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A new genus and four new species of onchidiid slugs from South-East Asia (Mollusca: Gastropoda: Pulmonata: Onchidiidae)

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ABSTRACT

The taxonomy of the Onchidiidae has remained extremely confusing for decades. As part of an on-going systematic revision of the entire family, a new genus, Melayonchis Dayrat and Goulding gen. nov., and four new species (Melayonchis eloisae Dayrat sp. nov., Melayonchis siongkiati Dayrat and Goulding sp. nov., Melayonchis annae Dayrat sp. nov., and Melayonchis aileenae Dayrat and Goulding sp. nov.) are described. Species are delineated using an integrative approach, based on morphological characters and DNA sequences. First-hand field observations and pictures of live animals are provided in order to help future species identification. All four Melayonchis species live in mangrove forests. The geographic distribution of Melayonchis ranges from the Andaman Sea to the South China Sea through the Strait of Malacca. Records are based on entirely new collections from the Andaman Islands, Peninsular Malaysia, Singapore, Brunei Darussalam and Vietnam. The nomenclature of all existing onchidiid species- and genus-group names from that region is addressed, as well as intraspecific character variation within Melayonchis.


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KEYWORDS

Biodiversity; Indo-West Pacific; integrative taxonomy; mangroves; systematics

Introduction

Onchidiid slugs belong to the crown group of the gastropods, Euthyneura, which includes the pulmonates (land snails and slugs) and the opisthobranchs (sea slugs). Like all other euthyneurans, onchidiids are hermaphroditic. Historically, it has been proposed that they could be related to sea slugs because they are true slugs (i.e. with no internal shell) and marine, or to pulmonates, because they breathe air with a lung, but modern phylogenetic studies all agree that they are related to land snails and slugs (Dayrat et al. 2011a; White et al. 2011). Onchidiid slugs are biologically fascinating because, even though they are undeniably marine (their larvae develop in the sea and they live in the intertidal zone), they die if they are submerged for more than a few hours because they breathe air. A few terrestrial species are known but they clearly are an exception (Dayrat 2010a).
They are also fascinating from a biogeographic perspective. Onchidiids are distributed worldwide (except in polar waters). One group has diversified in the tropical and subtropical Indo-West Pacific, especially South-East Asia, and another group has diversified in the rest of the world, especially temperate waters. These two groups—traditionally referred to as *Onchidium* Buchannan, 1800 and *Onchidella* JE Gray, 1850—only overlap in south-eastern Australia, South Africa and Japan.

However, the taxonomy of the onchidiid slugs has remained chaotic for decades. The species diversity and higher relationships have remained extremely confusing, despite the existence of nearly 150 species names in the literature (Dayrat 2009). Several reasons explain that regrettable situation, a few of which can be mentioned here: almost no new species descriptions have been based on live animals, and our data show that the colour of live animals is, in many cases, absolutely key for species identification; too many new species names were created without any consideration for individual variation and based on characters that highly vary within species; traditional species descriptions are most often lacking some critical anatomical information (e.g. intestinal loops, penial accessory gland); many types are lost or destroyed and their internal features cannot be checked; most museum specimens are of little value because we are lacking some important information (especially regarding the morphology and colour of live animals), not to mention that they cannot be sequenced; genera were based on characters that highly vary intraspecifically or that are even impacted by preservation (e.g. angle of the hyponotum with respect to the foot margin). So, as a result, until now, no onchidiid species could be properly identified in South-East Asia, where onchidiids are the most common and diverse (with dozens of species).

A few DNA sequences have been published in the past in the context of euthyneuran phylogenies (e.g. Klussmann-Kolb et al. 2008; Dayrat et al. 2011a), even though species identifications from those early molecular studies probably need to be revisited. A molecular study was also published on onchidiids from the coast of China (Sun et al. 2014) but these species were unfortunately all misidentified at the species level, the genus level, or both. The recent taxonomic revision of the genus *Onchidium* was the first study of the species diversity of onchidiids using both molecular and morphological data (Dayrat et al. 2016). We found no cryptic diversity in *Onchidium*: the three known species are clearly distinct morphologically and strongly supported by molecular data too. That is not to say that there is no cryptic diversity in onchidiids, though.

The Dayrat lab is in the process of revising the taxonomy of the entire family, using an integrative approach (Dayrat 2005). For the past 6 years, a considerable amount of time was spent in the field collecting fresh material, worldwide, especially in South-East Asia. Data on the natural history and colour variation of live animals were systematically gathered. Fresh specimens were used to build a phylogenetic tree which includes about 100 species (worldwide) divided into about 10 clades at the generic level. In addition, all available types were borrowed and most important museum collections were visited personally. Many species and genera are new to science, and the taxa with existing names are poorly known and need to be re-described.

A revision of *Onchidium*, the first genus ever named, was recently published, showing that the name *Onchidium* applies to a clade including only three species (Dayrat et al. 2016). In the present contribution, a new genus is described, including four new species. This new genus is restricted to a region that ranges from the Andaman Sea to the South
China Sea through the Strait of Malacca. Species are found from the Andaman Islands, the Strait of Malacca, Singapore, western Borneo (Brunei Darussalam) and southern Vietnam.

The four new species described here live in mangrove forests. They do not live directly on the surface of the mud (where many onchidiid species can be found) but primarily live on mangrove trees (stilt roots, bark, etc.) as well as in man-made cement ditches or on bridges bordering mangroves. Species are delineated using both morphological features (e.g. colour of live animals, reproductive system) and DNA sequences. Existing names are thoroughly examined to guarantee that the new taxa being described here were not already named.

That *Melayonchis* and its four species have not been discovered earlier can easily be explained by several factors. First, relatively few onchidiid species were described from that part of the world: for instance, only one species was ever described from the Strait of Malacca. Also, many of the species that were described from the geographic range of *Melayonchis* are either species that live on rocky shores (such as the *Peronia* species) or species that live in mangroves but are very abundant and common (such as some of the *Platevindex* species). Finally, before the present study, very few specimens were available in museum collections from that part of the world. So, overall, only intense field work could provide opportunities to discover those relatively uncommon species that live deep in the mangrove forests of South-East Asia.

**Materials and methods**

**Field collecting and sampling**

All specimens examined here were collected by us in the context of an exploration of mangrove snails and slugs in South-East Asia, which provided fresh material for DNA sequencing and invaluable natural history observations. We often were accompanied by local guides (local villagers or fishermen). Sites were accessed by car or by boat. Each site was explored for an average of 2 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 micro-habitats where specimens were collected.

In the field, specimens were individually numbered and photographed in their habitat (it is very important to take photographs before animals are touched because they retract when disturbed and do not relax again for a long time). Importantly, a piece of tissue was cut from all individually numbered specimens (for DNA extraction) and the rest of each specimen was relaxed and fixed for comparative anatomy.

In the field, detailed notes were written, commenting on whether specimens could be part of the same species or not. Specimens that looked similar were tentatively assigned to species units (e.g. ‘species 1’, ‘species 2’, ‘the small ball species’), acknowledging the presence of individual variation. DNA sequences and anatomical data gathered later allowed us to check whether or not, for instance, all the specimens labeled in the field as ‘the small ball species’ were together in a single unit. We tried our best to sample as much diversity as possible at each site. In addition to individually numbering the specimens that looked different, we also individually numbered many specimens that looked similar so that we could test for the presence of cryptic diversity.
Finally, in addition to individually numbered specimens, we also collected clusters of specimens that were not individually numbered. Those specimens could also be used for both DNA sequencing (as long as they were not fixed in formalin) and comparative anatomy (as long as they were preserved in 70% alcohol). However, connecting one picture to one specimen is only possible for specimens that were individually numbered and photographed. The number of individuals collected for the present study is (per species): 258 (M. siongkiati), 128 (M. eloisae), 30 (M. annae) and 23 (M. aileenae), acknowledging that species can be rare at some sites and abundant at other sites. The number of specimens individually numbered, photographed and sequenced is: 39 (M. siongkiati), 28 (M. eloisae), five (M. annae) and eight (M. aileenae). The number of specimens fully dissected is: 12 (M. siongkiati), 12 (M. eloisae), six (M. annae) and seven (M. aileenae).

