally thought by Plate and Labbé (both intra- and inter-specifically), and the differences between intestinal types must be clarified. Because we have examined the intestinal type of every known species (as well as its associated individual variation) in the context of our taxonomic revision of the whole family Onchidiidae, we can introduce here a new approach to reliably determine each intestinal type. Here, we most particularly focus on the intestinal types II and III, which are the only intestinal types found in *Melayonchis*.

The new taxon name *Melayonchis eberlyi* Dayrat & Goulding, new species is the responsibility of Benoît Dayrat and Tricia C. Goulding. The authorship of the new taxon name should be cited as *Melayonchis eberlyi* Dayrat & Goulding in Dayrat, Goulding, Bourke, Khalil & Tan, 2019.

The new taxon name *Melayonchis tillieri* Dayrat, new species is the responsibility of Benoît Dayrat. The authorship of the new taxon name should be cited as *Melayonchis tillieri* Dayrat in Dayrat, Goulding, Bourke, Khalil & Tan, 2019.

MATERIAL & METHODS

Collecting. All new specimens examined here were collected by us, which provided fresh material for DNA sequencing and natural history field observations. Specimens from Malaysia were collected by Benoît Dayrat and party. Specimens from Indonesia were collected by Munawar Khalil and party. Specimens from Australia were collected by Adam Bourke. Specimens were collected by hand at low tide. Sites were accessed by car or by boat. Each site was explored for an average of two hours, but the exact time spent at each site also depended on the time of the low tide, the weather, etc. At each site, photographs were taken to document the kind of mangrove being visited as well as the diverse microhabitats where specimens were collected. GPS coordinates are available for all collecting sites, each collecting site being associated with a unique station number. If the same site was visited on different days, it was associated with distinct station numbers (e.g., our stations 27, 257, 262, 263 correspond to the same site visited on four different days). A piece of tissue was cut from specimens for DNA extraction, the rest of the specimen being relaxed in magnesium chloride and preserved in formalin or 70% ethanol for comparative anatomy. Each specimen received a unique identifier from the moment it was photographed in the field.

Specimens. Twenty-two specimens were already in our original description of the genus *Melayonchis* and are included in the molecular analyses here to demonstrate the existence of new species and new records (Table 1). The mitochondrial COI and 16S sequences for those 22 specimens are from our previous *Melayonchis* study, but their nuclear ITS2 and 28S sequences are new. All mitochondrial and nuclear sequences for the 13 specimens representing new species or new records are new. Overall, mitochondrial COI and 16S sequences are provided for 35 individuals, and nuclear 28S and ITS2 sequences are provided for 27 of those 35 individuals (excluding outgroups). DNA extraction numbers used in phylogenetic analyses are indicated in the lists of material examined as well as in the figure captions (numbers are between brackets). Size (length/width) is indicated in millimeters (mm) for each specimen. Many additional specimens were examined in the context of our worldwide revision of the family, including all available types and hundreds of onchidiids representing all the known genera and nearly all known species. The 13 specimens representing new species and new records were deposited as vouchers in an institution of their country of origin: Universitas Malikussaleh, North Aceh, Sumatra (Indonesia); Universiti Sains Malaysia, Penang (Malaysia); Museum and Art Gallery of the Northern Territory (Australia).

Museum abbreviations. National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (NMNH); Museum and Art Gallery of the Northern Territory, Darwin, Northern Territory, Australia (NTM); Universitas Malikussaleh, North Aceh, Sumatra, Indonesia (UMIZ); Universiti Sains Malaysia, Mollusk Collection, Penang, Malaysia (USMMC); Museum für Naturkunde,

Table 1. DNA extraction numbers and Genbank accession numbers for all the specimens included in the present study. The letter H next to an extraction number indicates a holotype. *Melayonchis* sequences without an asterisk are new. Sequences marked with an asterisk (including all outgroup sequences) are from our former publications (Dayrat et al., 2011, 2016, 2017, 2018, 2019a, b; Dayrat & Goulding, 2017; Goulding et al., 2018a, b, c). Abbreviations: Australian Museum, Sydney (AM); Brunei Darussalam Museum of Natural History (BDMNH); Bombay Natural History Society, India (BNHS); Institute of Tropical Biology, Zoology Collection, Vietnam Academy of Science and Technology (ITBZC); Museum and Art Gallery of the Northern Territory, Darwin, Australia (NTM); Muséum national d'Histoire naturelle, Paris, France (MNHN); National Museum of the Philippines, Manila (PNM); University of Florida, Gainesville (UF); Universitas Malikussaleh, North Aceh, Sumatra, Indonesia (UMIZ); Universiti Sains Malaysia Mollusc Collection, Penang, Malaysia (USMMC); Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore (ZRC).

Species	DNA #	Voucher	Locality	COI	16S	ITS2	28S
Melayonchis aileenae	970 H	USMMC 00018	Peninsular Malaysia	KX240033*	KX240057*	MK122902*	MK125514*
	968	USMMC 00020	Peninsular Malaysia	KX240032*	KX240056*		
	1047	BDMNH	Brunei Darussalam	KX240034*	KX240058*	MN527537	MN527570
	5631	ITBZC IM 00007	Vietnam	KX240036*	KX240060*	MN527538	MN527571
	5606	ITBZC IM 00005	Vietnam	KX240035*	KX240059*	MN527539	MN527572
Melayonchis annae	1010 H	ZRC.MOL.6502	Singapore	KX240015*	KX240039*	MK122903*	MK122919*
	1008	ZRC.MOL.6503	Singapore	KX240013*	KX240037*	MN527540	MN527573
	1009	ZRC.MOL.6503	Singapore	KX240014*	KX240038*	MN527541	MN527574
	1045	BDMNH	Brunei Darussalam	KX240016*	KX240040*	MN527542	MN527575
	1046	BDMNH	Brunei Darussalam	KX240017*	KX240041*	MN527543	MN527576
Melayonchis eberlyi	950 H	USMMC 00066	Peninsular Malaysia	MN528030	MN528043	MN527544	MN527577
	6042	USMMC 00067	Peninsular Malaysia	MN528031	MN528044	MN527545	MN527578
	6044	USMMC 00068	Peninsular Malaysia	MN528032	MN528045	MN527546	MN527579
	6045	USMMC 00069	Peninsular Malaysia	MN528033	MN528046		
Melayonchis eloisae	1011 H	ZRC.MOL.6499	Singapore	KX240026*	KX240050*	MK122904*	MK125515*
	1003	ZRC.MOL.6500	Singapore	KX240025*	KX240049*	MN527547	MN527580
	1097	BNHS 49	Andaman, India	KX240028*	KX240052*		
	922	USMMC 00009	Peninsular Malaysia	KX240023*	KX240047*		
	951	USMMC 00007	Peninsular Malaysia	KX240024*	KX240048*	MN527548	MN527581
	1043	BDMNH	Brunei Darussalam	KX240027*	KX240051*	MN527549	MN527582
	5607	ITBZC IM 00009	Vietnam	KX240031*	KX240055*	MN527550	MN527583
	1775	UMIZ 00003	Sumatra	MN528034	MN528047		
Melayonchis siongkiati	1002 H	ZRC.MOL.6501	Singapore	KX240020*	KX240044*	MK122905*	MK122920*
	1052	BDMNH	Brunei Darussalam	KX240021*	KX240045*	MN527552	MN527585
	920	USMMC 00016	Peninsular Malaysia	KX240018*	KX240042*		
	947	USMMC 00016	Peninsular Malaysia	KX240019*	KX240043*	MN527551	MN527584
	5608	ITBZC IM 00010	Vietnam	KX240022*	KX240046*	MN527553	MN527586
	5875	NTM P.59483	Northern Territory, Australia	MN528035	MN528048	MN527554	MN527587
	5877	NTM P.59484	Northern Territory, Australia	MN528036	MN528049	MN527555	MN527588
	5878	NTM P.59485	Northern Territory, Australia	MN528037	MN528050		

| Species	DNA #	Voucher	Locality	COI	16S	ITS2	28S
Melayonchis tillieri	973 H	USMMC 00070	Peninsular Malaysia	MN528038	MN528051	MN527556	MN527589
	972	USMMC 00071	Peninsular Malaysia	MN528039	MN528052	MN527557	MN527590
	5957	USMMC 00072	Peninsular Malaysia	MN528040	MN528053		
	5970	USMMC 00073	Peninsular Malaysia	MN528041	MN528054	MN527558	MN527591
	5972	USMMC 00074	Peninsular Malaysia	MN528042	MN528055	MN527559	MN527592
Alionchis jailoloensis	5137	UMIZ 00117	Indonesia, Halmahera	MG953528*	MG953538*	MG953548*	MK122918*
Marmaronchis vaigiensis	1183	ZRC.MOL.3007	Singapore	MK122812*	MK122854*	MK122877*	MK122910*
Marmaronchis marmoratus	5409	MNHN-IM-2013-15764	PNG, Madang	MK122838*	MK122859*	MK122893*	MK122915*
Paromoionchis penangensis	957	USMMC 00061	Peninsular Malaysia	MH055078*	MH055137*	MH055255*	MH055293*
Paromoionchis tumidus	963	USMMC 00057	Peninsular Malaysia	MH054946*	MH055101*	MH055194*	MH055266*
Onchidella celtica	5013	MNHN-IM-2014-6891	France	MG958715*	MG958717*	MK122906*	MK122921*
Onchidella nigricans	1524	AM C468921.002	Australia, NSW	MG970878*	MG970944*	MK122908*	MK122923*
Onchidina australis	1523	AM C468918.002	Australia, NSW	KX179548*	KX179561*	MG958719*	MG971209*
Onchidium stuxbergi	5605	ITBZC IM 00002	Vietnam	KX179520*	KX179537*	MG958721*	MG971211*
Onchidium typhae	965	USMMC 00005	Peninsular Malaysia	KX179509*	KX179525*	MG958720*	MG971210*
Peronia sp.	706	UF 303653	USA, Hawaii	HQ660038*	HQ659906*	MG958722*	MG971212*
Peronia sp.	696	UF 352288	Japan, Okinawa	HQ660043*	HQ659911*	MG958871*	MG958883*
Peronina tenera	960	USMMC 00039	Peninsular Malaysia	MG958740*	MG958796*	MG958840*	MG958874*
Peronina zulfigari	924	USMMC 00048	Peninsular Malaysia	MG958760*	MG958816*	MG958853*	MG958876*
Platevindex luteus	1001	ZRC.MOL.10179	Singapore	MG958714*	MG958716*	MG958718*	MG958888*
Wallaconchis ater	3272	PNM 041222	Philippines, Bohol	MG970809*	MG970910*	MG971132*	MG971185*
Wallaconchis sinanui	2740	UMIZ 00059	Indonesia, Ambon	MG970713*	MG970881*	MG971093*	MG971161*

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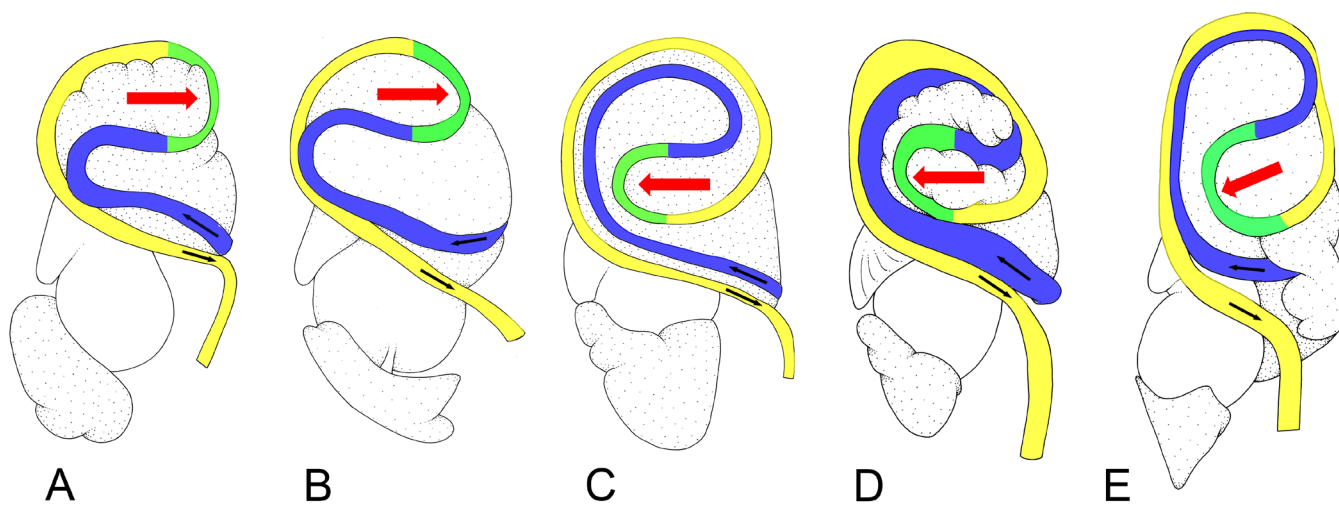
Fig. 1. Onchidiid intestinal types I and II. Small black arrows indicate the direction of the intestinal transport, which starts in the blue loop. A blue loop turns clockwise. A yellow loop turns counterclockwise. A green loop is transitional between a blue loop and a yellow loop. The orientation of the transitional (green) loop is indicated with a red arrow (at 3 o'clock in A and B, at 9 o'clock in C and D, and at 8 o'clock in E). A, Plate's (1893: pl. 8, fig. 29) original figure of a type I, redrawn (scale unclear); B, Type I, *Wallaconchis sinanui* (from Goulding et al., 2018b: fig. 8D), scale bar = 1 mm; C, Plate's (1893: pl. 8, fig. 31) original figure of a type II, redrawn (scale unclear); D, Type II, *Paromoionchis tumidus* (from Dayrat et al., 2019a: fig. 12A), scale bar = 2 mm; E. Type II, *Melayonchis annae* (from Dayrat et al., 2017: fig. 17a), scale bar = 3 mm.

Berlin, Germany (ZMB); Zoologisches Museum, Hamburg, Germany (ZMH).

DNA extraction and PCR amplification. DNA was extracted using a phenol-chloroform extraction protocol with cetyltrimethyl-ammonium bromide (CTAB). The mitochondrial cytochrome *c* oxidase I region (COI) and the 16S regions were amplified using the following primers (5′-3′): LCO1490 GGT CAA CAA ATC ATA AAG ATA TTG G and HCO2198 TAA ACT TCA GGG TGA CCA AAR AAY CA (Folmer et al., 1994), 16Sar-L CGC CTG TTT ATC AAA AAC AT (Palumbi, 1996), and 16S 972R CCG GTC TGA ACT CAG ATC ATG T (Dayrat et al., 2011). The nuclear ITS2 and 28S regions were amplified using the following primers (5′-3′): ITS2-LSU1 CTAGCTGCGAGAATTAATGTGA, ITS2-LSU-3 ACTTTCCCTCACGGTACTTG (Wade & Mordan, 2000), 28SC1 ACC CGC TGA ATT TAA GCA T (Hassouna et al., 1984), and 28SD3 GAC GAT CGA TTT GCA CGT CA (Vonnemann et al., 2005). The PCRs for COI and 16S were 25 µl reactions containing 15.8 µl of water, 2.5 µl of 10X PCR Buffer, 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of each 10 µM primer, 2 µl of dNTP mixture, 0.2 µl (1 unit) of TaKaRa Taq (Clontech, Cat No. R001A), 1 µl of 20 ng/µl template DNA, and 1 µl of 100X BSA (Bovine Serum Albumin). The PCRs for 28S differed by reducing the water to 14.3 µl, reducing the dNTP mixture to 1 µl, reducing the amount of template DNA reduced to 0.5 µl, and replacing the $MgCl_2$ and BSA with 5 µl of Q solution (QIAGEN, ID: 201203). The PCRs for ITS2 used the reagents in the same amounts as for COI and 16S, except that water was reduced to 14.8 µl, the amount of 100X BSA was increased to 2 µl, and the amount of template DNA reduced to 0.5 µl. The COI and 16S thermoprofile was: 5 minutes at 94°C; 35 cycles of 40 seconds at 94°C, 1 minute at 46°C, and 1 minute at 72°C; and 10 minutes at 72°C. The 28S thermoprofile was: 4 minutes at 94°C; 38 cycles of 30 seconds at 94°C, 50 seconds at 52°C, and 2 and a half minutes at 72°C; and 10 minutes at 72°C. The ITS2 thermoprofile was: 1 minute at 96°C; 35 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 1 minute at 72°C; and a final extension of 10 minutes at 72°C. The PCR products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) prior to sequencing. Sequenced fragments represented approximately 680 bp of COI, 530 bp of 16S, 1,000 bp of 28S, and 580 bp of ITS2. Finally, COI sequences were also translated into amino acid sequences in MEGA using the invertebrate mitochondrial genetic code to check for the presence of stop codons (no stop codon was found).

Phylogenetic analyses. Chromatograms were consulted to resolve rare ambiguous base calls. DNA sequences were aligned using Clustal W in MEGA 7 (Kumar et al., 2016). Alignments included 35 sequences for COI and 16S, and 27 sequences for 28S and ITS2 of *Melayonchis* individuals. Seventeen other onchidiid species were selected as outgroups from some of our previous studies to represent other onchidiid genera (Dayrat et al., 2011, 2016, 2017, 2018, 2019a, b; Dayrat & Goulding, 2017; Goulding et al., 2018a, b, c): *Alionchis jailoloensis* Goulding & Dayrat *in* Goulding et al., 2018a, *Marmaronchis marmoratus* (Lesson, 1831), *Marmaronchis vaigiensis* (Quoy & Gaimard, 1825), *Onchidella celtica* (Cuvier *in* Audouin & Milne-Edwards, 1832), *Onchidella nigricans* (Quoy & Gaimard, 1832), *Onchidina australis* (Semper, 1880), *Onchidium typhae* Buchannan, 1800, *Onchidium stuxbergi* (Westerlund, 1883), *Paromoionchis tumidus* (Semper, 1880), *Paromoionchis penangensis* Dayrat & Goulding *in* Dayrat et al., 2019a, *Platevindex luteus* (Semper, 1880), *Peronia* sp. (Okinawa), *Peronia* sp. (Hawaii), *Peronina tenera* (Stoliczka, 1869),
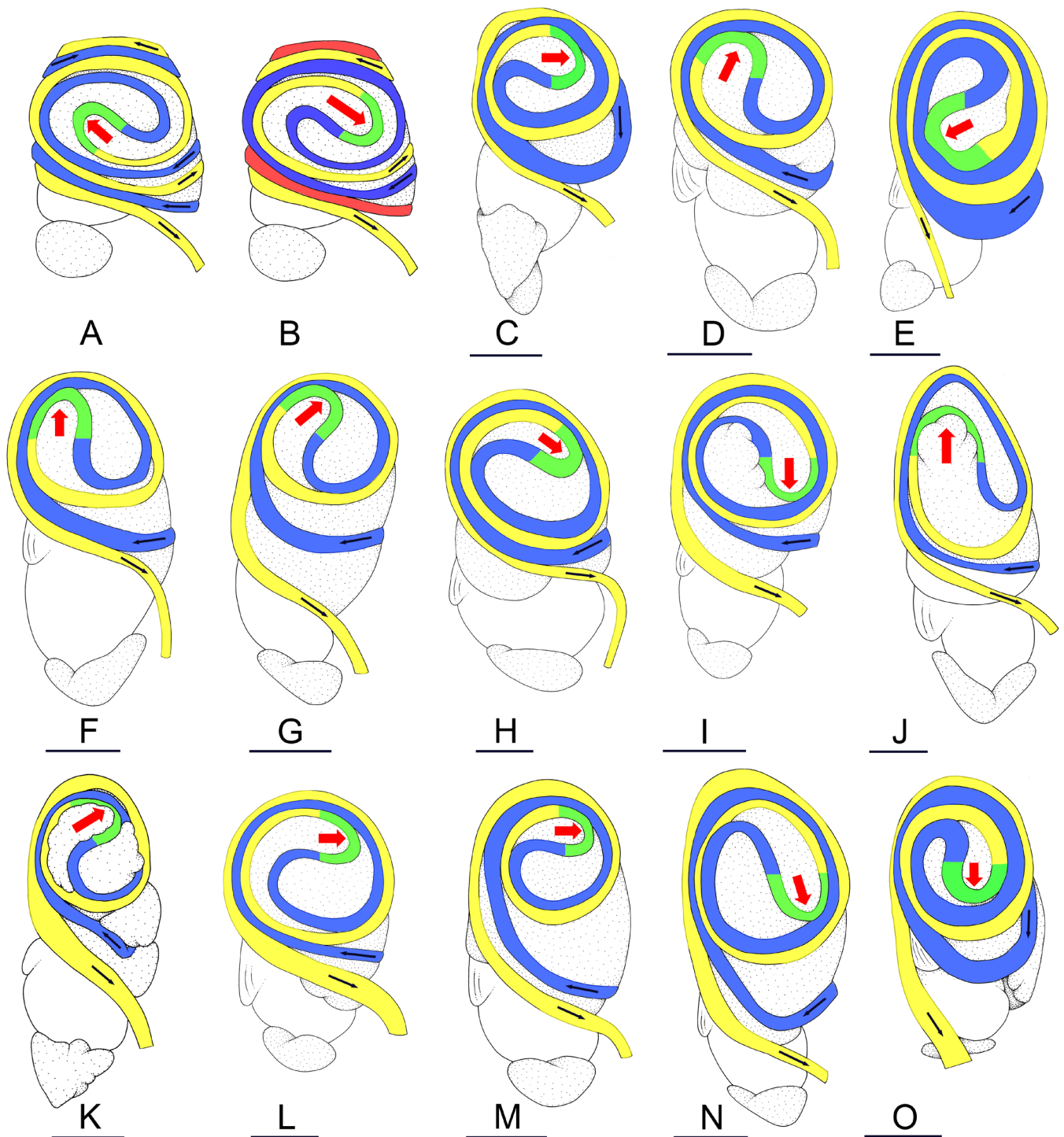
Fig. 2. Onchidiid intestinal type III. Small black arrows indicate the direction of the intestinal transport, which starts in the blue loop. A blue loop turns clockwise. A yellow loop turns counterclockwise. A green loop is transitional between a blue loop and a yellow loop. The orientation of the transitional (green) loop is indicated with a red arrow (e.g., at 3 o'clock in C, at 6 o'clock in I, and at 12 o'clock in J). For details about the *Melayonchis* and *Onchidium* individuals illustrated here, see Dayrat et al. (2016, 2017). A, Plate's (1893: pl. 8, fig. 31a) original figure of a type III, *Onchidium nigrum* (junior synonym of *Onchidium stuxbergi*), redrawn (scale unclear), colours indicate Plate's interpretation; B, same as A, colours indicate a new interpretation based on two parts (in red) drawn by mistake by Plate (see the text); C, *O. stuxbergi* (from Dayrat et al., 2016: fig. 11B), scale bar = 5 mm; D, *O. stuxbergi*, (PNM 041200), scale bar = 4 mm; E, *O. stuxbergi*, [5605], scale bar = 5 mm; F, *Melayonchis eloisae*, [1011], scale bar = 2 mm; G, *M. eloisae*, [922], scale bar = 2 mm; H, *M. eloisae*, [5607], scale bar = 1 mm; I, *M. eloisae*, [951], scale bar = 2 mm; J, *M. aileenae*, [5631], scale bar = 2 mm; K, *M. aileenae* (from Dayrat et al., 2017: fig. 23a), scale bar = 3 mm; L, *M. aileenae*, [970], scale bar = 2 mm; M, *M. siongkiati*, [1052], scale bar = 2 mm; N, *M. siongkiati*, [5608], scale bar = 3 mm; O, *M. siongkiati* (from Dayrat et al., 2017: fig. 12a), scale bar = 4 mm.

Peronina zulfigari Goulding & Dayrat *in* Goulding et al., 2018c, *Wallaconchis ater* (Lesson, 1831), and *Wallaconchis sinanui* Goulding & Dayrat *in* Goulding et al., 2018b.

DNA sequences were all deposited in GenBank and vouchers deposited in museum collections (Table 1). The ends of each alignment were trimmed and sequences were concatenated. The concatenated mitochondrial alignment included 1,035 nucleotide positions: 614 (COI) and 421 (16S). The concatenated nuclear alignment included 1,497 nucleotide positions: 505 (ITS2) and 992 (28S).

Pairwise genetic distances between COI sequences were calculated in MEGA 7. Mitochondrial and nuclear sequences were analysed separately to test whether they support the same phylogenetic relationships or not. Prior to phylogenetic analyses, the best-fitting evolutionary model was selected for each locus using the Model Selection option from Topali v2.5 (Milne et al., 2004). A GTR + G model was selected for COI and 16S, and a HKY + G model was independently selected for ITS2 and 28S. Maximum Likelihood analyses were performed using PhyML (Guindon & Gascuel, 2003) as implemented in Topali v2.5. Node support was evaluated using bootstrapping with 100 replicates. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) as implemented in Topali v2.5, with five simultaneous runs of 1.5×10^6 generations each, sample frequency of 100, and burn in of 25% (and posterior probabilities were also calculated). Topali did not detect any issue with respect to convergence. Other (unpublished) analyses were performed using different combinations of outgroups, which all yielded identical results.

Comparative anatomy. The detailed anatomy of the type species, *M. eloisae*, can be found in our previous *Melayonchis* study (Dayrat et al., 2017). The description of the anatomical features that are virtually identical between species (nervous system, heart, stomach, etc...) is not repeated here, to avoid unnecessary repetition. Also, some traits that are identical between the species described here (peripodial groove, shape of radular teeth) are only mentioned once, in the first species of the systematic account, *M. eberlyi*. However, all the characters that are useful for species comparison (colour of live animals, size, radular formulae, intestinal loops, reproductive system, etc.) are described for the two new species as well as for the specimens representing a new record of *M. siongkiati* in the Northern Territory given that those specimens are quite far from the rest of its distribution (Brunei, Peninsular Malaysia, Singapore, and Vietnam). Special attention has been paid to illustrating the holotype of each new species and the plate illustrating the species habitats also include at least one picture from the type locality.

Types of intestinal loops. In onchidiid slugs, the types of intestinal loops are based on the dorsal pattern of the intestine. Plate (1893: pl. 8, figs. 29–32) first distinguished four types of intestinal loops (types I to IV) and Labbé (1934: 177–178, fig. 3) later added a type V. However, the pattern of intestinal loops varies far more than what was originally thought by Plate and Labbé (both intra- and inter-specifically), and the

differences between intestinal types must be clarified. A new approach is introduced here to help reliably determine each intestinal type (Dayrat et al., 2019). This new approach is based on recognising three different sections in intestinal loops, each section being coloured differently: a clockwise loop is coloured in blue, a counterclockwise loop in yellow, and a transitional loop between them in green (Figs. 1, 2). For the sake of clarity, Plate's (1893: pl. 8, figs. 29, 31, 31a) original illustrations of his types I, II, and III are reproduced here (Figs. 1A, C, 2A, B).

The intestine first appears dorsally on the right side. In a type I (Fig. 1A, B), the intestine starts by forming a clockwise (blue) loop. The latter, however, is not completed, i.e., it does not form a full circle, and soon transitions into a counterclockwise (yellow) loop. As a result, the transitional (green) loop between the clockwise and counterclockwise loops is oriented to the right, typically at 3 o'clock (horizontal red arrow). Intestinal loops of type I are not found in *Melayonchis*. In a type II (Fig. 1C–E), the clockwise (blue) loop is longer and rotates more than in a type I. As a result, the transitional (green) loop is oriented to the left, typically at 9 o'clock (horizontal red arrow). So, simply put, intestinal loops are of type I if the transitional (green) loop is oriented to the right and of type II if the transitional (green) loop is oriented to the left. In *Melayonchis*, intestinal loops of type II are found only in *M. annae*, in which the orientation of the transitional (green) loop varies approximately between 8 and 9 o'clock (Fig. 1E).

According to Plate (1893: 189, pl. 8, fig. 31a) and Labbé (1934: 177–178, fig. 3), the type III is restricted to *Onchidium nigrum* Plate, 1893 —Labbé did not examine any new material for that species and simply repeated Plate's information. *Onchidium nigrum* is a junior synonym of *Onchidium stuxbergi* (Westerlund, 1883) and, more importantly, its intestinal loops (Fig. 2C–E) differ from what was originally illustrated by Plate (Fig. 2A, B). Plate's description of the type III was based on a single specimen, the holotype of *O. nigrum* (ZMB/Moll 22749), of which the digestive system was completely destroyed (Dayrat et al., 2016: 23). The intestinal loops of *O. stuxbergi* are characterised by four parallel ducts on the left, not five (Fig. 2C–E) and so it is clear that, in his original illustration of a type III (Fig. 2A), Plate drew one loop which does not exist (coloured in red in Fig. 2B). Indeed, the orientation of the transitional loop in *O. stuxbergi* varies from 1 to 8 o'clock (Fig. 2C–E) and so it is unlikely that the only one specimen examined by Plate (the holotype of *O. nigrum*) was characterised by a transitional loop at 11 o' clock (Fig. 2A), way outside the variation range observed in *O. stuxbergi*. Plus, the transitional loop of any of the species of *Onchidium*, *Melayonchis*, *Platevindex* Baker, 1938, and *Alionchis* Goulding & Dayrat *in* Goulding et al., 2018a, with intestinal loops of type III never goes beyond an orientation at 8 o'clock, so an orientation at 11 o'clock, as suggested by Plate's original drawing (Fig. 2A), is unlikely.