Museum vouchers and collections

All specimens were deposited as vouchers in institutions of the country where they were collected. All measurements of individual specimens are provided as length/width in mm. Types of existing species names were borrowed on loan. Acronyms of collections are: Bombay Natural History Society, Mumbai, India (BNHS); Brunei Museum, Natural History, Brunei Darussalam (BDMNH); Institute of Tropical Biology, Zoology Collection, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam (ITBZC); Natural History Museum, London, United Kingdom (NHMUK); Universiti Sains Malaysia, Mollusk Collection, Penang, Malaysia (USMMC); Zoologisches Museum, Berlin, Germany (ZMB); Zoologisches Museum, Hamburg, Germany (ZMH); Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore (ZRC).

Animal preparation and anatomical description

All anatomical observations were made under a dissecting microscope and drawn with a camera lucida. In addition, organs were prepared for scanning electron microscopy (SEM). Radulae were cleaned in 10% NaOH for a week, rinsed in distilled water for at least a week, briefly cleaned in an ultrasonic water bath (less than a minute), sputter-coated with gold-palladium, and examined by SEM. Soft parts (penis and penial hooks) were dehydrated in ethanol and critical point dried before coating. When a lot included several specimens, all pieces of the dissected specimens were carefully numbered, both inside the jar and on the SEM stubs. A range of minimum to maximum animal size is provided for each lot of specimens. In addition, individualised numbers and measurements are provided for the specimens illustrated here or part of the molecular data set.

The anatomy of M. eloisae, the type species, is fully detailed. The written description of the many anatomical features that are virtually identical between species (nervous system, heart, etc.) is given only for the type, to avoid repeating the information four times. So, any feature that is mentioned in M. eloisae is also present and identical in the three other species, such as, for instance, the position of the male aperture or the anatomy of the stomach. As expected, differences between species are found in the reproductive system as well as in some minute details of the radula (although, overall, the radulae are not distinguishable and could not be used for species identification). The
morphology and colour of live animals are described in detail for all four species because they are highly informative to identify and distinguish species.

**DNA extraction and PCR amplification**

DNA was extracted using the phenol-chloroform extraction protocol with cetyltrimethylammonium bromide (CTAB). Portions of the two mitochondrial genes Cytochrome Oxidase 1 (COI) and 16S were amplified using the following universal primers: COIF (5’–3’) GGT CAA CAA ATC ATA AAG ATA TTG G, and COIR (5’–3’) TAA ACT TCA GGG TGA CCA AAR AAY CA (Folmer et al. 1994); 16Sar (5’–3’) CGC CTG TTT ATC AAA AAC AT (Palumbi 1996), and 16S 972R (5’–3’) CCG GTC TGA ACT CAG ATC ATG T (Dayrat et al. 2011a). The 25 μL Polymerase Chain Reaction (PCR) reactions contained 15.8 μL of water, 2.5 μL of 10X PCR buffer, 1.5 μL of 25 mM MgCl₂, 0.5 μL of each 10 μM primer, 2 μL of dNTP mixture, 0.2 μL (1 unit) of TaKaRa Taq (Code No. R001A), 1 μL of 20 ng/μL template DNA, and 1 μL of 100X bovine serum albumin (BSA). The thermoprofile used for COI and 16S was: 5 minutes at 94°C; 30 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 PCR products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) prior to sequencing. Untrimmed sequence fragments represented ~680 bp of COI, and ~530 bp of 16S.

**Phylogenetic analyses**

Alignments were obtained using Clustal W in MEGA 6 (Tamura et al. 2013). Chromatograms were consulted to resolve rare ambiguous base calls. DNA sequences were all deposited in Genbank and vouchers clearly identifiable in museum collections (Table 1). The ends of each alignment were trimmed and sequences were concatenated. The concatenated alignment included 980 nucleotide positions: 578 COI and 402 16S. Prior to phylogenetic analyses, the best-fitting evolutionary model was selected using the Model Selection option from Topali v. 2.5 (Milne et al. 2004). A Generalised Time-Reversible + Gamma + Invariable sites (GTR + G + I) model was selected. Other (unpublished) analyses were performed using different models, which all yielded identical results. Maximum likelihood analyses were performed using PhyML (Guindon and Gascuel 2003) as implemented in Topali v. 2.5. Node support was evaluated using bootstrapping with 100 replicates. Bayesian analyses were performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) as implemented in Topali v. 2.5, with four simultaneous runs of 10⁶ generations each, sample frequency of 100 and burn-in of 25% (and posterior probabilities were also calculated). Six other onchidiid species and their corresponding COI and 16S sequences were selected from previous studies from our lab as outgroups (Dayrat et al. 2011a, 2016; Dayrat and Goulding submitted): Onchidella floridana (Dall 1885), Peronia sp. (Okinawa), Peronia sp. (Hawaii), Onchidina australis Semper, 1885, Onchidium stuxbergi (Westerlund 1883) and Onchidium typhae Buchannan, 1800. Analyses were not run enforcing the a priori monophyly of the Melayonchis sequences (i.e. outgroups were not designated a priori, and trees were rooted using Onchidella floridana). Other (unpublished) analyses were performed using different combinations of outgroups, which all yielded identical results.
In addition to analyses with the two concatenated markers, another set of analyses was performed with only COI sequences. Pairwise genetic distances between COI sequences were calculated in MEGA 6. BEAST (Drummond et al. 2012) was used to generate trees for a Generalized Mixed Yule Coalescent (GMYC) analysis run in R using the Splits package with a single threshold (Fujisawa and Barraclough 2013). An Automatic Barcode Gap Discovery (ABGD) analysis was also performed to detect the barcode gap (Puillandre et al. 2011).

**Phylogenetic results**

**Molecular phylogenetic analyses**

The primary purpose of using DNA sequences here is to test the species limits within *Melayonchis*. The monophyly of *Melayonchis*, supported very strongly in our comprehensive data set including a much larger taxon sampling (ongoing study), is also strongly supported here (bootstrap value of 98 and posterior probability of 0.97). The phylogenetic analyses yielded four species units that are all reciprocally monophyletic and strongly supported (Figure 1). Each species is supported by a bootstrap support of (or close to) 100 and posterior probabilities of 1. There also is some

<table>
<thead>
<tr>
<th>Species</th>
<th>Individual (DNA)</th>
<th>Locality</th>
<th>Genbank COI</th>
<th>Genbank 16S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onchidella floridana</em></td>
<td>Tobago</td>
<td>HQ660035</td>
<td>HQ659903</td>
<td></td>
</tr>
<tr>
<td><em>Peronia</em> sp.</td>
<td>Okinawa, Japan</td>
<td>HQ660043</td>
<td>HQ659911</td>
<td></td>
</tr>
<tr>
<td><em>Peronia</em> sp.</td>
<td>Hawaii</td>
<td>HQ660038</td>
<td>HQ659906</td>
<td></td>
</tr>
<tr>
<td><em>Onchidina australis</em></td>
<td>New South Wales</td>
<td>KX179548</td>
<td>KX179561</td>
<td></td>
</tr>
<tr>
<td><em>Onchidium stuxbergi</em></td>
<td>Vietnam</td>
<td>KX179520</td>
<td>KX179537</td>
<td></td>
</tr>
<tr>
<td><em>Onchidium typhae</em></td>
<td>Peninsular Malaysia</td>
<td>KX179509</td>
<td>KX179525</td>
<td></td>
</tr>
<tr>
<td><strong>M. siongkiant</strong></td>
<td>1052</td>
<td>Brunei Darussalam</td>
<td>KX240021</td>
<td>KX240045</td>
</tr>
<tr>
<td>- 920</td>
<td>Peninsular Malaysia</td>
<td>KX240018</td>
<td>KX240042</td>
<td></td>
</tr>
<tr>
<td>- 947</td>
<td>Peninsular Malaysia</td>
<td>KX240019</td>
<td>KX240043</td>
<td></td>
</tr>
<tr>
<td>- 5608</td>
<td>Vietnam</td>
<td>KX240022</td>
<td>KX240046</td>
<td></td>
</tr>
<tr>
<td>- 1002 H</td>
<td>Singapore</td>
<td>KX240020</td>
<td>KX240044</td>
<td></td>
</tr>
<tr>
<td><strong>M. aileenae</strong></td>
<td>968</td>
<td>Peninsular Malaysia</td>
<td>KX240032</td>
<td>KX240056</td>
</tr>
<tr>
<td>- 970 H</td>
<td>Peninsular Malaysia</td>
<td>KX240033</td>
<td>KX240057</td>
<td></td>
</tr>
<tr>
<td>- 1047</td>
<td>Brunei Darussalam</td>
<td>KX240034</td>
<td>KX240058</td>
<td></td>
</tr>
<tr>
<td>- 5631</td>
<td>Vietnam</td>
<td>KX240036</td>
<td>KX240060</td>
<td></td>
</tr>
<tr>
<td>- 5606</td>
<td>Vietnam</td>
<td>KX240035</td>
<td>KX240059</td>
<td></td>
</tr>
<tr>
<td><strong>M. annae</strong></td>
<td>1045</td>
<td>Brunei Darussalam</td>
<td>KX240016</td>
<td>KX240040</td>
</tr>
<tr>
<td>- 1046</td>
<td>Brunei Darussalam</td>
<td>KX240017</td>
<td>KX240041</td>
<td></td>
</tr>
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<td>- 1008</td>
<td>Singapore</td>
<td>KX240013</td>
<td>KX240037</td>
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</tr>
<tr>
<td>- 1009</td>
<td>Singapore</td>
<td>KX240014</td>
<td>KX240038</td>
<td></td>
</tr>
<tr>
<td>- 1010 H</td>
<td>Singapore</td>
<td>KX240015</td>
<td>KX240039</td>
<td></td>
</tr>
<tr>
<td><strong>M. eloiseae</strong></td>
<td>1097</td>
<td>Andaman, India</td>
<td>KX240028</td>
<td>KX240052</td>
</tr>
<tr>
<td>- 922</td>
<td>Peninsular Malaysia</td>
<td>KX240023</td>
<td>KX240047</td>
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<td>- 951</td>
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<td>KX240024</td>
<td>KX240048</td>
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<tr>
<td>- 1043</td>
<td>Brunei Darussalam</td>
<td>KX240027</td>
<td>KX240051</td>
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<tr>
<td>- 5607</td>
<td>Vietnam</td>
<td>KX240031</td>
<td>KX240055</td>
<td></td>
</tr>
<tr>
<td>- 1003</td>
<td>Singapore</td>
<td>KX240025</td>
<td>KX240049</td>
<td></td>
</tr>
<tr>
<td>- 1011 H</td>
<td>Singapore</td>
<td>KX240026</td>
<td>KX240050</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Phylogenetic tree. Relationships within the genus *Melayonchis* based on COI and 16S sequences. Numbers above branches are the bootstrap values (maximum likelihood analysis, ML) and below are the posterior probabilities (Bayesian analysis); only numbers > 60% (ML) and > 0.9 (Bayesian) are indicated. Numbers for each individual correspond to unique identifiers for DNA extraction. All sequences of *Melayonchis* specimens are new. Information on individually identified specimens can be found in the lists of material examined and in Table 1.

Genetic structure within three of the species where some subunits are well supported (*M. aileenae*, *M. eloisae* and *M. annae*), mostly based on geographic distribution: for example, in *M. annae*, the specimens from Singapore and those from Brunei cluster in different subunits. This genetic structure, however, does not warrant ascribing species status to the subunits, because the genetic divergences between subunits are too low (see below) and because no morphological differences could be found between subunits (see the species descriptions). Finally, there is virtually no phylogenetic structure in *M. siongkiati*. 
Pairwise genetic divergences

The analyses of the pairwise genetic distances also strongly support the existence of four species of *Melayonchis* (Table 2). There is a wide and unambiguous gap between intra- and interspecific distances (Figure 2). All intraspecific genetic distances are below 5.2%, and all interspecific genetic distances are minimally 14.1% (between *M. eloisa* and *M. siongkiati*) and as high as 21.4% (between *M. aileenae* and *M. annae*). Importantly, there is only one single, obvious and large barcode gap within the data, between 5 and 14%, which can thus be used as a threshold for species limits (Figure 2). Also, the intraspecific distances form a continuum from 0 to 5% (Figure 2). The GMYC analyses recover four clusters that correspond to our four species.

**Table 2.** Intra- and interspecies pairwise genetic distances. Ranges of minimum to maximum distances are indicated (as percentages). For instance, within *Melayonchis siongkiati*, individual sequences are between 0.2 and 0.9% divergent, and individual sequences between *M. siongkiati* and *M. aileenae* are minimally 16.1 and maximally 17.2% divergent.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>M. siongkiati</em></th>
<th><em>M. aileenae</em></th>
<th><em>M. annae</em></th>
<th><em>M. eloisa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. siongkiati</em></td>
<td>0.2–0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. aileenae</em></td>
<td>16.1–17.2</td>
<td>0.0–5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. annae</em></td>
<td>17.8–18.6</td>
<td>19.7–21.4</td>
<td>0.0–1.9</td>
<td></td>
</tr>
<tr>
<td><em>M. eloisa</em></td>
<td>14.1–15.5</td>
<td>14.3–17.4</td>
<td>15.9–17.0</td>
<td>0.3–3.7</td>
</tr>
</tbody>
</table>

**Figure 2.** Histogram showing the distribution of pairwise genetic distances within the data, obtained using ABGD. The unique, large and obvious barcode gap between 5 and 14% is used here as an objective separation between intraspecific (< 5%) and interspecific (> 14%) divergences. There is a continuum within both intraspecific and interspecific divergences (i.e. no additional gap can be detected within intraspecific or interspecific divergences, even though there is a peak of intraspecific divergences < 1% and a peak of interspecific divergences between 16 and 17%).
Table 3. Summary of traits that can help identify *Melayonchis* species. Observations in parentheses are only occasional. All traits may be subject to individual variation. Traits are described in detail in the corresponding species descriptions and compared at the end of the Discussion.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dorsal colour</th>
<th>Foot colour</th>
<th>Coiling up in a ‘ball’ when disturbed</th>
<th>Secreting oily mucus when disturbed</th>
<th>Type of intestinal loops</th>
<th>Accessory gland</th>
<th>Penis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. eloisae</em></td>
<td>Black, brown (light brown), mottled by white</td>
<td>Orange (yellow)</td>
<td>Always and completely</td>
<td>No</td>
<td>Between II and III</td>
<td>No</td>
<td>Elongated, slightly curved</td>
</tr>
<tr>
<td><em>M. siongkiati</em></td>
<td>Brown, greyish, mottled by dark brown (whitish)</td>
<td>Grey, creamish (darker)</td>
<td>Occasionally and partly</td>
<td>Always and abundantly</td>
<td>III</td>
<td>Yes</td>
<td>Short, straight papilla</td>
</tr>
<tr>
<td><em>M. annae</em></td>
<td>Irregularly mottled with cream, light brown, and dark brown</td>
<td>Cream</td>
<td>No</td>
<td>Occasionally, and mildly</td>
<td>II</td>
<td>No</td>
<td>Short, slightly conical papilla</td>
</tr>
<tr>
<td><em>M. aileenae</em></td>
<td>Brown (light brown, black) with minute white dots and linear markings</td>
<td>Whitish to light yellow</td>
<td>Occasionally and partly</td>
<td>No</td>
<td>Between II and III</td>
<td>No</td>
<td>Short, slightly conical papilla</td>
</tr>
</tbody>
</table>
In addition to detecting the obvious barcode gap (Figure 2), the ABGD analyses recognise from four to eight units, depending on the maximum prior intraspecific divergence that is being used. However, dividing up those four species into a higher number of units is rejected here, for several compelling reasons: (1) our four species are very distinct morphologically, but no morphological differences could be found which would support more than four units (Table 3, and species descriptions); (2) it would yield units exclusively based on geographic distribution (e.g. M. annae would be divided into a species in Singapore and another species in Brunei), when genetic distances could be easily explained by the geographic distance between localities; and (3) it would mean that a distinct threshold for species limits would have to apply for each species (e.g. the threshold between the two units within M. annae – one unit from Brunei and one from Singapore – is somewhere between 0.2 and 1.7%, whereas it is somewhere between 3.2 and 4.6% between the two units within M. aileenae – one unit from Vietnam and Brunei, and one unit from Malaysia). So, overall, we regard the large barcode gap within sequence divergences as a valid and objective threshold between intraspecific and interspecific divergences (Figure 2).