In a type III (Fig. 2C–E), the clockwise (blue) loop is longer and rotates more than in a type II. As a result, the transitional
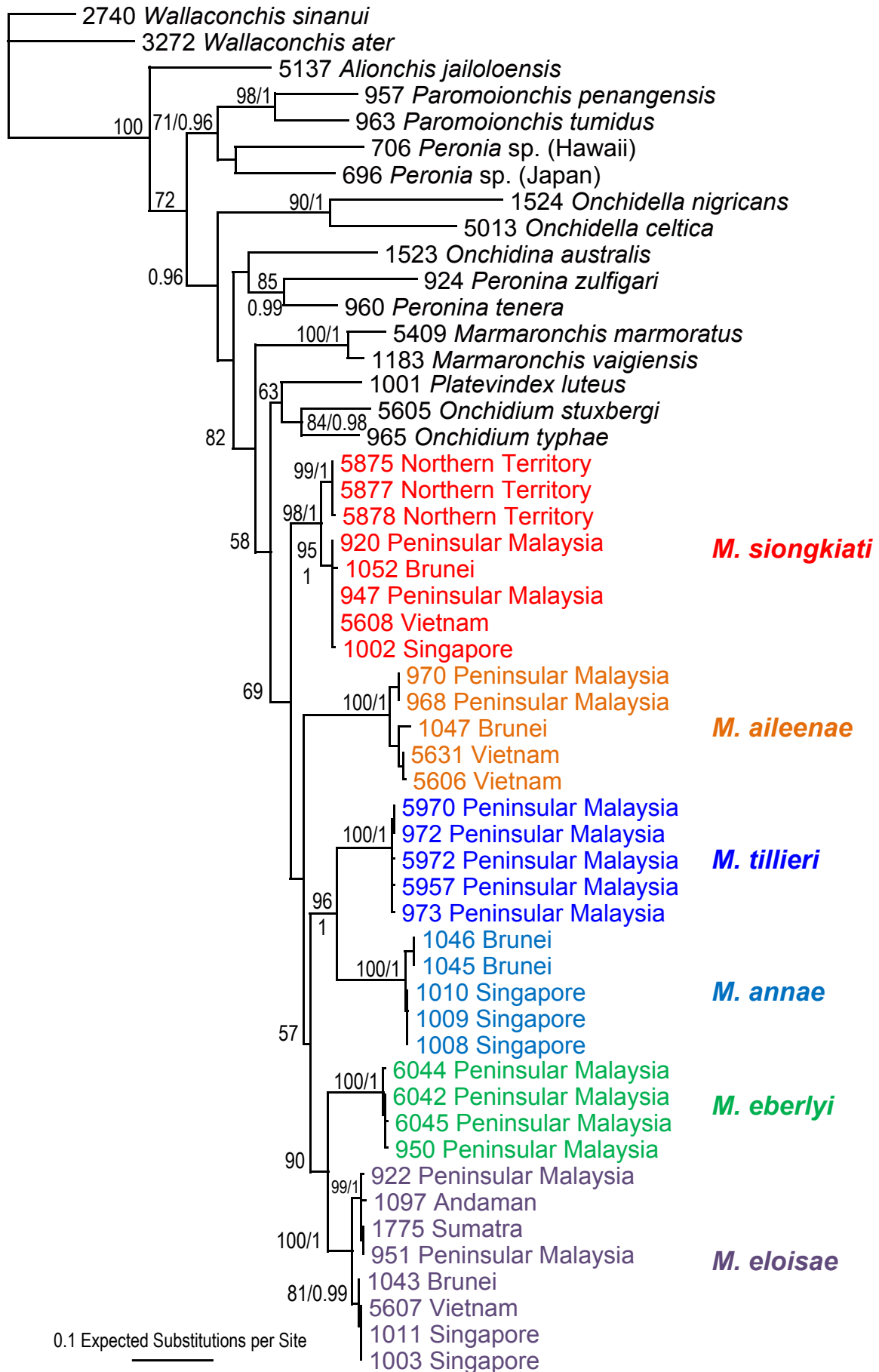
Fig. 3. Phylogenetic tree showing relationships between *Melayonchis* individuals based on mitochondrial COI and 16S sequences. Numbers by the nodes are the bootstrap values (Maximum Likelihood analysis) and the posterior probabilities (Bayesian analysis); only significant numbers (>50% and >0.9) are indicated. All other sequences serve as outgroups. Information on individually-identified *Melayonchis* specimens can be found in the lists of material examined and Table 1.
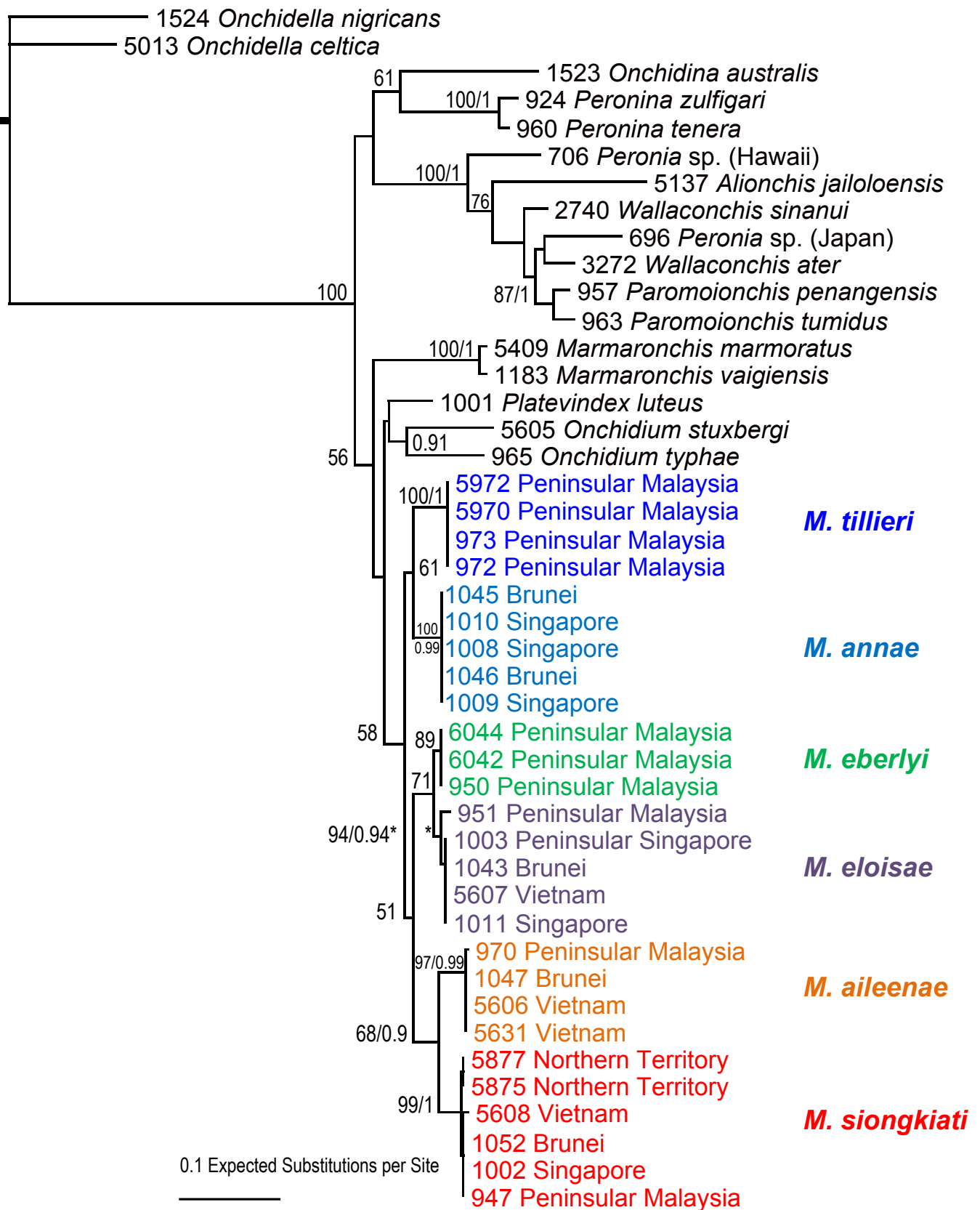
Fig. 4. Phylogenetic tree showing relationships between *Melayonchis* individuals based on concatenated nuclear ITS2 and 28S sequences. Numbers by the nodes are the bootstrap values (Maximum Likelihood analysis) and the posterior probabilities (Bayesian analysis); only significant numbers (>50% and >0.9) are indicated. All other sequences serve as outgroups. Information on individually-identified *Melayonchis* specimens can be found in the lists of material examined and Table 1.

Table 2. Pairwise genetic distances between mitochondrial COI sequences in *Melayonchis*. Ranges of minimum to maximum distances are indicated (in percentage). For instance, the intra-specific divergences within *M. annae* are between 0 and 1.9%, while the inter-specific divergences between *M. annae* and *M. siongkiati* are between 19.5 and 21.2%.

Species	*M. annae*	*M. siongkiati*	*M. eloisae*	*M. eberlyi*	*M. aileenae*	*M. tillieri*
M. annae	0.0–1.9					
M. siongkiati	19.5–21.2	0.2–4.3				
M. eloisae	18.2–19.2	14.9–17.2	0.3–3.6			
M. eberlyi	19.5–20.5	16.0–17.6	13.2–14.1	0.2–1.0		
M. aileenae	22.5–24.7	17.9–19.9	16.0–19.6	18.7–20.5	0.0–5.3	
M. tillieri	17.7–19.4	19.1–20.9	19.1–19.9	22.5–23.0	21.4–23.6	0.2–1.0
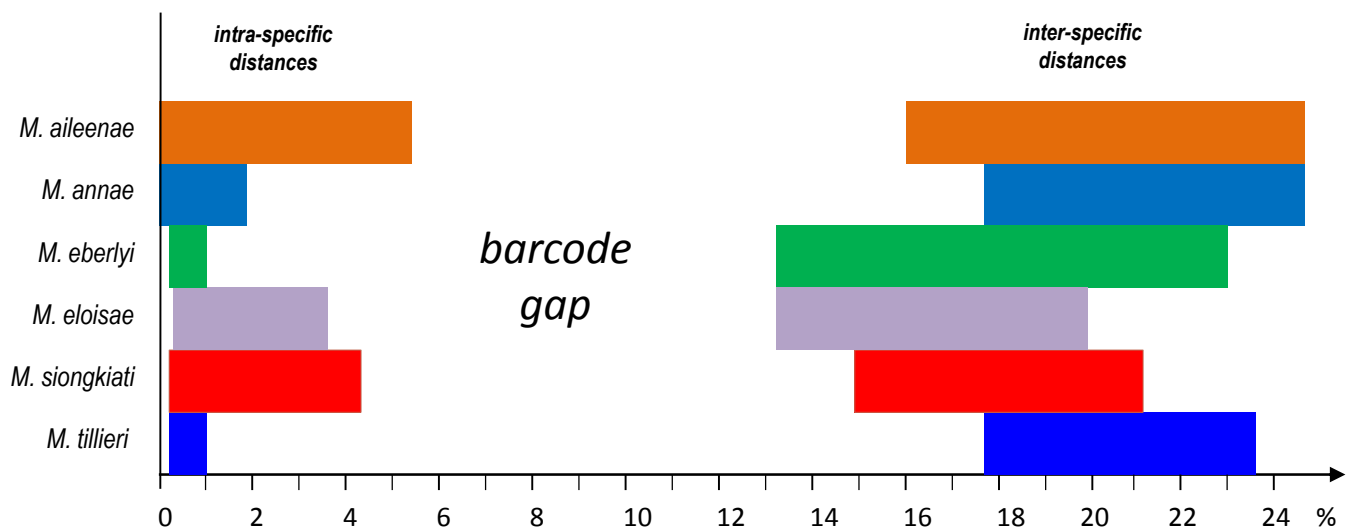
Fig. 5. Diagram that helps visualize the data on pairwise genetic distances between COI sequences within and between *Melayonchis* species (Table 2). Ranges of minimum to maximum distances are indicated (in percentages). For instance, within *M. tillieri*, individual sequences are between 0.2 and 1% divergent; individual sequences between *M. tillieri* and the other species are minimally 17.7% and maximally 23.6% divergent; overall, the distance gap between *M. tillieri* and the five other species is between 1 and 17.7%. The colours used for each species are the same as those used in Figs. 3, 4, and 6.

(green) loop is oriented to the right, typically at 3 o'clock (horizontal red arrow in Fig. 2C). In *Melayonchis eloisae*, the transitional loop is not always perfectly horizontal at 3 o'clock but its orientation varies between 12 and 6 o'clock (Fig. 2F–I). In *M. aileenae* and the new species *M. tillieri*, the orientation of the transitional loop varies between 12 and 3 o'clock (Fig. 2J–L). In *M. siongkiati*, the orientation of the transitional loop varies between 3 and 6 o'clock (Fig. 2M–O). In the new species *M. eberlyi*, the orientation of the transitional loop varies between 6 and 8 o'clock. Note that, although the intestinal loops of two species may be of type III, their transitional loop may display a significantly distinct pattern of variation, at least based on present data (e.g., between 12 and 3 o'clock *in M. aileenae* and between 6 and 7 o'clock in *M. eberlyi*).

PHYLOGENETIC RESULTS

Molecular phylogenetic analyses. DNA sequences are used here to help determine the species diversity in *Melayonchis*. Six highly-supported, reciprocally-monophyletic species are consistently recovered in all mitochondrial and nuclear analyses (Figs. 3, 4). Also, analyses agree on two pairs of sister species: *M. eloisae* and *M. eberlyi*, and *M. annae* and *M. tillieri*. Finally, the monophyly of *Melayonchis* is recovered in all analyses even though it is not strongly supported (with bootstrap values of 69 and 58).

Pairwise genetic divergences. Pairwise genetic distances between COI sequences also support the existence of six *Melayonchis* species (Fig. 5, Table 2). Intra-specific divergences are all lower than 5.3% (within *M. aileenae*) and inter-specific divergences are all higher than 13.2% (between *M. eloisae* and *M. eberlyi*), with a large and obvious barcode gap (from 5.3% to 13.2%) separating the *Melayonchis* species.
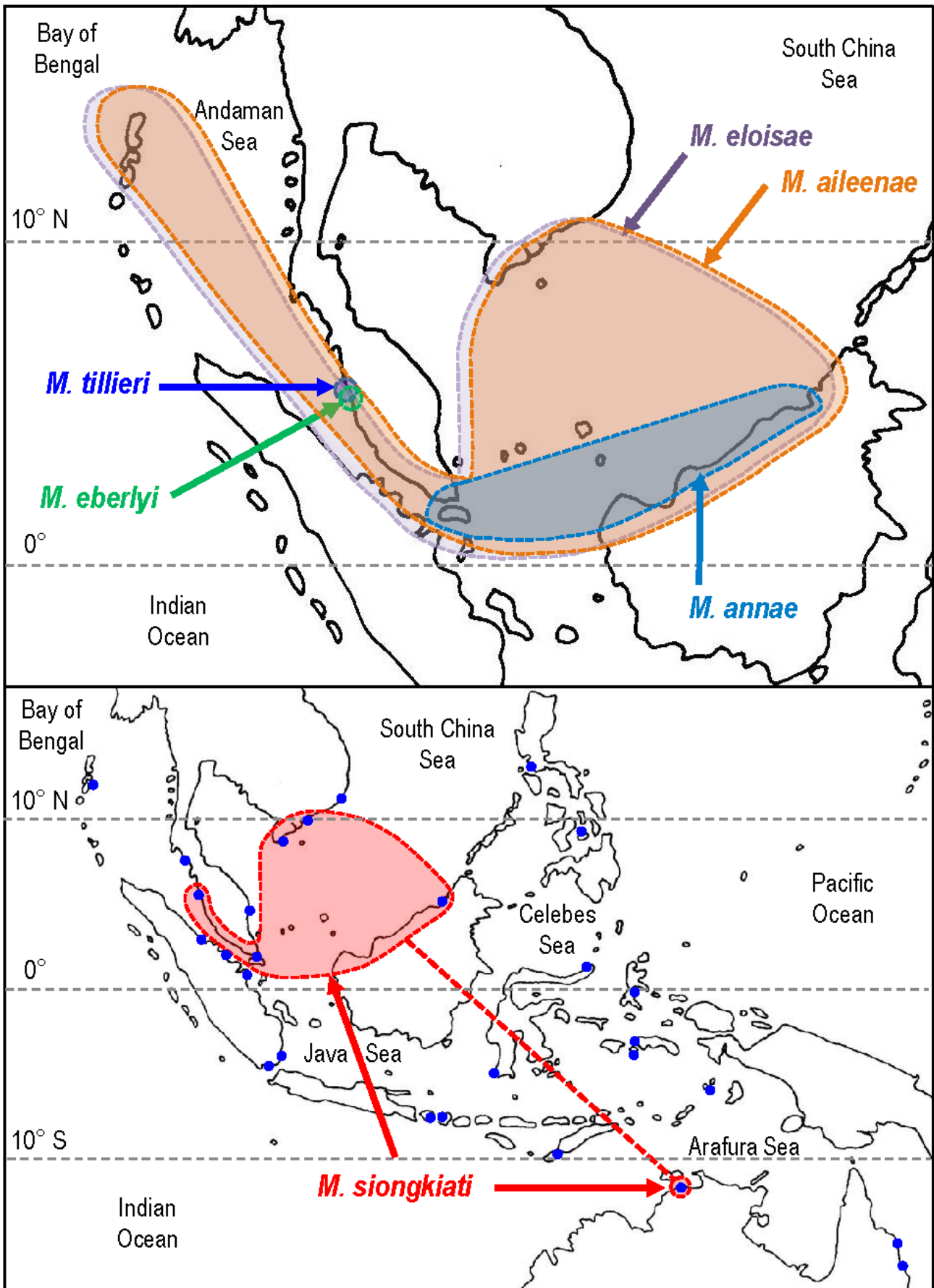
Fig. 6. Geographic distribution of the six known *Melayonchis* species. The many blue dots correspond to regions where we collected gastropods since 2010 (with several sites per dot), clearly indicating where we have not found *Melayonchis* (e.g., Philippines, Sulawesi, Queensland). So far, *Melayonchis* was only found in the Andaman Islands, on both sides of the Strait of Malacca (western Peninsular Malaysia and eastern Sumatra), Singapore, southern Vietnam, Brunei Darussalam, and Northern territory. The colours used for each species are the same as those used in Figs. 3–5.

Table 3. Morphological differences among *Melayonchis* species. Observations between parentheses are only occasional. All traits are subject to individual variation. Information regarding *M. aileenae*, *M. annae*, *M. eloisae*, and *M. siongkiati* is from Dayrat et al. (2017). The orientation of the transitional loop (TL) is provided along with the type of intestinal loops. For the radular formulae, the range of number of rows (e.g., 70 to 80 rows in *M. eberlyi*) and the range of number of lateral teeth per half row (e.g., 140 to 180 in *M. eberlyi*) are provided.