**Testing species boundaries**

The four species recognised here based on DNA sequences and comparative anatomy, and described below, were originally recognised as four distinct species in the field. The first intuition of naturalists was correct. A table is provided here, summarising all the features that distinguish all four species (Table 3). In the field, M. eloisa had been tentatively called ‘small ball species’ (because it always coils up into a tight ball when disturbed), M. siongkiai ‘the oily species’ (because it secretes an abundant oily mucus when disturbed), M. annae ‘the big black-and-white species’ (because of the colour of its dorsal notum) and M. aileenae ‘the smooth and white hyponotum species’ (because of its exceptionally smooth dorsal notum and white hyponotum). There is, naturally, individual variation, especially regarding the dorsal colour, but the present results show that this variation is intraspecific.

**Systematics and anatomical descriptions**

**ONCHIDIIDAE** Rafinesque, 1815

_Melayonchis_ Dayrat and Goulding gen. nov.

**Type species**  
_Melayonchis eloisa_, designated here.

**Etymology**  
Combination of Melayu and _Onchis_. Melayu is the Malay word for Malays. It is selected here because the core of the known distribution of the new genus described here mostly corresponds to the historical Malay world – that is, Peninsular Malaysia (including Singapore) and coastal Borneo. _Onchis_ is one of the names used for onchidiid slugs.

**Diagnosis**  
Body not flattened. No marginal glands in the notum. No dorsal gills. Dorsal eyes present on notum. Fully retractable, central papilla (with three dorsal eyes) present. Short eye
tentacles. Male opening below the right ocular tentacle, slightly to its left. Pneumostome median. Intestine of types II and III (and intermediary between types II and III). Rectal gland present. Accessory penial gland and hollow spine present or absent. Penis with no hooks.

Remarks
A new generic name is needed because no existing name could apply to the clade described here. We provide here a few remarks on the status of the generic names that are potentially valid in Onchidiidae (Dayrat 2009). Our remarks are based on the examination of all the type specimens available, especially those of all the type species, as well as the careful analysis of all the original descriptions (especially when no type specimens were available). More details will be provided in our revisions of the corresponding genera. However, here we do not comment on the generic names that refer to Onchidella J.E. Gray, 1850 and Hoffmannola Strand, 1932, which are not represented in the tropical Indo-West Pacific (Dayrat 2009; Dayrat et al. 2011b; Avila-Poveda et al. 2014).

Several generic names apply to a clade including all the onchidiid slugs with dorsal gills—that is, Peronia Fleming, 1822; Eudrastus Gistel, 1848; Lessonina Starobogatov, 1976; Onchis d’Audebard de Férussac, 1821; Paraperonia Labbé, 1934; Quoyella Starobogatov, 1976; and Scaphis Labbé, 1934. The generic name Labella Starobogatov, 1976 is a junior synonym of Onchidium Buchanan, 1800 which applies to a different clade including the type species Onchidium typhae Buchanan, 1800, and is characterised by large conical papillae on the dorsal notum (Dayrat et al. 2016). The generic name Paraoncidium Labbé, 1934 is a junior synonym of Onchidina Semper, 1885 which applies to a species with no dorsal eyes (Dayrat and Goulding submitted). Peronina von Plate, 1893 applies to its type species Peronina alta von Plate, 1893, characterised by a pneumostome distinctly and unusually located on the margin of the notum (a feature that is present in the type material of Peronina alta). Platevindex Baker, 1938 applies to a clade including species with a distinctly flattened body and narrow foot, such as the type species Platevindex coriaceum (Semper 1885). Finally, Semperoncis Starobogatov, 1976 applies to species that are adapted to terrestrial life in the Philippines (Dayrat 2010a). As no other generic name exists for the taxon described here, a new name is needed.

Distinctive diagnostic features
No external diagnostic feature seems to unambiguously distinguish Melayonchis from all other genera (which is not surprising because many onchidiid species from different genera look very similar externally). Internally, however, the lateral radular teeth of Melayonchis are characterised by a protuberance on their inner lateral margin (see arrows in Figures 6, 13, 18, 24 and 25) which is a distinctive feature that has not been observed in any other species so far (e.g. Dayrat 2010a, 2010b; Dayrat et al. 2011b, 2016).

Melayonchis eloisa Dayrat sp. nov. (Figures 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]. By definition, the type locality is the locality of the holotype, but the paratype is also from the same locality.
Type material
Holotype: 95% alcohol [DNA 1011], 15/10 mm. One paratype: formalin, 22/13 mm. The holotype and the paratype are both designated here, leg. B. Dayrat and S.K. Tan (ZRC. MOL.6499).

Additional material examined
India, Andaman Islands, Middle Andaman, Rangat, Shyamkund, 12°28.953′ N, 092°50.638′ E, 11 January 2011, 4 specimens (17/12 [DNA 1097] to 7/5 mm), leg. B. Dayrat and V. Bhave [station 57, by a large river, deep mangrove with tall Rhizophora trees, small creeks, and plenty of dead muddy logs, next to a road and a small cemented bridge for creek] (BNHS 49); India, Andaman Islands, South Andaman, Bamboo Flat, Shoal Bay, 11°47.531′ N, 092°42.577′ E, 13 January 2011, 9 specimens (22/12 to 5/3 mm), leg. B. Dayrat and V. Bhave [station 59, open mangrove with medium trees, hard mud, dead logs, next to a road and a small cemented bridge for creek] (BNHS 51); Singapore, Lim Chu Kang, 01°26.785′ N, 103°42.531′ E, 5 April 2010, 1 specimen (21/12 [DNA 1003] mm), leg. B. Dayrat and S.K. Tan [station 9, mangrove east of the jetty; open forest with medium trees and medium mud; ended on sun-exposed
mudflat outside the mangrove with soft mud; very polluted with trash] (ZRC.MOL.6500). **Malaysia**, Peninsular Malaysia, Merbok, 05°39.035’ N, 100°25.782’ E, 12 July 2011, 31 specimens (22/12 [#1] to 5/3 mm; 20/13 [#2], 10/7 [#3], 10/8 [DNA 951] mm), leg. B. Dayrat and T. Goulding [station 21, deep *Rhizophora* forest with old, tall trees, hard mud, many small creeks and many dead logs] (USMMC 00007); Malaysia, Peninsular Malaysia, Merbok, 05°40.143’ N, 100°26.178’ E, 12 July 2011, 3 specimens (22/14 to 12/10 mm), leg. B. Dayrat and T. Goulding [station 22, mostly *Rhizophora*, soft mud and some very soft mud near creek] (USMMC 00008); Malaysia, Peninsular Malaysia, Kuala Sepatang, 04°50.434 N, 100°38.176 E, 18 July 2011, 8 specimens (23/14 to 7/6 mm; 12/10 [DNA 922] mm), leg. B. Dayrat and T. Goulding [station 27, old forest with old, tall *Rhizophora* trees, high in the tidal zone (ferns), following boardwalk in educational preserve, reached a creek lower in the tidal zone, with mud] (USMMC 00009); Malaysia, Peninsular Malaysia, Matang, off Kuala Sepatang, Crocodile River, Sungai Babi Manpus, 04°49.097’ N, 100°37.370’ E, 19 July 2011, 3 specimens (19/10 to 6/5 mm), leg. B. Dayrat and T. Goulding [station 28, old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many dead logs] (USMMC 00010); Malaysia, Peninsular Malaysia, Matang, close to the jetty, facing the fisherman’s village on the other side of the river, 04°50.154’ N, 100°36.368’ E, 20 July 2011, 6 specimens (21/15 to 8/6 mm), leg. B. Dayrat and T. Goulding [station 29; oldest and open *Rhizophora* forest of tallest and beautiful trees, with hard mud, many creeks and many dead logs] (USMMC 00011); **Brunei Darussalam**, Mentiri, Jalan Batu Marang, 04°59.131 N, 115°01.820 E, 29 July 2011, 67 specimens (24/12 [#1] to 6/5 mm; 7/5 [DNA 1043] mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 34, old mangrove with tall *Rhizophora* trees with high roots and *Thalassina* mounds] (BDMNH); **Vietnam**, Can Gio, 10°27.803’ N, 106°53.288’ E, 16 July 2015, 4 specimens (20/12 to 7/5 mm; 13/8 [DNA 5607]), leg. T. and J. Goulding [station 228, high intertidal, open forest of tall *Rhizophora* trees, hard mud not deep, near a small river] (ITBZC IM 00009).

**Distribution**

Singapore (type locality), India (Andaman Islands), Peninsular Malaysia (Strait of Malacca), Brunei Darussalam, Vietnam.

**Etymology**

*Melayonchis eloisae* is dedicated to Eloïse Dayrat, for the time that her father (the first author) has to spend away from her, exploring mangroves and missing her.

**Habitat (Figure 3)**

*Melayonchis eloisae* is mostly found in the high intertidal, and it especially favours old *Rhizophora* forests with *Thalassina* mounds. It lives on trunks and roots of mangrove trees, often not muddy but covered with algae instead. It can also be found on dead logs and even, occasionally, on cemented walls (of bridges or ditches near mangroves). It is not found directly on mud.

**Abundance**

In the right habitat, *M. eloisae* can be abundant (for instance, we found dozens of specimens in Brunei). However, even when the habitat seems perfect for it, one may not find it or may find just a few individuals. So, unlike some species of *Platevindex* which are almost always found, one cannot really predict whether *M. eloisae* will be found.
Figure 4. Live specimens, *Melayonchis eloisae*. (a) Holotype, dorsal view, undisturbed, 15 mm long [DNA 1011], Singapore (ZRC.MOL.6499). – (b) Same as (a), washed, dorsal view (left) and ventral view (right). (c) Dorsal view, undisturbed, 20 mm long, Andaman Islands, station 59 (BNHS 51). (d) Dorsal view, washed, from 5 to 22 mm, Andaman Islands, station 59 (BNHS 51). (e) Dorsal view, undisturbed, 13 mm long [DNA 5607], Vietnam (ITBZC IM 00009). (f) Dorsal view, 17 mm long [DNA 1097], Andaman Islands, station 57 (BNHS 49). (g) Dorsal view, washed, 20 mm long, Malaysia (USMMC 00007). (h) Same as F, rolled into a ball (left), ventral view (right).
**Colour and morphology of live animals (Figure 4)**

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. That thin layer of mucus makes them very cryptic. It may also help prevent desiccation. The colour of the dorsal notum appears after the thin muddy layer is removed. The background of the notum is usually black or dark brown, but it can exceptionally be light brown. The background is mottled by white areas. Most typically, those white areas form two irregular longitudinal lines, one either side of the central axis. Additional, irregular white areas can exist as well. The colour of the hyponotum varies between light greyish and dark brown but is always marked by a significantly lighter ring at its margin. The foot is orange, occasionally yellow. The ocular tentacles are short and extend for only a few millimetres beyond the notum margin when the animal is crawling undisturbed. The head is small and remains covered by the dorsal notum as the animal crawls.

The body is not flattened. The dorsal notum is elongated, oval. The dorsal notum is thick. Its surface, when the animal is undisturbed, is not smooth. Dorsal gills are absent. Large papillae are absent but small conical papillae are present. About 10 of those papillae bear a black ‘dorsal eye’. A larger, central papilla bears three black ‘dorsal eyes’. In addition, the notum is finely granular. When the animal is disturbed (typically, if one touches its dorsum), it forms an almost perfect sphere, with a smooth dorsal notum. In fact, in the field, we called this species ‘black and white small ball’. Animals that are preserved without first being relaxed remain coiled up in a sphere. Crawling individuals can measure up to 25 mm, but most of them measure about 15 mm on average.

**External morphology (Figure 5a, b)**

Preserved specimens no longer display the distinct colour traits of live animals. The ventral colour, in particular, is homogeneously whitish or creamish. The width of the hyponotum relative to the width of the pedal sole varies among individuals and ranges from about 1/3 to 3/5 of the total width (in ventral view). The anus is posterior, median and close to the edge of the pedal sole (Figure 5a). 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 millimetres from the anus and the pneumostome (Figure 5b). The pneumostome is median. Its position on the hyponotum relative to the notum margin and the edge of 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 (Figure 5b). In the anterior region, the left and right ocular tentacles are superior to the mouth. They are outside the body if specimens were relaxed before preservation; otherwise, they are retracted. 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 bump, likely with sensitive receptors. The male aperture (opening of the copulatory complex) is located below the right ocular tentacle, slightly to its left (internal) side (Figure 5a).

**Visceral cavity and pallial complex**

Marginal glands (found in *Onchidella*) are absent. The anterior pedal gland is oval and flattened, lying free on the floor of the visceral cavity below the buccal mass. The visceral cavity is not pigmented internally and not divided (the heart is not separated from the
Figure 5. *Melanochis eloisae*, Malaysia (USMMC 00007, #1). (a) Anterior region, ventral view, scale bar = 3.5 mm. (b) Posterior region, ventral view. (c) Digestive system, dorsal view. (d) Stomach, ventral view. (e) Stomach, dorsal view. (f) Nervous system, dorsal view. Abbreviations: a, anus; ddg, dorsal lobe of digestive gland; f, foot; fo, female opening; h, hyponotum; i, intestine; lcg, left cerebral ganglion; ldg, lateral lobe of the digestive gland; lplg, left pleural ganglion; m, mouth; mo, male opening; oddg, opening of the dorsal lobe of the digestive gland; ol, oral lobe; oldg, opening of the lateral lobe of the digestive gland; opdg, opening of the posterior lobe of the digestive gland; ot, ocular tentacle; pdg, posterior lobe of the digestive gland; pn, pneumostome; ppg, peripodial groove; rcg, right cerebral ganglion; rg, rectal gland; rplg, right pleural ganglion; st, stomach; st1, stomach chamber 1; st2, stomach chamber 2; st3, stomach chamber 3; st4, stomach chamber 4; vg, visceral ganglion. Scale bar: a = 3.5 mm; b = 4 mm; c = 2.8 mm; d, e = 2 mm; f = 0.5 mm.
visceral organs by a thick, muscular membrane). The heart, enclosed in the pericardium, is on the right side of the visceral cavity, slightly posterior to the middle. The ventricle, anterior, gives an anterior vessel supporting several anterior organs such as the buccal mass, the nervous system and the copulatory complex. The auricle is posterior. The kidney is more or less symmetrical, the right and left parts being equally developed. Occasionally, the right part is slightly longer than the left part. The kidney is intricately attached to the respiratory complex. The lung is in two left and right, equally developed, more or less symmetrical parts. Occasionally, the right part is slightly longer than the left part.