Species	*M. aileenae*	*M. annae*	*M. eberlyi*	*M. eloisae*	*M. siongkiati*	*M. tillieri*
Size	13–32 mm	10–33 mm	25–40 mm	5–25 mm	8–40 mm	33–52 mm
Dorsal colour	Brown (light brown, black) with minute white dots & linear markings	Irregularly mottled with cream, light brown, and dark brown	Black, dark or light brown, mottled with white	Black, brown (light brown), mottled with white	Brown, greyish, mottled with dark brown (whitish)	Homogenously beige
Foot colour	Whitish to light yellow	Cream	Yellow to bright orange	Orange (yellow)	Grey, creamish (darker); Darwin: pale orange	Pure white to beige
Papillae with dorsal eyes	~20	8 to 12	15–20	~10	~15	15–25
Coiling up into a ball	Occasionally (partly)	No	Always and completely	Always and completely	Occasionally (partly or completely)	No
Secreting mucus	No	Occasionally, and mildly	No	No	Always and abundantly	No
Intestinal loops	III, TL at 12 to 3 o'clock	II, TL at 8 to 9 o'clock	III, TL at 6 to 8 o'clock	III, TL at 12 to 6 o'clock	III, TL at 3 to 6 o'clock	III, TL at 12 to 3 o'clock
Radular formulae	95/140, 250/380	65/100, 185/290	70/80, 140/180	45/66, 75/110	40/80, 80/165; Darwin: 75/90, 160/170	105/135, 310/420
Accessory penial gland	No	No	No	No	Yes	No
Penis papilla length	0.5–0.8 mm	0.4 mm	0.8 mm	0.7 mm	0.5–1 mm; Darwin: 0.4–0.5 mm	0.6–0.9
Penis papilla shape	Short, slightly conical	Short, slightly conical	Club-shaped tip	Elongated and narrow, 35–40 μm in diameter, slightly curved, rim at the tip with ~9 teeth	Short, straight	Short, straight, slightly conical, tip with 5 folds and 5 deep grooves

SYSTEMATICS

Family Onchidiidae Rafinesque, 1815

Melayonchis Dayrat & Goulding *in* Dayrat et al., 2017

Type species. *Melayonchis eloisae* Dayrat *in* Dayrat et al., 2017, by original designation.

Etymology. Combination of Melayu, the Malay word for Malays, and *Onchis*, one of the old names used for onchidiid slugs (Dayrat et al., 2017: 1860).

Gender. Masculine, gender of *onchis* (ICZN, 1999; Article 30.1.1), a word derived from the masculine Greek word ὁ ὄγκος (*onchos*), which means 'mass' or 'tumour.'

Diagnosis. Body not flattened. No dorsal gills. Dorsal eyes present on notum. Retractable, central papilla present or absent. Foot wide. Eye tentacles short and narrow. Eyes at tip of ocular tentacles. Elongated, transversal protuberance on each oral lobe. Male opening below and on left of right ocular tentacle (in dorsal view). Pneumostome median. Female opening close to anus. Intestinal loops of types II and III. Rectal gland present. Lateral radular teeth with a strong protuberance on their inner lateral margin. Accessory penial gland and hollow spine present or absent. When present,

Fig. 7. Habitats, *Melayonchis eberlyi*, unique site (type locality) where *Melayonchis eberlyi* was found and which corresponds to the stations 27 (in 2011), 262 (in 2016), and 263 (in 2016) (A, B). Old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in the educational mangrove preserve near Kuala Sepatang, Peninsular Malaysia.

accessory penial gland with no muscular sac. Penis short (< 1 mm), soft papilla with no hooks.

Distinctive features. Externally, *Melayonchis* slugs could be easily confused with *Marmaronchis* Dayrat & Goulding in Dayrat et al., 2018, or *Wallaconchis* Goulding & Dayrat in Goulding et al., 2018b, even though the colour pattern of some *Melayonchis* species is very distinctive and unmistakable. Internally, however, the short (< 1 mm), the soft papilla with no hooks is unique to *Melayonchis* slugs. In addition, the lateral radular teeth of *Melayonchis* are characterised by a strong protuberance on their inner lateral margin. A similar protuberance is also present in radular teeth of *Platevindex* and *Marmaronchis* slugs. However, the protuberance of *Melayonchis* radular teeth is much stronger than in *Platevindex* and *Marmaronchis*.

Distribution (Fig. 6). *Melayonchis* is known from the Andaman Islands, both sides of the Strait of Malacca (western Peninsular Malaysia and eastern Sumatra), the South China Sea (southern Vietnam, Brunei Darussalam), and the Northern Territory, Australia. The Strait of Malacca is the core of the geographic distribution of *Melayonchis*: the Matang mangrove, near Kuala Sepatang, western Peninsular Malaysia, hosts all but one species.

Remarks. Differences among *Melayonchis* species are summarised in Table 3. Both the diagnosis and the distinctive features above are slightly updated from our previous description (Dayrat et al., 2017: 1860) to incorporate new findings: the retractable central papilla is absent in some species (described as present in the 2017 diagnosis) and, when present, is only slightly larger than the other dorsal papillae; there are no intestinal loops "intermediary between types II and III" and intestinal loops are clearly of type II or of type III (Fig. 2); finally, in 2017, the protuberance on the inner lateral margin of the lateral radular teeth was

mentioned as a distinctive feature unique to *Melayonchis*, but, after dissecting many other onchidiid slugs since then (Dayrat et al., 2018, 2019a, b; Goulding et al., 2018a, b, c), we know now that a smaller but similar protuberance is found in *Platevindex* and *Marmaronchis*, but that the short (< 1 mm), soft penial papilla is truly unique to *Melayonchis*.

Melayonchis eberlyi Dayrat & Goulding, new species
(Figs. 7–11)

Type locality (Fig. 7). Malaysia, Peninsular Malaysia, Kuala Sepatang, 04°50.434′N, 100°38.176′E, 18 July 2011, station 27, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in educational mangrove preserve.

Type material. Holotype, 40/20 mm [950], designated here (USMMC 00066).

Additional material examined. Malaysia, Peninsular Malaysia, Kuala Sepatang, 04°50.605′N, 100°38.133′E, 2 August 2016, 1 specimen 30/17 [6042], station 262, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in educational mangrove preserve (USMMC 00067); Peninsular Malaysia, Kuala Sepatang, 04°50.605′N, 100°38.133′E, 3 August 2016, 1 specimen 33/15 [6044], station 263, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in educational mangrove preserve (USMMC 00068); Peninsular Malaysia, Kuala Sepatang, 04°50.605′N, 100°38.133′E, 3 August 2016, 1 specimen 25/14 [6045], station 263, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in educational mangrove preserve (USMMC 00069).

Distribution (Fig. 6). Malaysia, Peninsular Malaysia, Malacca Strait, Kuala Sepatang (known from only one site).

Etymology. *Melayonchis eberlyi* is dedicated to the Eberly College of Science at the Pennsylvania State University

Fig. 8. Live animals, *Melayonchis eberlyi*, dorsal (A–C) and ventral (D–F) views. A, Holotype, 40 mm long [950] (USMMC 00066); B, 30 mm long [6042] (USMMC 00067); C, 33 mm long [6044] (USMMC 00068); D, same as A; E, 25 mm long [6045] (USMMC 00069); F, same as B.

for being understanding and supportive of our biodiversity exploration.

Habitat (Fig. 7). *Melayonchis eberlyi* is found in the high intertidal. It lives on mangrove tree trunks and roots. It is not found directly on mud.

Abundance. *Melayonchis eberlyi* is known from one only site where it is hard to find. We found only one individual in 2011 (station 27). We went back four times to that site in 2016 (stations 257, 258, 262, and 263) and we found only three additional individuals, even though we were

specifically looking for that species. At that site, other onchidiid species are common, including *M. eloisae*, the sister species of *M. eberlyi*.

Live animals (Fig. 8). Even though they are not found directly on mud, live animals are covered dorsally with a thin layer of muddy mucus and the colour of their dorsal notum can hardly be seen. The background of the notum is usually dark (black or brown), but it can also be light brown. The background is mottled with white areas, some of which may form two irregular longitudinal lines on either side of the medial axis. The colour of the hyponotum varies
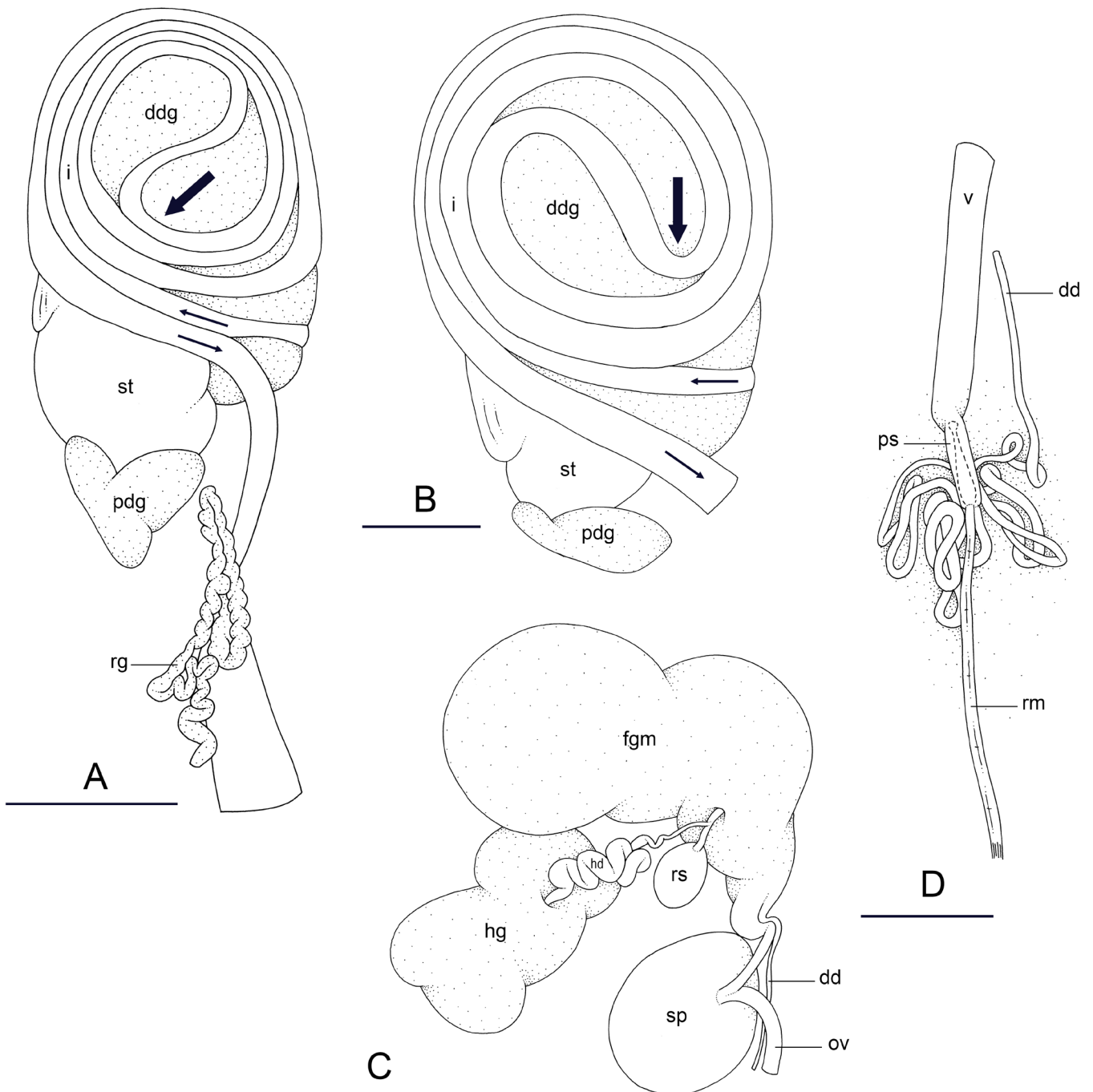
Fig. 9. Digestive system and reproductive system, *Melayonchis eberlyi*. Small arrows indicate the direction of the intestinal transport. Large arrows indicate the orientation of the transitional loop. A, Digestive system (type III, with a transitional loop at 8 o'clock), dorsal view, scale bar = 4 mm, [950] holotype (USMMC 00066); B, Digestive system (type III, with a transitional loop at 6 o'clock), dorsal view, scale bar = 2 mm, [6045] (USMMC 00069); C, Posterior reproductive parts, scale bar = 3 mm, same as A; D, Anterior male copulatory parts, scale bar = 2 mm, same as A. Abbreviations: dd, deferent duct; ddg, dorsal lobe of digestive gland; fgm, female gland mass; hd, hermaphroditic duct; hg, hermaphroditic gland; i, intestine; ov, oviduct; pdg, posterior lobe of the digestive gland; ps, penial sheath; rg, rectal gland; rm, penial retractor muscle; rs, receptaculum seminis; sp, spermatheca; st, stomach; v, vestibule.

between light grayish and black but is always marked by a white ring at the margin. The colour of the foot varies from yellow to bright orange. When the animal crawls undisturbed, the ocular tentacles are short and extend for only a few millimeters beyond the notum margin, and the head is small and remains covered by the dorsal notum.

The body is oval, not flattened. The dorsal notum is thick. Its surface, when the animal is undisturbed, is not smooth.

Dorsal gills and large papillae are absent, but small conical papillae are present. Between 15 and 20 of those papillae bear a single black dorsal eye. A slightly larger, central papilla bears three black dorsal eyes. In addition, the notum is finely granular. When the animal is disturbed, however, it forms an almost perfect ball, and its dorsal notum becomes completely smooth. Animals preserved without first being relaxed remain coiled up into a ball. Live animals are from 25 to 40 mm long.

Table 4. Radular formulae for the two new *Melayonchis* species as well as the specimens of *M. siongkiati* from the Northern Territory. Each formula follows the same format: number of rows × number of lateral teeth per left half row - 1 (rachidian tooth) - number of lateral teeth per right half row. Each DNA extraction number corresponds to one individual. The letter H next to an extraction number indicates a holotype.

Species	Radular formula	Spm length (mm)	Voucher	DNA extraction number
M. eberlyi	80 × 180-1-180	40	USMMC 00066	950 H
	75 × 160-1-160	30	USMMC 00067	6042
	70 × 140-1-140	33	USMMC 00068	6044
M. siongkiati	90 × 170-1-170	33	NTM P.59483	5875
	80 × 160-1-160	35	NTM P.59484	5877
	75 × 160-1-160	28	NTM P.59485	5878
M. tillieri	125 × 360-1-360	50	USMMC 00070	973 H
	105 × 310-1-310	36	USMMC 00072	5957
	135 × 420-1-420	52	USMMC 00074	5972
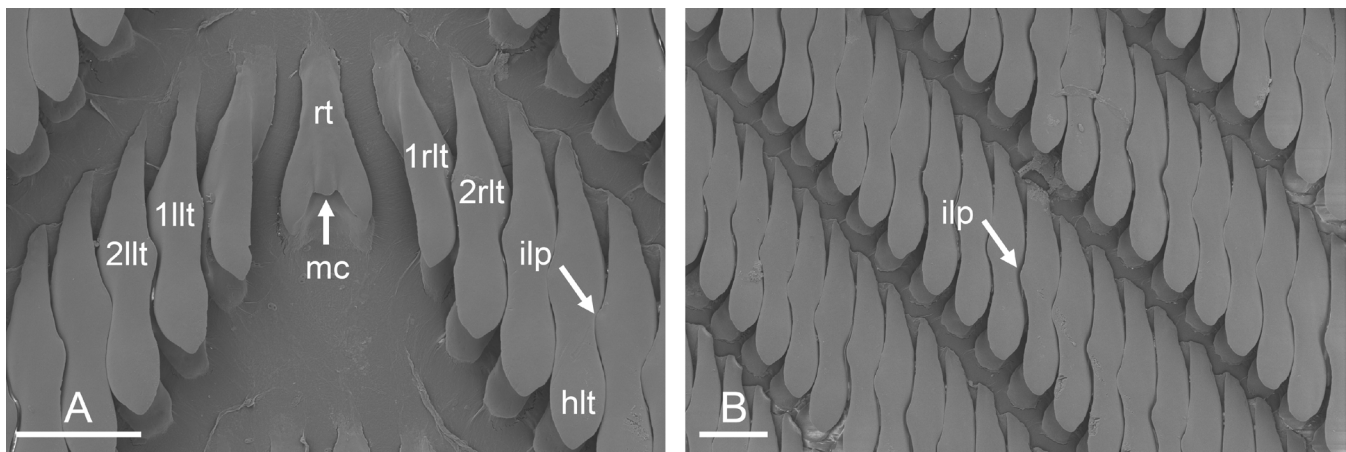
Fig. 10. Radula, *Melayonchis eberlyi*, [6042] (USMMC 00067). A, Rachidian and innermost lateral teeth, scale bar = 20 μm; B, Right lateral teeth, scale bar = 20 μm. Abbreviations: 1llt, first, left, lateral tooth; 1rlt, first, right, lateral tooth; 2llt, second, left, lateral tooth; 2rlt, second, right, lateral tooth; hlt, hook of lateral tooth; ilp, inner lateral protuberance; mc, median cusp of rachidian tooth; rt, rachidian tooth.