**Digestive system (Figures 5c–e, 6)**

There are no jaws. The left and right salivary glands, heavily branched, join the buccal mass dorsally, on either side of the esophagus. The radula is between two large postero-lateral muscular masses. Each radular row contains a rachidian tooth and two half rows of lateral teeth. Examples of radular formulae are: $66 \times (110–1–110)$ in BDMNH station 34 #1 (24 mm long), $55 \times (110–1–110)$ in USMMC 00007 #1 (22 mm long), $65 \times (95–1–95)$ in USMMC 00007 #2 (20 mm long), and $45 \times (75–1–75)$ in USMMC 00007 #3 (10 mm long). 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 (Figure 6). The length of the rachidian teeth is about 30 µm, significantly less than the length of the lateral teeth. The lateral aspect of the base of the rachidian teeth is straight (not concave). The half rows of lateral teeth form an angle of 45° with the rachidian axis. Along a half row, all the lateral teeth do not have exactly the same length and shape. The lateral teeth seem to be unicuspid with a flattened and curved hook, but there also is an outer pointed spine on the lateral expansion of the base. In most cases, the basal lateral spine cannot be observed because it is hidden below the hook of the next, outer lateral tooth. It can only be observed when the teeth are not too close or when teeth are placed in an unusual position. The length of the hook of the lateral teeth gradually increases (from innermost to outermost) from about 40–50 µm to 60–70 µm, excluding the first few (about 5) innermost and outermost lateral teeth which are significantly smaller than the rest of the lateral teeth. 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. This protuberance diminishes gradually and is inconspicuous in the outermost teeth (of which, as a result, the lateral aspect is almost straight). The outermost teeth are much closer to one another than the innermost teeth and, as a result, seem narrower. Finally, the tip of the hook gradually changes from pointed to round from the innermost to outermost teeth.

The esophagus is narrow and straight, with thin internal folds. The esophagus enters the stomach anteriorly (Figure 5d, e). Only a portion of the posterior aspect of the stomach can be seen in dorsal view because it is partly covered by the lobes of the digestive gland (Figure 5c). The dorsal lobe is mainly on the right. The left, lateral lobe is mainly ventral. The posterior lobe covers the posterior aspect of the stomach. The stomach is a U-shaped sac divided into four chambers (Figure 5d, e). The first chamber, which receives the esophagus, is delimited by a thin layer of tissue, and receives the ducts of the dorsal and lateral lobes of the digestive gland. The second chamber, posterior, is delimited by a thick muscular tissue and receives the duct of the posterior lobe of the digestive gland. It appears divided externally but consists of only one internal chamber. The third, funnel-shaped chamber is delimited by a thin layer of tissue with high ridges internally. The fourth chamber is continuous and externally similar to the third, but it bears only low, thin ridges internally.
The intestine is long and narrow (Figure 5c). The pattern of its loops is intermediary between types II and III. A rectal gland is present (Figure 5c). It is a long, narrow and coiled tube that opens in the left portion of the pulmonary complex. Its function is unknown.

**Nervous system (Figure 5f)**

The circum-esophageal nerve ring is post-pharyngeal and pre-esophageal. The cerebral commissure between the two cerebral ganglia is short but its length does vary among individuals. Pleural and pedal ganglia are also all distinct. The visceral commissure is short but distinctly present and the visceral ganglion is more or less median. Cerebro-pleural and pleuro-pedal connectives are very short and pleural and cerebral ganglia touch each other. Nerves from the cerebral ganglia innervate the buccal area and the

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**Figure 6.** Radula, *Melayonchis eloisae*. (a) Innermost lateral and lateral teeth, Malaysia (USMMC 00007, #1). (b) Lateral teeth, Brunei Darussalam (BDMNH, #1). (c) Rachidian tooth, Malaysia (USMMC 00007, #1). (d) Outermost lateral teeth, Malaysia (USMMC 00007, #1). (e) Lateral teeth, detail, Brunei Darussalam (BDMNH, #1). The arrows indicate the lateral protuberance which is characteristic of the teeth in *Melayonchis*. Scale bars: a = 40 mm; b = 30 μm; c = 10 μm; d = 20 μm; e = 5 μm.

The intestine is long and narrow (Figure 5c). The pattern of its loops is intermediary between types II and III. A rectal gland is present (Figure 5c). It is a long, narrow and coiled tube that opens in the left portion of the pulmonary complex. Its function is unknown.
ocular tentacles, and, on the right side, the penial complex. Nerves from the pedal ganglia innervate the foot. Nerves from the pleural ganglia innervate the lateral and dorsal regions of the mantle. Nerves from the visceral ganglia innervate the visceral organs.

Reproductive system (Figure 7a)
Sexual maturity is correlated with animal length. Mature individuals have large female organs (with a large female gland mass) and fully developed, anterior male copulatory parts. Immature individuals may have inconspicuous female organs (or simply none) and rudimentary anterior male parts. The hermaphroditic gland is a single mass, joining the spermoviduct through the hermaphroditic duct (which conveys the eggs and the autosperm). There is a large and approximately spherical receptaculum seminis (caecum) along the hermaphroditic duct. The female gland mass contains various glands (mucous and albumin) which can hardly be

Figure 7. Reproductive system, *Melayonchis eloisae*, Malaysia (USMMC 00007, #1). (a) Anterior, male apparatus. (b) Posterior hermaphroditic (female) reproductive system. Abbreviations: dd, deferent duct; fgm, female gland mass; hd, hermaphroditic duct; hg, hermaphroditic gland; ov, oviduct; ps, penial sheath; rm, retractor muscle; rs, receptaculum seminis; sp, spermatheca; spv, spermoviduct; v, vestibule. Scale bar: a = 2 mm; b = 1.5 mm.
separated by dissection and of which the exact connections remain uncertain. The hermaphroditic duct becomes the spermoviduct (which conveys eggs, exosperm and autosperm) which is not divided proximally, at least not externally. The spermoviduct is embedded within the female gland mass, at least proximally. Distally, the spermoviduct branches into the deferent duct (which conveys the autosperm up to the anterior region, running through the body wall) and the oviduct. The free oviduct conveys the eggs up to the female opening and the exosperm from the female opening up to the fertilisation chamber, which should be near the proximal end of the spermoviduct. The spermatheca (for the storage of exosperm) is nearly spherical and connects to the oviduct through a very short duct. The oviduct is narrow and straight. The vaginal gland is absent.

**Copulatory apparatus (Figures 7b, 8)**

The male anterior organs consist of the penis, penial sheath, vestibule, deferent duct and retractor muscle (Figure 7b). There is no penial accessory gland. The penial sheath is short (less than 2 mm in length) and straight. The penial sheath protects a penis which consists of a short, elongated and slightly curved tube, about 0.7 mm long and 35 μm in diameter (Figure 8). There are no penial hooks. The vestibule is approximately as long as the penial sheath, but much larger (Figure 7b). The insertion of the retractor muscle marks the separation between the penial sheath and the deferent duct (Figure 7b). The retractor muscle is much longer than the penial sheath and inserts at about half the length of the visceral cavity. The deferent duct also is highly convoluted with many loops (in immature specimens, the deferent duct is significantly less convoluted).

**Distinctive field diagnostic features**

A table at the end of the introduction summarises the most important features that can help distinguish and identify *Melayonchis* species (Table 3). In the field, individuals of *M. eloisae* can easily be recognised because they coil up into a sphere when disturbed. The presence of an orange foot and a notum with a black background and two longitudinal, irregular, white lines can also help identify live specimens in the field (even though the dorsal colour pattern does vary among individuals and the two white lines are not always present). When animals are observed without being disturbed while crawling and still covered with a thin layer of muddy mucus, then they are much harder to identify.

*Figure 8.* Penis, *Melayonchis eloisae*. (a) Malaysia (USMMC 00007, #2). (b) Malaysia (USMMC 00007, #1). – (c) Same as (b) detail. Scale bars: a, b = 100 μm; c = 20 μm.
**Melayonchis siongkiati** Dayrat and Goulding sp. nov.  
(Figures 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 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, designated here: one specimen 32/20 [DNA 1002] mm, in formalin and 70% alcohol, with a piece in 95% alcohol, leg. B. Dayrat and S.K. Tan (ZRC.MOL.6501).

**Additional material examined**
**Malaysia.** Peninsular Malaysia, Matang, Crocodile River, 04°49.521’ N, 100°37.630’ E, 11 July 2011, 5 specimens (28/17 to 17/12 mm), leg. B. Dayrat and T. Goulding [station 16, tall *Rhizophora* trees with soft and hard mud] (USMMC 00012); Malaysia, Peninsular Malaysia, Merbok, 05°39.035’ N, 100°25.782’ E, 12 July 2011, 1 specimen (32/15 mm),

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**Figure 9.** Habitats for *Melayonchis siongkiati*. (a) Type locality, Singapore, Mandai River, open mangrove forest with tall trees and soft mud (station 6). (b) Malaysia, Peninsular Malaysia, Matang, old and open *Rhizophora* forest with tall trees, hard mud, creeks and many dead logs (station 28). (c) Brunei Darussalam, Pulau Pyatan, open mangrove with a few sparse old trees, and large old logs, by the river (station 32). (d) Vietnam, Can Gio, open mangrove with large *Avicennia* trees, soft mud, some dead logs (station 231).
leg. B. Dayrat and T. Goulding [station 21, deep *Rhizophora* forest with old, tall trees, hard mud, many small creeks and dead logs] (USMMC 00013); Malaysia, Peninsular Malaysia, Merbok, 05°40.143′ N, 100°26.178′ E, 12 July 2011, 1 specimen (8/6 mm), leg. B. Dayrat and T. Goulding [station 22, mostly *Rhizophora*, soft mud and some very soft mud near creek] (USMMC 00014); Malaysia, Peninsular Malaysia, Kuala Sepatang, 04° 50.434 N, 100°38.176 E, 18 July 2011, 2 specimens (28/15 and 25/16 mm), leg. B. Dayrat and T. Goulding [station 27, old forest with tall, old *Rhizophora* trees, high in the tidal zone (ferns), following boardwalk in educational preserve, reached a creek lower in the tidal zone, with mud] (USMMC 00015); Malaysia, Peninsular Malaysia, Matang, off Kuala Sepatang, Crocodile River, Sungai Babi Manpus, 04°49.097′ N, 100°37.370′ E, 19 July 2011, 19 specimens (40/25 [#2] to 11/9 mm; 34/20 [#1], 26/20 [DNA 920] and 17/11 [DNA 947] mm), leg. B. Dayrat and T. Goulding [station 28, old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many dead logs] (USMMC 00016); Malaysia, Peninsular Malaysia, Matang, close to the jetty, facing the fishermen’s village on the other side of the river, 04°50.154′ N, 100°36.368′ E, 20 July 2011, 4 specimens (25/16 to 16/12 mm), leg. B. Dayrat and T. Goulding [station 29, oldest and open *Rhizophora* forest of tallest and beautiful trees, with hard mud, many creeks, and many dead logs] (USMMC 00017); Brunei Darussalam, Pulau Siaru, Temburung, 04°49.066′ N, 115°02.250′ E, 26 July 2011, 38 specimens (35/21 [#1] to 10/6 [#2] mm; 12/9 [#3]), leg. B. Dayrat, T. Goulding and S. Calloway [station 30, mostly *Nypa* palms, by the river] (BDMNH); Brunei Darussalam, Sungai Brunei, 04°53.756′ N, 114°59.496′ E, 26 July 2011, 3 specimens (27/14 to 10/10 mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 31, very large, tall *Rhizophora* trees, soft mud] (BDMNH); Brunei Darussalam, Pulau Pyatan, Teluk Brunei, 04°55.246′ N, 115°02.764′ E, 27 July 2011, 46 specimens (35/22 [DNA 1052] to 10/6 mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 32, open mangrove with a few sparse old trees, and large old logs, by the river] (BDMNH); Brunei Darussalam, Pulau Kaingara, 04°57.020′ N, 115°01.785′ E, 28 July 2011, 23 specimens (33/17 to 12/8 mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 33, open mangrove with medium *Rhizophora* trees and logs, by the river] (BDMNH); Brunei Darussalam, Mentiri, Jalan Batu Marang, 04°59.131′ N, 115°01.820′ E, 29 July 2011, 12 specimens (28/17 to 17/10 mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 34, old mangrove with tall *Rhizophora* trees with high roots and *Thalassina* mounds] (BDMNH); Vietnam, Can Gio, 10°27.803′ N, 106°53.288′ E, 16 July 2015, 2 specimens (31/18 and 30/20 [DNA 5608] mm), leg. T. and J. Goulding [station 228, high intertidal, open forest of tall *Rhizophora* trees, hard mud, near a small river] (ITBZC IM 00010); Vietnam, Can Gio, 10°27.620′ N, 106°53.316′ E, 17 July 2015, 1 specimen (37/25 mm), leg. T. and J. Goulding [station 231, open mangrove with large *Avicennia* trees, soft mud, some dead logs] (ITBZC IM 00011).

**Distribution**

Singapore (type locality), Peninsular Malaysia, Brunei Darussalam, Vietnam.

**Etymology**

*M. siongkiati* is dedicated to Siong Kiat Tan, from the Lee Kong Chian Natural History Museum (National University of Singapore), who kindly spent some time with the first author in the mangroves of Singapore and generously shared his excellent field knowledge of the local fauna, especially snails and slugs.
**Habitat (Figure 9)**

*Melayonchis siongkiati* is mostly found in the high intertidal, and it especially favours old and open *Rhizophora* forests with dead logs. It lives on trunks and roots of mangrove trees, often not muddy but covered with algae instead. It can also be found on dead logs. Occasionally, when abundant, it can also be found on muddy rocks and cemented walls at the margin of a mangrove. It is not found directly on mud.

**Abundance**

*Melayonchis siongkiati* was abundant only in Brunei, where we found many specimens at almost every site that we visited. In Malaysia, we found a few individuals in most sites that seemed to be the right habitat, and a large population was found only at one site. It is uncommon in Singapore and Vietnam.

**Colour and morphology of live animals** *(Figures 10, 11)*

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. That thin layer of mucus makes them very cryptic. It may also help prevent desiccation. The colour of the dorsal notum appears after the thin muddy layer is removed. The background of the notum, light brown or greyish, is irregularly mottled by dark brown areas. In addition, the background can occasionally be mottled with whitish, irregular areas also. The colour of the hyponotum varies from light grey to dark brown or dark blue but is always marked by a significantly lighter ring at its margin. The foot is grey (light to dark). The brown ocular tentacles are short and extend for only a few millimetres beyond the notum margin when the animal crawls undisturbed. The head is small and remains covered by the dorsal notum as the animal crawls.