External morphology. Preserved specimens tend to lose their distinct, live colour traits. The ventral colour of preserved specimens, in particular, is homogenously whitish. The width of the pedal sole ranges from approximately a third to half of the total width. The anus is posterior, median, close to the edge of the pedal sole. On the right side (to the left in ventral view), a peripodial groove is present at the junction between the pedal sole and the hyponotum, running longitudinally from the buccal area to the posterior end, a few millimeters from the anus and the pneumostome. The pneumostome is median. Its position on the hyponotum relative to the notum margin and the pedal sole varies among individuals but averages in the middle. The position of the female pore (at the posterior end of the peripodial groove) does not vary much among individuals. In the anterior region, the left and right ocular tentacles are superior to the mouth. Eyes are at the tip of the ocular tentacles. Inferior to the ocular tentacles, superior to the mouth, the head bears a pair of oral lobes. On each oral lobe, there is an elongated, transversal protuberance, likely with sensitive receptors. The male aperture (opening of the copulatory apparatus) is located below the right ocular tentacle, slightly to its left side, in dorsal view.

Digestive system (Figs. 9A, B, 10, Table 4). Examples of radular formulae are presented in Table 4. The rachidian teeth are unicuspid: the median cusp is always present; there are no distinct lateral cusps on the lateral sides of the base of the rachidian tooth. The length of the rachidian teeth is approximately 25 to 35 μm, significantly less than the lateral teeth. The lateral aspect of the base of the rachidian teeth is straight, or very slightly concave. The length of the hook of the lateral teeth gradually and slightly increases (from innermost to outermost) from approximately 50 μm to 65 μm, excluding the first few (about 5) innermost and outermost lateral teeth which are significantly smaller. The inner lateral aspect of the hook of the lateral teeth is not straight. It is marked by a strong protuberance placed over the preceding adjacent tooth. The tip of the hook of the lateral teeth varies from (slightly) pointed to round. The intestinal loops are of type III, with a transitional loop oriented between 6 and 8 o'clock. A rectal gland is present.
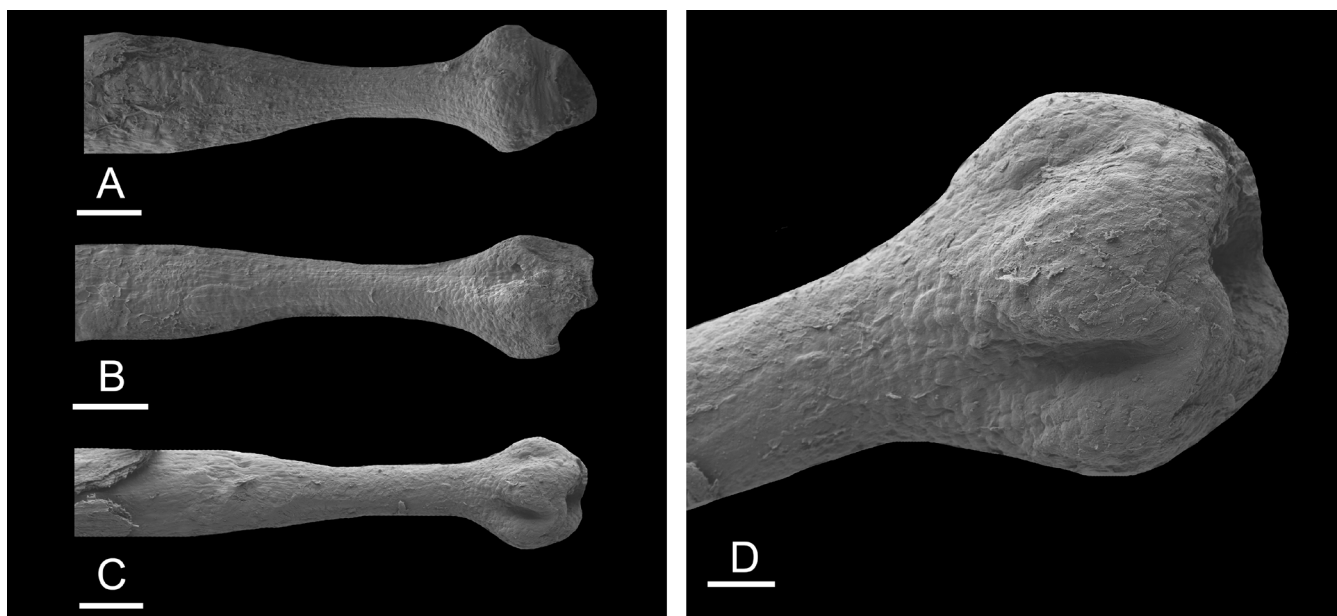
Fig. 11. Penial papilla, *Melayonchis eberlyi*. A, Scale bar = 100 μm, [950] holotype (USMMC 00066); B, Scale bar = 100 μm, [6045] (USMMC 00069); C, Scale bar = 100 μm, [6042] (USMMC 00067); D, Tip of the papilla, scale bar = 30 μm, same as C.

Reproductive system (Fig. 9C). The receptaculum seminis (caecum) is spherical to ovate. The spermatheca is nearly spherical and connects to the oviduct through a very short duct. The oviduct and the deferent duct are narrow and straight. A vaginal gland is absent.

Copulatory apparatus (Figs. 9D, 11). The male anterior organs consist of the penial papilla, the penial sheath, the vestibule, the deferent duct, and the retractor muscle. There is no accessory penial gland. The penial sheath is short (approximately 1 mm long) and straight, followed distally by a longer vestibule (approximately 4 mm long). Given that there is no accessory penial gland, the penial sheath and the vestibule are continuous. Both could be described as a penial sheath of approximately 5 mm long. Proximally, the penial sheath protects a penial papilla which consists of a short tube, conical at its base and club-shaped at its tip. Its tip is marked by a distinct pattern of five protuberances alternating with five grooves. Its length is approximately 0.8 mm. Its diameter is approximately 200 μm at the base and at the tip, and 70 μm in between. There are no penial hooks. The retractor muscle is longer than the penial sheath and inserts at about half the length of the floor of the visceral cavity. The deferent duct is highly convoluted with many loops.

Remarks. Even though we found only one individual in 2011, we immediately recognised that *Melayonchis eberlyi* was a distinct species. We also immediately realised that it was closely related to *M. eloisae* because individuals of both species share exactly the same colour pattern and coil up into a ball when disturbed. However, individuals of *M. eberlyi* are significantly larger (Table 3) and so we originally called this species the "big, black and white ball" species in the field. Internally, *M. eberlyi* also differs from *M. eloisae* (Table 3): for instance, the penial papilla of *M. eloisae* is narrow and elongated and its tip is marked by a rim with approximately 9 teeth, while the penial papilla of *M. eberlyi*

is not narrow and marked by a distinctive, club-shaped tip. That being said, the largest individuals of *M. eloisae* (~ 25 mm long) could easily be confused with the small individuals of *M. eberlyi* (~ 25 mm long).

Melayonchis tillieri Dayrat, new species
(Figs. 12–16)

Type locality (Fig. 12E). Malaysia, Peninsular Malaysia, Matang, off Kuala Sepatang, Crocodile River, Sungai Babi Manpus, 04°49.097′N, 100°37.370′E, 19 July 2011, station 28, old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many dead logs.

Type material. Holotype, 50/20 [973] mm, designated here (USMMC 00070).

Additional material examined. Malaysia, Peninsular, Malaysia, Matang, facing the fishermen's village on the other side of the river, 04°50.154′N, 100°36.368′E, 20 July 2011, 1 specimen 33/17 [972] mm, station 29 (same site as station 256 in 2016), oldest and open *Rhizophora* forest of tall and beautiful trees, with hard mud, many creeks and logs (USMMC 00071); Peninsular Malaysia, Kuala Sepatang, 04°50.217′N, 100°36.826′E, 26 July 2016, 1 specimen 36/18 [5957] mm, station 256 (same site as station 29 in 2011), oldest and open *Rhizophora* forest of tall and beautiful trees, with hard mud, many creeks and logs (USMMC 00072); Peninsular Malaysia, Kuala Sepatang, 04°50.605′N, 100°38.133′E, 27 July 2016, 1 specimen 36/14 [5970] mm, station 257, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in the educational mangrove preserve (USMMC 00073); Peninsular Malaysia, Kuala Sepatang, 04°50.605′N, 100°38.133′E, 27 July 2016, 1 specimen 52/20 [5972] mm, station 257, old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in the educational mangrove preserve (USMMC 00074).

Fig. 12. Habitats, *Melayonchis tillieri*, at the only three sites where *M. tillieri* is known, near Kuala Sepatang, Peninsular Malaysia. A, From the boat, view of station 256 (in 2016, same site as station 29 in 2011), old and open *Rhizophora* forest of tall and beautiful trees, with hard mud, many creeks and logs; B. Landing place to access station 256; C, Station 256, inside the forest; D, same as C; E, Station 28 (in 2011, type locality), old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many logs; F, Station 257 (in 2016), old forest with tall *Rhizophora* trees, high in the tidal zone (ferns), in the educational mangrove preserve.

Distribution (Fig. 6). Malaysia, Peninsular Malaysia, Malacca Strait, Kuala Sepatang (known from only three sites very close to each other).

Etymology. *Melayonchis tillieri* is dedicated to Simon Tillier, former PhD advisor of the first author at the Muséum national d'Histoire naturelle (Paris, France), for all the support and freedom he provided during those important years.

Habitat (Fig. 12). *Melayonchis tillieri* lives in high intertidal, well-preserved, old mangrove forests. It crawls on tree trunks and roots as well as logs and even young stems. It is not found directly on the mud. It is the only onchidiid species that we ever found crawling on a green stem, high above ground (4 feet).
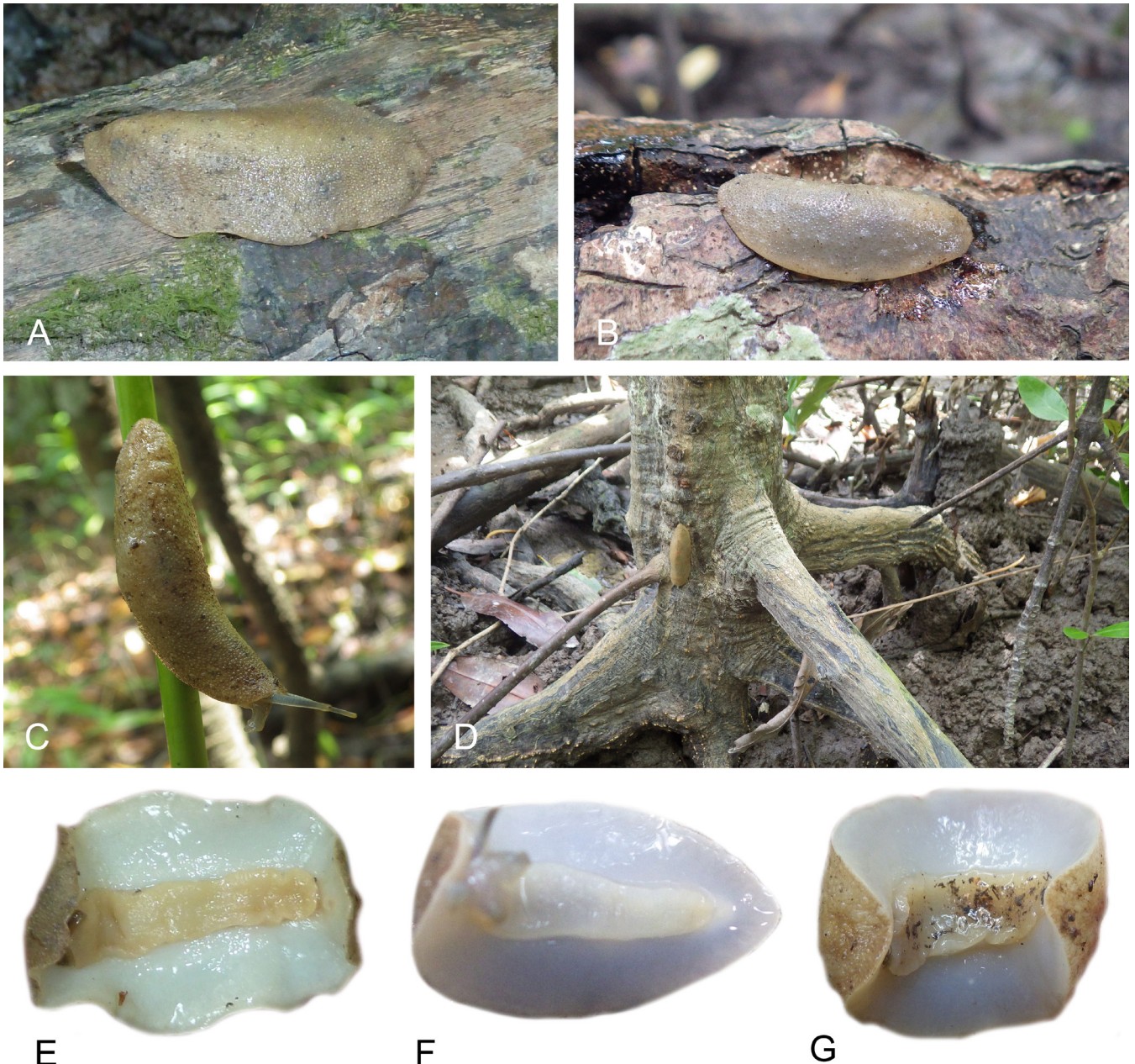
Fig. 13. Live animals, *Melayonchis tillieri*, dorsal (A–D) and ventral (E–G) views. A, Holotype, 50 mm long [973] (USMMC 00070); B, 36 mm long [5957] (USMMC 00072); C, 36 mm long [5970] (USMMC 00073); D, 52 mm long [5972] (USMMC 00074); E, same as A; F, same as B; G, same as D.

Abundance. *Melayonchis tillieri* is a rare species. We found only five individuals at three different sites in the Matang mangrove, near the fishermen's village of Kuala Sepatang, western Peninsular Malaysia. Two sites are facing the village on the other side of the large river and are only accessible by boat: the site of station 28 (2011) and the site of stations 29 (2011) and 256 (2016). The third site is the mangrove educational preserve accessible by the road (station 257, in 2016). Only one individual was found at station 28, one at station 29, one at station 256, and two at station 257. The site of station 257 was visited four other times (station 27 in 2011 and stations 258, 262, and 263 in 2016) but *M. tillieri* was not found there.

Live animals (Fig. 13). Live animals are not covered dorsally with a thin layer of mud. The notum is homogenously beige.

The hyponotum is pure white. The foot varies from pure white to beige. When the animal crawls undisturbed, the ocular tentacles extend normally for only a few millimeters beyond the notum margin but exceptionally for up to one centimeter, and the head is small and remains covered by the dorsal notum.

The body is elongated, not flattened. The dorsal notum is moderately thick and flexible. Its surface, when the animal is undisturbed, is finely granular, but not completely smooth. Dorsal gills and papillae are absent. There are between 15 and 25 single black dorsal eyes and a central group of three dorsal eyes. There is no retractable central papilla. When the animal is disturbed, it does not become a ball, and its dorsal notum does not become completely smooth. Live animals are from 33 to 52 mm long.
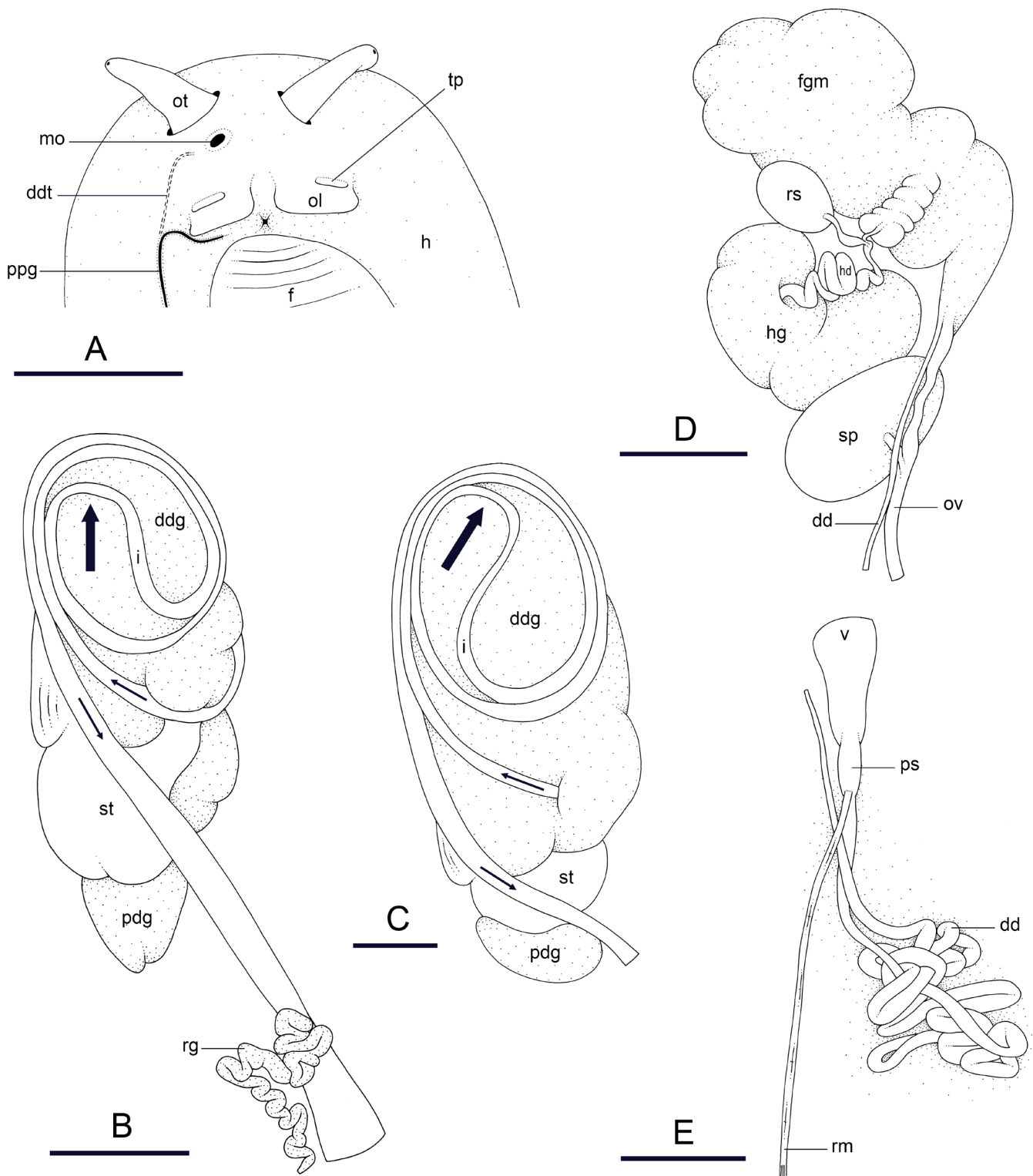
Fig. 14. External morphology, digestive system, and reproductive system, *Melayonchis tillieri*. Small arrows indicate the direction of the intestinal transport. Large arrows indicate the orientation of the transitional loop. A, Ventral, anterior view, scale bar = 5 mm, [5972] (USMMC 00074); B, Digestive system (type III, with a transitional loop at 12 o'clock), dorsal view, scale bar = 5 mm, holotype [973] (USMMC 00070); C, Digestive system (type III, with a transitional loop at 1 o'clock), dorsal view, scale bar = 2 mm, same as A; D, Posterior reproductive parts, scale bar = 4 mm, same as B; E, Anterior male copulatory parts, scale bar = 3 mm, same as B. Abbreviations: dd, deferent duct; ddg, dorsal lobe of digestive gland; ddt, deferent duct (by transparency); f, foot (pedal sole); fgm, female gland mass; h, hyponotum; hd, hermaphroditic duct; hg, hermaphroditic gland; i, intestine; mo, male opening; ol, oral lobe; ot, ocular tentacle; ov, oviduct; pdg, posterior lobe of the digestive gland; ppg, peripodial groove; ps, penial sheath; rg, rectal gland; rm, penial retractor muscle; rs, receptaculum seminis; sp, spermatheca; st, stomach; tp, transversal protuberance (on oral lobe); v, vestibule.
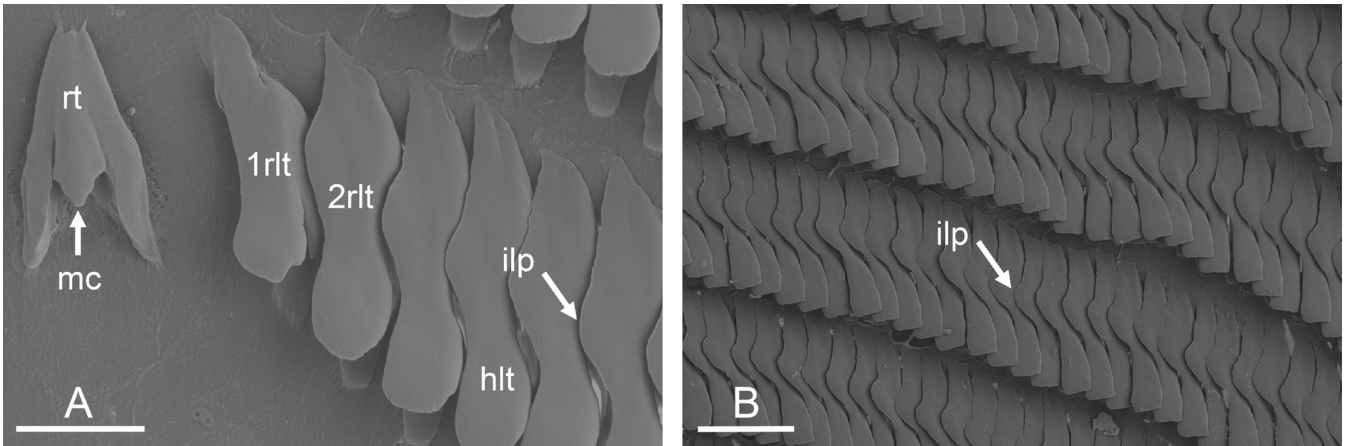
Fig. 15. Radula, *Melayonchis tillieri*. A, Rachidian and innermost lateral teeth, scale bar = 10 μm, [973] holotype (USMMC 00070); B, Right lateral teeth, scale bar = 20 μm, [5972] (USMMC 00074). Abbreviations: 1rlt, first, right, lateral tooth; 2rlt, second, right, lateral tooth; hlt, hook of lateral tooth; ilp, inner lateral protuberance; mc, median cusp of rachidian tooth; rt, rachidian tooth.
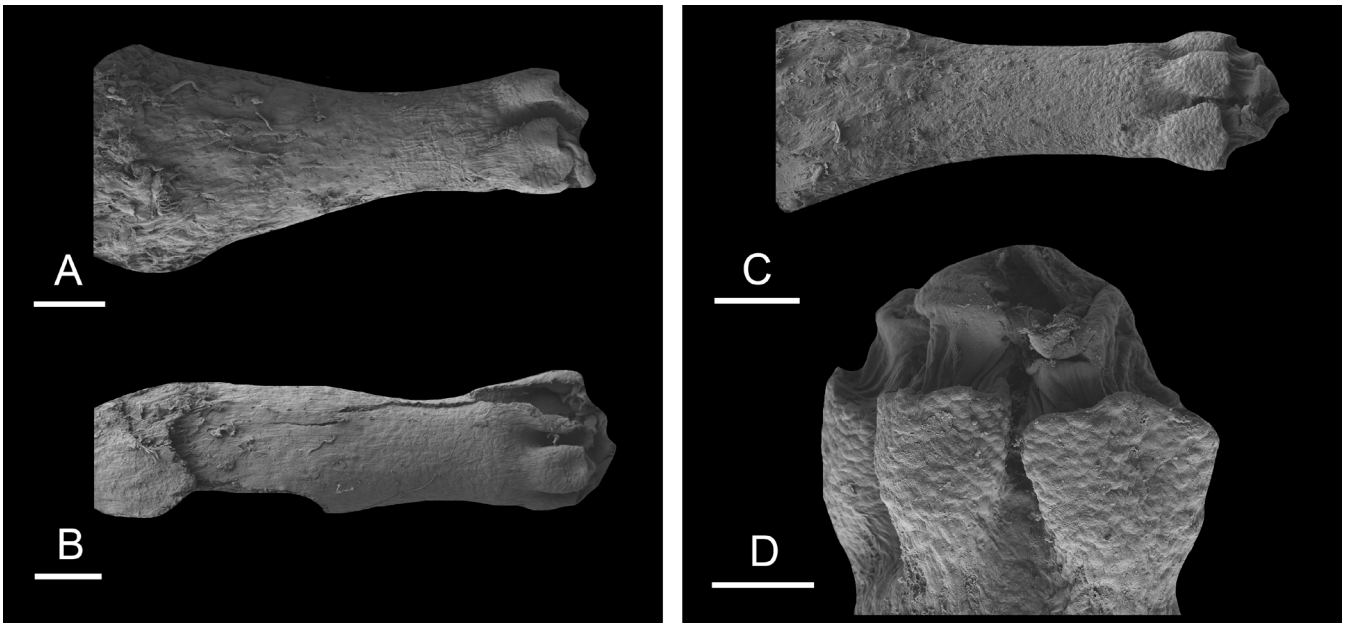

Fig. 16. Penial papilla, *Melayonchis tillieri*. A, Scale bar = 100 μm, [973] holotype (USMMC 00070); B, Scale bar = 100 μm, [5972] (USMMC 00074); C, Scale bar = 100 μm, [972] (USMMC 00071); D, Tip of the papilla, scale bar = 40 μm, same as C.

External morphology (Fig. 14A). The colour of preserved specimens is similar to live animals (homogenously beige dorsally and whitish ventrally). The width of the pedal sole is approximately a third of the total width. The pneumostome is median. Its position on the hyponotum relative to the notum margin and the pedal sole varies among individuals (from very close to the pedal sole to about half way between the pedal sole and the notum margin). The male aperture (opening of the copulatory complex) is located below the right ocular tentacle, slightly to its left side in dorsal view. In one individual, the deferent duct was observed (by transparency) following the peripodial groove from its anterior end all the way to its posterior end.

Digestive system (Figs. 14, 15, Table 4). Examples of radular formulae are presented in Table 4. The length of the rachidian teeth is approximately 15 to 25 μm, significantly less than the lateral teeth. The length of the hook of the lateral teeth is approximately 40 μm, excluding the first few

(about 5) innermost and outermost lateral teeth which are significantly smaller. The inner lateral aspect of the hook of the lateral teeth is not straight. It is marked by a strong protuberance placed over the preceding adjacent tooth. The tip of the hook of the lateral teeth is mostly tapered (square). The intestinal loops are of type III, with a transitional loop oriented between 12 and 3 o'clock. A rectal gland is present.

Reproductive system (Fig. 14D). The receptaculum seminis (caecum) is spherical to ovate. The spermatheca is ovate and connects to the oviduct through a very short duct. The oviduct and the deferent duct are narrow and straight. A vaginal gland is absent.

Copulatory apparatus (Figs. 14E, 16). The male anterior organs consist of the penial papilla, the penial sheath, the vestibule, the deferent duct, and the retractor muscle. There is no accessory penial gland. The penial sheath is short (approximately 1 mm long) and straight, followed by a longer
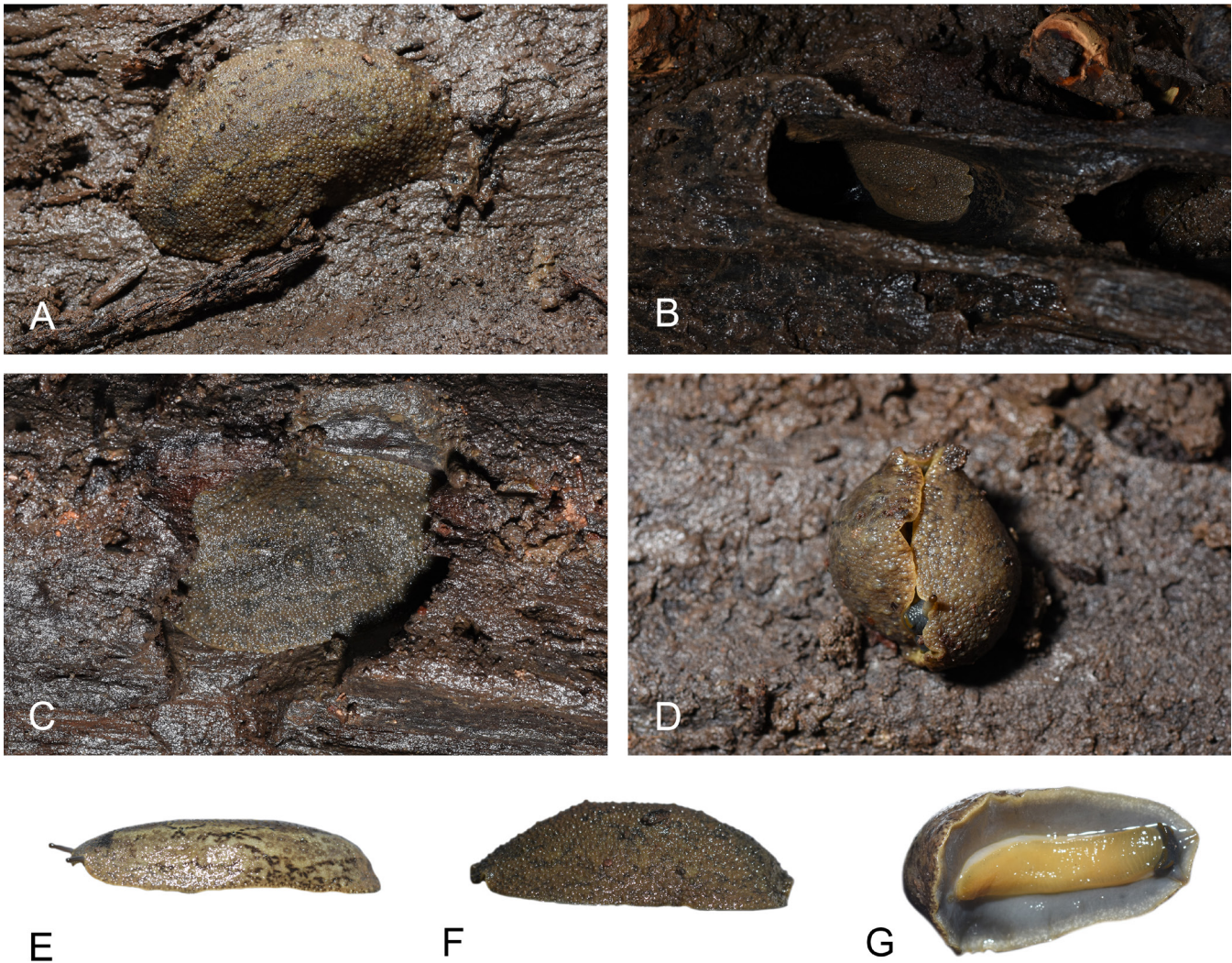
Fig. 17. Live animals, *Melayonchis siongkiati*, Darwin, Northern Territory, Australia, dorsal (A–C), ventral (D, G), and lateral (E, F) views. A, 33 mm long [5875] (NTM P.59483); B, 35 mm long [5877] (NTM P.59484); C, 28 mm long [5878] (NTM P.59485); D, same as C; E, same as A; F, same as B; G, same as A.

vestibule (approximately 3 mm long). Given that there is no accessory penial gland, the penial sheath and the vestibule are continuous. Both could be described as a penial sheath of approximately 4 mm long. Proximally, the penial sheath protects a penial papilla which consists of a short tube, slightly conical at its base. Its tip is marked by a distinct pattern of five longitudinal folds separated by deep grooves. Its length ranges between 0.6 and 0.9 mm. Its diameter is approximately 250 to 300 μm at the base and 200 μm at the tip. There are no penial hooks. The retractor muscle is longer than the penial sheath and inserts at about the anterior third of the floor of the visceral cavity. The deferent duct is highly convoluted with many loops.

Remarks. *Melayonchis tillieri* is rare but it is one of the few unmistakable onchidiid species. In the field, we immediately recognised it as a distinct species and we called it the "white, smooth species" because of its pure white hyponotum and very finely granular dorsum. Internally, *M. tillieri* also differs from other *Melayonchis* species, especially by its distinctive penial papilla (Table 3).

Melayonchis siongkiati Dayrat & Goulding *in* Dayrat et al., 2017
(Figs. 17–20)

Melayonchis siongkiati Dayrat & Goulding *in* Dayrat et al., 2017: 1871–1878, figs. 9–15.

Type locality. Singapore, Mandai River, 01°26.237′N, 103°45.730′E, 2 April 2010, station 6, following the river from the railroad towards the sea, open mangrove forest with tall trees and soft mud, ending on sun-exposed mudflat outside the mangrove with very soft mud.

Type material. Holotype, by original designation, 32/20 [1002] mm (ZRC.MOL.6501).

Additional material examined. Australia, Northern Territory, upper Darwin Harbour, Elisabeth River, upstream Bennetts Creek, 12°35.26740′, 131°01.31160′, 7 September 2016, 1 specimen 33/22 [5875] mm, station 265, at mid-tide level, on bank of *Rhizophora*-dominated tidal creek, deeply buried within moist woody debris and decaying stumps (NTM P.59483); Australia, Northern Territory, upper
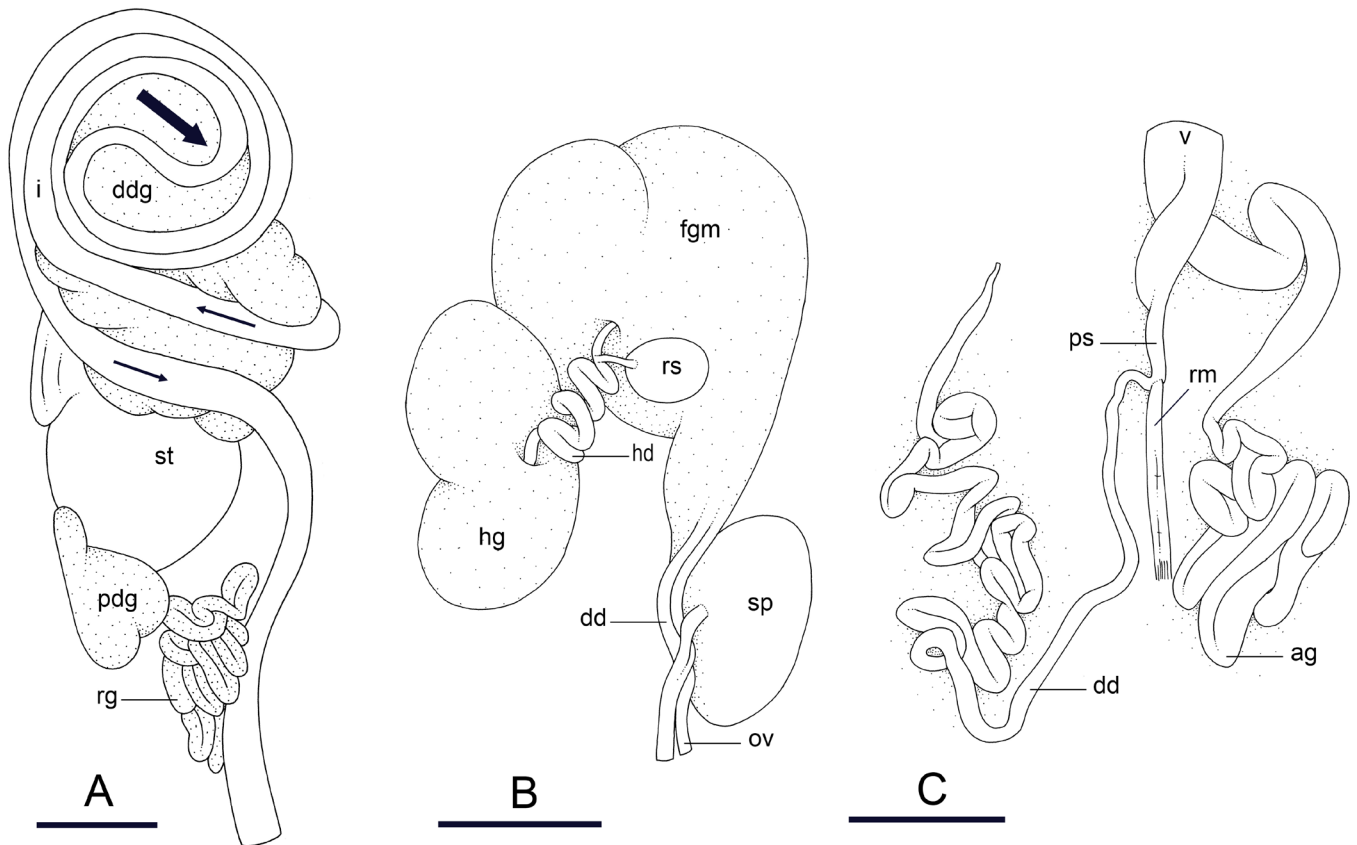
Fig. 18. Digestive system, and reproductive system, *Melayonchis siongkiati*, [5878] (NTM P.59485), Darwin, Northern Territory, Australia. Small arrows indicate the direction of the intestinal transport. Large arrows indicate the orientation of the transitional loop. A, Digestive system (type III, with a transitional loop at 4 o'clock), dorsal view, scale bar = 2 mm; B, Posterior reproductive parts, scale bar = 2 mm; C, Anterior male copulatory parts, scale bar = 2 mm. Abbreviations: ag, accessory penial gland; dd, deferent duct; ddg, dorsal lobe of digestive gland; fgm, female gland mass; hd, hermaphroditic duct; hg, hermaphroditic gland; i, intestine; ov, oviduct; pdg, posterior lobe of the digestive gland; ps, penial sheath; rg, rectal gland; rm, penial retractor muscle; rs, receptaculum seminis; sp, spermatheca; st, stomach; v, vestibule.

Darwin Harbour, Elisabeth River, upstream Bennetts Creek, 12°35.26740′, 131°01.31160′, 7 September 2016, 1 specimen 35/20 [5877] mm, station 265, at mid-tide level, on bank of *Rhizophora*-dominated tidal creek, deeply buried within moist woody debris and decaying stumps (NTM P.59484); Australia, Northern Territory, upper Darwin Harbour, Elisabeth River, upstream Bennetts Creek, 12°35.26740′, 131°01.31160′, 7 September 2016, 1 specimen 28/20 [5878] mm, station 265, at mid-tide level, on bank of *Rhizophora*-dominated tidal creek, deeply buried within moist woody debris and decaying stumps (NTM P.59485).

Distribution (Fig. 6). Australia, Northern Territory (new record). Other records are in Brunei Darussalam, Peninsular Malaysia, Singapore (type locality), and Vietnam (Dayrat et al., 2017: 1872).

Habitat. In the Northern Territory, *M. siongkiati* was found at one site deeply buried within moist woody debris and decaying stumps, on the bank of a tidal creek, in a *Rhizophora*-dominated forest. In the rest of its distribution, *M. siongkiati* usually lives in old forests where it is found on tree trunks and roots as well as logs, though it can occasionally be found on cemented walls of ditches or bridges near mangroves (Dayrat et al., 2017: 1873).

Abundance. *Melayonchis siongkiati* is extremely rare in the Northern Territory. Only a few specimens at one site were found in years of mangrove exploration by Adam Bourke. In the rest of its distribution, *M. siongkiati* is abundant only in Brunei and a large population was found at one site in Malaysia (Dayrat et al., 2017: 1873).

Live animals (Fig. 17). The beige background of the notum is irregularly mottled with darker areas, both light and dark brown. The colour of the hyponotum varies between light to dark gray and is marked by a significantly lighter ring at the margin. The foot is pale orange. When the animal crawls undisturbed, the brown ocular tentacles are short and extend for only a few millimeters beyond the notum margin, and the head is small and remains covered by the dorsal notum. The body is elongated, oval, usually not flattened, though animals may seem flattened when crawling. The dorsal notum is not particularly thick. Its surface, when the animal is undisturbed, is not smooth. Dorsal gills and large papillae are absent, but small conical papillae are present. About 15 of those papillae bear a black dorsal eye. A very slightly larger, central papilla bears three black dorsal eyes. In addition, the notum is finely granular. When disturbed, the animal can coil up, and occasionally even form a complete ball. Also, when disturbed, the dorsal notum immediately secretes an abundant oily and shiny mucus. Finally, the dorsal
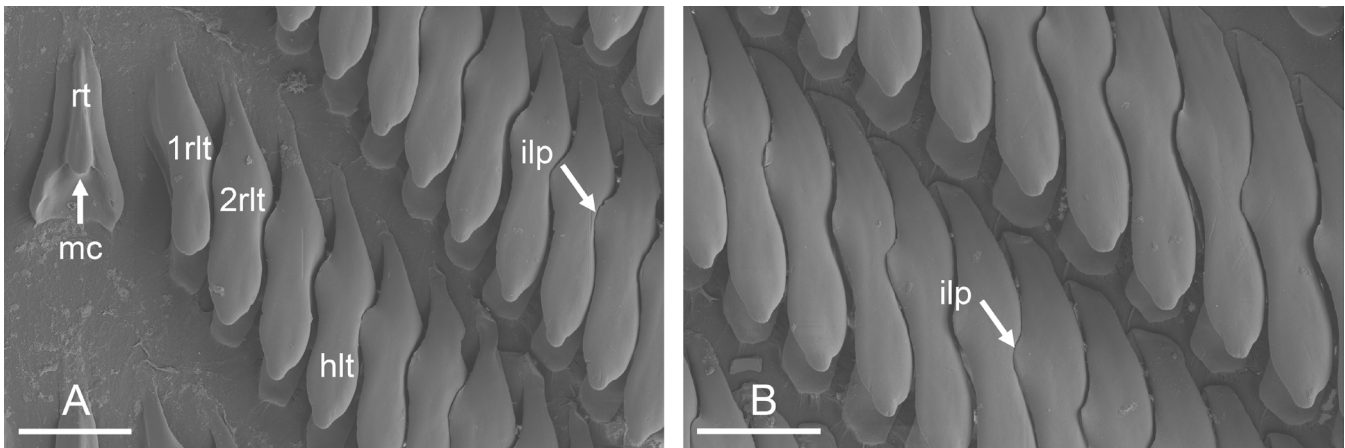
Fig. 19. Radula, *Melayonchis siongkiati*, [5875] (NTM P.59483), Darwin, Northern Territory, Australia. A, Rachidian and innermost lateral teeth, scale bar = 20 μm; B, Right lateral teeth, scale bar = 20 μm. Abbreviations: 1rlt, first, right, lateral tooth; 2rlt, second, right, lateral tooth; hlt, hook of lateral tooth; ilp, inner lateral protuberance; mc, median cusp of rachidian tooth; rt, rachidian tooth.
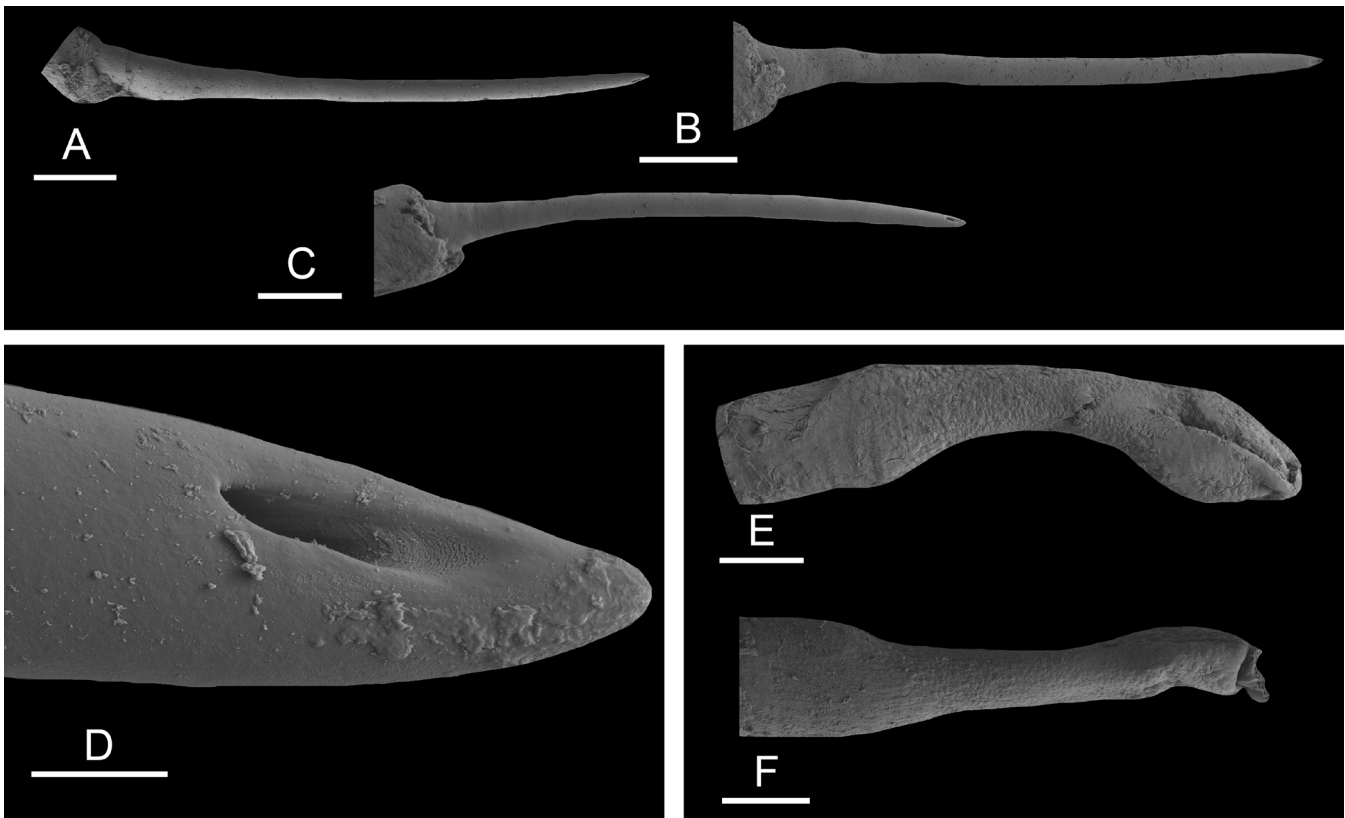

Fig. 20. Hollow spine of the accessory penial gland (A–D), and penial papilla (E, F), *Melayonchis siongkiati*, Darwin, Northern Territory, Australia. A, Scale bar = 100 μm, [5878] (NTM P.59485); B, Scale bar = 100 μm, [5877] (NTM P.59484); C, Scale bar = 100 μm, [5875] (NTM P.59483); D, Spine tip, scale bar = 8 μm, same as C; E, Scale bar = 100 μm, same as A; F, Scale bar = 100 μm, same as B.

notum of disturbed animals tends to be smooth (instead of finely granular). Live animals are from 28 to 35 mm long.

Digestive system (Figs. 18, 19, Table 4). Examples of radular formulae are presented in Table 4. The length of the rachidian teeth is approximately 30 μm, significantly less than the lateral teeth. The length of the hook of the lateral teeth is approximately 60 μm, excluding the first few (about 5) innermost and outermost lateral teeth which are significantly smaller. The inner lateral aspect of the hook of the lateral teeth is not straight. It is marked by a strong protuberance placed over the preceding adjacent tooth. The tip of the hook is similar across the half row, although it tends to be slightly more pointed in the innermost and slightly more round in the outermost teeth. The intestinal loops are of type III, with a transitional loop oriented at approximately 4 o'clock. A rectal gland is present.

Reproductive system (Fig. 18B). The receptaculum seminis (caecum) is spherical ovate. The spermatheca is ovate and connects to the oviduct through a very short duct. The oviduct and the deferent duct are narrow and straight. A vaginal gland is absent.

Copulatory apparatus (Figs. 18C, 20). The male anterior organs consist of the penial complex (penial papilla, penial sheath, deferent duct, and retractor muscle) and the accessory penial gland (flagellum and hollow spine). The penial complex and the accessory penial gland share the same vestibule and male opening. The flagellum of the penial gland is coiled. Distally, it ends in a hard, hollow spine. The hollow spine is narrow, elongated, and slightly curved. It measures about 60 μm in diameter at its conical base and narrows down to 15 to 20 μm distally, for a length that varies from 0.6 to 0.7 mm. The hollow spine opens into the proximal region of the vestibule.

The penial sheath protecting the penial papilla is straight and short (approximately 1 mm long) and joins distally the accessory penial gland in the vestibule. The total length from the retractor muscle attachment (proximal end of the penial sheath) to the opening of the vestibule is about 4 mm. The penial papilla consists of a short tube, slightly conical at its base and a slightly enlarged tip. Its length ranges between 0.4 and 0.5 mm. Its diameter is approximately 130 to 150 μm at the base, 150 μm at the tip, and 60 μm in between. There are no penial hooks. The retractor muscle is longer or shorter than the penial sheath and inserts at about half the length of the floor of the visceral cavity. The deferent duct is highly convoluted with many loops.

Remarks. The description of the individuals from the Northern Territory matches perfectly the original description of *M. siongkiati* from the Strait of Malacca and the South China Sea (Dayrat et al., 2017). Most especially, they share the most distinctive trait of the species, i.e., an abundant secretion of oily mucus when animals are disturbed, which is convenient to identify *M. siongkiati* in the field. Individuals can also coil up into a ball, but not as systematically and completely as *M. eloisae* or *M. eberlyi*. The dorsal colour of live animals, which is quite variable, is harder to use for identification. When crawling animals are observed without being disturbed, they could easily be confused with other species, including species from other genera (e.g., *Platevindex*, *Wallaconchis*). The only differences observed between individuals from the Northern Territory and the rest of the species are minor and can easily be explained by geographic distance and the fact that all three Australian specimens come from the same population (Table 3): the pedal sole is orange in Australian individuals and grey in the rest of the species; the penial papilla is slightly shorter in Australian individuals (0.4 to 0.5 mm long) compared to the rest of the species (0.5 to 1 mm long).

Melayonchis eloisae Dayrat *in* Dayrat et al., 2017

Melayonchis eloisae Dayrat *in* Dayrat et al., 2017: 1861–1870, figs. 3–8.

Type locality. Singapore, Pasir Park, 01°22.840′N, 103°57.224′E, 1 April 2010, station 5, mangrove forest with rich litter, lobster mounds, dead logs.

Type material. Holotype, 15/10 [1011] mm, and one paratype 22/13 mm, by original designation (ZRC.MOL.6499).

Additional material examined. Indonesia, Sumatra, Dumai, 01°42.838′N, 101°23.286′E, 9 October 2012, 1 specimen 23/14 mm [1775], station 74, mangrove forest just behind abandoned buildings, high intertidal, with many *Thalassina* mounds and small creeks in between (UMIZ 00003).

Distribution (Fig. 6). Sumatra (new record). Other records are in Brunei Darussalam, India (Andaman Islands), Peninsular Malaysia, Singapore (type locality), and Vietnam (Dayrat et al., 2017: 1863).

Habitat. In Sumatra, *M. eloisae* was found in a mangrove forest, at high intertidal, with many *Thalassina* mounds and small creeks in between, which is its most favorite habitat (Dayrat et al., 2017: 1863). Individuals were found on tree trunks and roots.

Abundance. *Melayonchis eloisae* is rare in Sumatra where we only found a few specimens at one site. It is hard to predict whether *M. eloisae* is present or not, even in what seems a perfect habitat (Dayrat et al., 2017: 1863).

Remarks. *Melayonchis eloisae* was expected to be present in eastern Sumatra, given its known records on the other side of the Strait of Malacca (western Peninsular Malaysia) and Singapore. *Melayonchis eloisae* in Sumatra is completely indistinguishable from the individuals found elsewhere. Also, the DNA sequences of the individual from eastern Sumatra are nested within the rest of the species (Figs. 3, 4).

DISCUSSION

Nomenclature. Existing species names have already been reviewed by Dayrat et al. (2017). No existing species name applies to *Melayonchis* species. Two brief, additional comments are needed here regarding *Onchidium pallidipes* Tapparone-Canefri, 1889, with a type locality in Myanmar, and *Onchidium pallidum* Stoliczka, 1869, with a type locality in the Ganges Delta.

Dayrat et al. (2017) suggested that the application of *Onchidium pallidipes* was unclear but that it could apply to a *Melayonchis* species. Now, after examining the anatomy of far more onchidiid species in the past few years, we think that *Onchidium pallidipes* likely refers to an *Onchidium* species. The original description of *Onchidium pallidipes* is useless but three syntypes were located (Dayrat et al., 2017). One syntype, 10/5 mm (ZMB/Moll 47190), is completely destroyed, and another syntype, 12/9 mm (ZMH 27467/1), is an immature specimen with no reproductive system (male or female). Those two syntypes could basically belong to any onchidiid species. The third syntype (NMNH 127328) was examined again. Its penial sheath is coiled exactly as in *Onchidium typhae* and *O. stuxbergi*. The NMNH syntype of *O. pallidipes* is likely a very poorly-preserved individual of *Onchidium* (there are some very faint traces of what

could have been dorsal papillae similar to those found in *Onchidium*). At any rate, *Onchidium pallidipes* does not apply to a *Melayonchis* species.

No type material was located for *Onchidium pallidum*. The original description of *O. pallidum* contains a mix of traits which are both compatible and incompatible with *Melayonchis* and it was very likely based on individuals of different species and even genera. This possibility, which was not considered in our previous review (Dayrat et al., 2017), makes most sense. Based on the number of teeth per half row (250, according to Stoliczka), we know that Stoliczka very likely examined at least one *Melayonchis* individual because such large numbers of teeth per half row are only known in *Melayonchis*. Stoliczka describes an accessory penial gland, which he calls a "supplementary albuminous string." An accessory penial gland is found in only one *Melayonchis* species, *M. siongkiati*, and a body generally covered with "copious mucous" is also compatible with *M. siongkiati*. The problem is that several other traits from Stoliczka's description are completely incompatible with *M. siongkiati* and even *Melayonchis*: a pale dorsum with a blackish, longitudinal stripe is similar to some colour variants found in *M. aileenae* but has never been observed in *M. siongkiati*; most importantly, Stoliczka describes a thick, 20 mm long penis, while the penial papillae of *Melayonchis* species are all shorter than 1 mm; finally, Stoliczka reports that "larger tubercles were in one specimen arranged in two longitudinal rows on either side of the dorsal [medial] stripe", but there are no such tubercles in *Melayonchis*. So, overall, it is most likely that, under the name *O. pallidum*, Stoliczka actually described individuals of different species, possibly *M. siongkiati* or *M. aileenae*, as well as species of other genera such as *Onchidium*, *Paromoionchis* Dayrat & Goulding *in* Dayrat et al., 2019a, and even *Peronina* Plate, 1893. Hence the confirmation that *Onchidium pallidum* is a nomen *dubium*, as proposed earlier (Dayrat et al., 2017). Stoliczka originally combined the specific name *pallidum* with *Onchidium* because he thought that *Onchidium* was the only genus in the Onchidiidae, but *pallidum* cannot be confidently combined with any onchidiid generic name.

Species identification in *Melayonchis*. In most onchidiid genera, species are cryptic externally but can be identified using DNA sequences or internal anatomy. *Melayonchis*, in that sense, is unusual because most species can be reliably and easily identified externally (Table 3). *Melayonchis tillieri* cannot be confused with any other known onchidiid species because its colour is very distinctive. The same is also true for *M. aileenae*. *Melayonchis siongkiati* can be identified by its abundant oily secretion when disturbed (some mucous secretion was also observed in *M. annae* but not as abundantly). *Melayonchis eloisae* and *M. eberlyi*, which share a similar and distinctive dorsal colour, differ in size. *Melayonchis annae*, however, is much harder to identify externally because its colour, which is highly variable, could easily be confused with some *Wallaconchis* species, especially *W. nangkauriensis* (Plate, 1893), even though the known geographic distribution of *W. nangkauriensis* does not overlap with that of *M. annae* and even though they live in quite different habitats (Goulding et al., 2018b).

Internally, *Melayonchis* species are all easily distinguishable (Table 3). *Melayonchis annae* is the only species with intestinal loops of type II (Fig. 1). *Melayonchis siongkiati* is the only species with an accessory penial gland (Fig. 18C). *Melayonchis eloisae*, *M. eberlyi*, and *M. tillieri* are each characterised by a distinctive penial papilla. Finally, *M. aileenae* is characterised by a unique combination of internal traits (e.g., type of intestinal loops and shape of penial papillae).

Species geographic distribution and abundance. Finding *M. siongkiati* in the Northern Territory was a surprise, to say the least, because Australia is really far from the core of the distribution of *Melayonchis* in the Andaman Sea, the Strait of Malacca, and the South China Sea (Fig. 6). It shows that *Melayonchis* should be expected in other regions, especially around the Java Sea. That we did not find *Melayonchis* in various places like the southeastern end of Sumatra, Lombok, Bali, and Timor, could easily be explained by the fact that some *Melayonchis* species are rare and can only be found if a site is explored several times: *M. siongkiati* was found only once by Adam Bourke in years of mangrove exploration around Darwin. It is possible that some of the species that are thought to be endemic or rare now will be found to be locally abundant in new places that have not been visited, such as the Nicobar Islands, Myanmar, northeastern Sumatra, southern Peninsular Malaysia, and southeastern and southwestern Borneo. *Melayonchis aileenae* was first discovered in the field in the Andaman Islands in January 2011 with only one individual. It was originally thought to be rare but it was later collected at 12 other sites (Fig. 6). Finally, given that the genus *Melayonchis* was only discovered a few years ago, it is possible that new species will still be discovered, whether in its diversity center (Strait of Malacca and South China Sea) or elsewhere.

Intestinal loops. Intestinal loops of type I (Fig. 1) are found in *Wallaconchis*, *Marmaronchis*, as well as some species of *Platevindex* and some species of *Peronia* Fleming, 1822. Because intestinal loops of type I are not found in *Melayonchis*, they are not discussed in detail here. Recently, a new type of intestinal loops was described as perfectly intermediate between type I and type II, i.e., with a transitional loop on average at 6 o'clock: it is found in the slugs of the genus *Laspionchis* Dayrat & Goulding *in* Dayrat et al., 2019b.

Intestinal loops of type II are found in *Paromoionchis* (all five species), *Peronina* (both species), some *Platevindex* species, as well as *Alionchis jailoloensis* (monotypic genus), *Onchidina australis* (monotypic genus), *Onchidium typhae* and *Melayonchis annae*. In most of those species, the transitional loop is oriented between 6 and 9 o'clock (see the descending red arrow in Fig. 1E). However, in *Paromoionchis*, the orientation of the transitional loop varies from 6 up to 11 o'clock (e.g., Dayrat et al., 2019a: fig. 12).

The orientation of the transitional loop of *M. annae* varies very little in comparison to other onchidiid species. In most onchidiid species, regardless of the intestinal type, the orientation of the transitional loop varies within a range of approximately 90 degrees, i.e., 45 degrees on either side of a mean axis, which is perfectly illustrated by the variation observed in *Melayonchis aileenae*, with a transitional loop oriented between 12 and 3 o'clock (Fig. 2J–L). In *M. annae*, the orientation of the transitional loop varies only between 8 and 9 o'clock (Fig. 1E). It is possible, however, that a higher variation will be found in *M. annae* when new populations are discovered (*M. annae* is known from only two sites in Singapore and one site in Brunei). Among species with intestinal loops of type II, the highest variation is found in *Paromoionchis tumidus*: the orientation of its transitional loop is not always perfectly horizontal at 9 o'clock (Fig. 1D) and varies approximately from 7 to 11 o'clock (Dayrat et al., 2019a: fig. 12). Among species with intestinal loops of type III, the highest variation is found in *Melayonchis eloisae*, in which the orientation of the transitional loop varies within a range of 180 degrees, from 12 to 6 o'clock (see the red arrow in Fig. 2F–I).

Intestinal loops of type III are found in two *Onchidium* species (*O. stuxbergi* and *O. reevesii*), some *Platevindex* species, *Alionchis jailoloensis*, and five of the six known *Melayonchis* species (only *M. annae* is characterised by intestinal loops of type II). Some of those species are characterised by a transitional loop oriented between 12 and 3 o'clock (e.g., see the ascending red arrow in Fig. 2J–L). Some other species are characterised by a transitional loop oriented between 3 and 6 o'clock and even up to 8 o'clock (e.g., see the descending red arrow in Fig. 2M–O). Finally, in *M. eloisae* and *O. stuxbergi*, the intestinal loops vary greatly and the transitional loop can be oriented from 12 to 6 o'clock (*M. eloisae*) and from 1 to 8 o'clock (*O. stuxbergi*), covering a range of 180 and 210 degrees respectively (Fig. 2C–I). So far, overall, the most highly coiled intestinal loops of type III are found in *O. stuxbergi* and *M. eberlyi*, in which, in some individuals, the clockwise loop makes two complete circles (blue loop in Fig. 2E) and the transitional loop is exceptionally oriented to the left (and not to the right as in a regular type III), up to 8 o'clock (Figs. 2E, 9A).

In our recent studies of *Onchidium*, *Melayonchis*, and *Alionchis*, we originally described the intestinal loops of *O. reevesii*, *M. eloisae*, *M. aileenae*, and *A. jailoloensis*, as being in between types II and III (Dayrat et al., 2016: fig. 14A; Dayrat et al., 2017: figs. 5c, 23a; Goulding et al., 2018a: fig. 5F). Now that the differences between intestinal types have been clarified (Figs. 1, 2), it is obvious that these intestinal loops are of type III, i.e., with a transition loop oriented to the right. These intestinal loops were a bit confusing because their transitional loop is not exactly at 3 o'clock but rather at 12 o'clock, or close to it. When individual variation is carefully taken into account, these intestinal loops clearly are of type III, as, for instance, in *M. eloisae*, the orientation of the transitional loop varies from 12 to 6 o'clock (Fig. 2F–I).

Each onchidiid species is characterised by only one type of intestinal loops, even though the intestinal loops vary between individuals, which is best illustrated by the variation of the orientation of the transitional loop (Fig. 2). There are only two known exceptions to that rule: *Alionchis jailoloensis*, characterised by both types II and III, and one *Platevindex* species, *P. luteus*, characterised by both types I and II (a taxonomic revision of *Platevindex* is in preparation). In *A. jailoloensis*, it is interesting to notice that a type II with a transitional loop at 7 o'clock (Goulding et al., 2018a: fig. 5E) and a type III with a transitional loop at 1 o'clock (Goulding et al., 2018a: fig. 5C) were collected at the same station (station 219, Halmahera), which is truly exceptional because this kind of individual variation has not been documented in any other onchidiid species so far.

Let us now briefly comment on the variation of the intestinal type within non-monotypic genera. Some genera are characterised by only one intestinal type: *Marmaronchis* and *Wallaconchis* are characterised by a type I (Dayrat et al., 2018; Goulding et al., 2018b), *Paromoionchis* and *Peronina* by a type II (Goulding et al., 2018c; Dayrat et al., 2019a), *Laspionchis* by a type between types I and II (Dayrat et al., 2019b). The other genera are characterised by more than one type: *Peronia* by types I and V (a taxonomic revision of *Peronia* is in preparation), *Platevindex* by types I, II, and III (a taxonomic revision of *Platevindex* is in preparation), and *Onchidium* and *Melayonchis* by types II and III. That kind of variation is not unusual. Other traits vary in a similar way among genera. For instance, an accessory penial gland is either always present (e.g., *Onchidium*), always absent (e.g., *Wallaconchis*), or both present and absent (e.g., *Peronina*). Some characters, however, are more stable within each genus, such as the rectal gland which is either always absent (e.g., *Wallaconchis*) or always present (e.g., *Onchidium*).

Finally, exceptionally high numbers of teeth per half row (more than 200 teeth) are only found in *Melayonchis*, even though not in all *Melayonchis* species (Table 3). In most onchidiid species, radulae contain less than 100 lateral teeth per half row.

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