The body is usually not flattened, but some animals may seem flattened, especially when crawling. The dorsal notum is elongated, oval. The dorsal notum is not particularly thick. Its surface, when the animal is undisturbed, is not smooth. Dorsal gills are absent. Large papillae are absent but small conical papillae are present. About 15 of those papillae bear a black ‘dorsal eye’ (occasionally two or three). A larger, central papilla bears three black ‘dorsal eyes’. In addition, the notum is finely granular. When the animal is disturbed (typically, if one touches its dorsum), it can coil up but does not form a complete sphere *(Figure 10b, d)*. Also, its dorsal notum immediately secretes a distinct oily mucus that makes the dorsum shiny and oily. In fact, in the field, we called this ‘the oily species’ *(Figures 10a, 11c)*. Also, the dorsum of disturbed animals tends to be smooth (instead of finely granular), but the small conical papillae inflate into slightly larger bumps. Animals that are preserved without first being relaxed remain coiled up into a sphere. Crawling individuals can measure up to 40 mm, but most of them measure about 20 to 25 mm on average. Preserved specimens no longer display the distinctive colour traits of live animals. The ventral colour, in particular, is homogeneously whitish or creamish, occasionally dark *(Figure 11b, d, f)*.

**Digestive system** *(Figures 12a, 13)*

Examples of radular formulae are *(Figure 13):* $80 \times (165–1–165)$ in BDMNH station 30 #1 (35 mm long), $40 \times (80–1–80)$ in BDMNH station 30 #2 (10 mm long), and $85 \times (160–1–160)$ in USMMC 00016 #2 (40 mm long). The length of the rachidian teeth is about 30 µm, significantly less than that of the lateral teeth. Along a half row, all the lateral teeth do not
have exactly the same shape. Their length (about 60 µm) is similar, although there seems to be a slight, gradual increase from innermost to outermost teeth (excluding the first few innermost and outermost lateral teeth which are significantly smaller than the rest of the lateral teeth). Finally, the tip of the hook is fairly 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 pattern of its loops is of type III (Figure 12a).
Reproductive system (Figures 12b, 14, 15)

There is a small and ovate receptaculum seminalis (caecum) along the hermaphroditic duct. The spermatheca (for the storage of exosperm) is large and elongated, and connects to the oviduct through a very short and narrow duct (Figure 12b).

The male anterior organs (Figure 14) consist of the penial complex (penis, penial sheath, vestibule, deferent duct, retractor muscle) and penial accessory gland (flagellum, duct and hollow spine). The penial complex and the penial accessory gland share distally the same vestibule and same anterior male opening (Figure 14). The length of the flagellum of the penial gland varies among individuals. However, in all specimens dissected, the flagellum of the penial gland is coiled. The gland is a tube with a dead end proximally: it is characterised by two portions of distinct diameter: the proximal portion, or flagellum, is slightly wider than the distal portion or gland duct. That distinction in diameter, however, does vary among
individuals. In immature specimens, the flagellum and the duct may not be distinguishable. Distally, the duct of the gland ends in a hard, hollow spine (Figure 15a, b), which conveys the secretion from the flagellum to the outside (and the partner). The hollow spine is narrow and elongated, slightly curved. It measures about 100 μm in diameter at its conical base and narrows down to 20 μm distally, for a length from 0.8 to 0.9 mm. The hollow spine opens into the proximal region of the vestibule.

The penial sheath is short (less than 4 mm in length) and straight. The penial sheath protects a penis which consists of a short, straight papilla, from 100 to 130 μm in diameter and from 0.5 to 1 mm in length (Figure 15c, d). There are no penial hooks. The insertion of the retractor muscle marks the separation between the penial sheath and the deferent duct (Figure 14). The retractor muscle is longer or shorter than the penial sheath but
inserts at about half the length of the visceral cavity. The deferent duct is convoluted with many loops (in immature specimens, the deferent duct is significantly less convoluted).

**Distinctive field diagnostic features**

A table at the end of the introduction summarises the most important features that can help distinguish and identify *Melayonchis* species (Table 3). In the field, individuals of *M. siongkiati* can easily be identified because their dorsal notum secretes a distinctive oily mucus as soon as they are disturbed, which makes their dorsum shiny and oily. They can also coil up into a sphere, but not as systematically and completely as *M. eloisae*. The dorsal colour of live animals, which is quite variable, is harder to use for identification. When animals are observed without being disturbed while crawling, then they are much
harder to identify and could easily be confused with other species, including species from other genera (such as Platevindex).

**Melayonchis annae** Dayrat sp. nov.
(Figures 16–19)

**Type locality**
Singapore, Lim Chu Kang, 01°26.785’ N, 103° 42.531’ E, 5 April 2010 [station 9, mangrove East of the jetty; open forest with medium trees and medium mud, ending on sun-exposed mudflat outside the mangrove with soft mud].

**Type material**
Holotype, designated here: one specimen 32/20 [DNA 1010] mm, in formalin and 70% alcohol, with a piece in 95% alcohol, leg. B. Dayrat and S.K. Tan (ZRC.MOL.6502).
Additional material examined

Singapore, Mandai, 01°26.237 N, 103° 45.730 E, 2 April 2010, 13 specimens (33/22 [#1] to 18/14 mm [#2]; 18/15 [DNA 1008] and 22/15 [DNA 1009] mm), leg. B. Dayrat and S.K. Tan [station 6, following the river from the railroad towards sea, open mangrove forest with tall trees and soft mud, ending on sun-exposed mudflat outside the mangrove with very soft mud, heavily polluted with trash] (ZRC.MOL.6503);

Brunei Darussalam, Mentiri, Jalan Batu Marang, 04°59.131’N, 115°01.820’E, 29 July 2011, 16 specimens (32/19 [#1] to 10/7 [#2] mm; 26/15 [DNA 1046] and 20/12 [DNA 1045] mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 34, old mangrove with tall Rhizophora trees with high roots and Thalassina mounds, but specimens collected on cemented walls at the margin of the mangrove] (BDMNH).
Figure 16. Habitat and live specimens, *Melayonchis annae*. (a) Singapore, Mandai River, open mangrove forest with tall trees and soft mud (station 6). (b) Holotype, dorsal view, 32 mm long [DNA 1010], Singapore (ZRC.MOL.6502). (c) Two individuals, dorsal view, 30 mm long, Singapore (ZRC.MOL.6503). (d) Two individuals, dorsal view, 30 mm long, Singapore, (ZRC.MOL.6503). (e) Ventral view, 25 mm long, Singapore (ZRC.MOL.6503). (f) Dorsal view, 20 mm long [DNA 1045], Brunei Darussalam (BDMNH). (g) Dorsal view, 26 mm long [DNA 1046], Brunei Darussalam (BDMNH).
**Distribution**
Singapore (type locality) and Brunei Darussalam.

**Etymology**
*Melayonchis annae* is dedicated to Anna Dayrat, for the time that her father (the first author) has to spend away from her, exploring mangroves and missing her.

**Habitat (Figure 16a)**
*Melayonchis annae* is found in the high intertidal. In Singapore, it was found quite high on tree trunks. In Brunei Darussalam, it was found on rocks and cemented walls at the margin of an old *Rhizophora* forest. So far, it has not been found directly on mud, or on muddy dead logs.

**Abundance**
*Melayonchis annae* is a rare species. We found it only at three of the dozens of mangrove sites that we visited in the region. However, in two of three sites, we found a reasonable number of specimens (more than 10) without difficulty (animals which live high on trunks and on rocks just outside mangroves are not cryptic).

**Colour and morphology of live animals (Figure 16b–g)**
Live animals are not covered dorsally with a thin layer of muddy mucus and the colour of their dorsal notum can readily be seen. The dorsal notum is irregularly mottled with cream, light brown, and dark brown areas. The colour of the hyponotum varies between cream to light grey. Because it is lightly coloured, its margin is not marked by a significantly lighter ring. The foot is cream (Figure 16e). The brown ocular tentacles are short and extend for only a few millimetres beyond the notum margin when the animal crawls undisturbed. The head is small and remains covered by the dorsal notum as the animal crawls.

The body is not flattened. The dorsal notum is elongated, oval. The dorsal notum is not particularly thick. Its surface, when the animal is undisturbed, is not smooth. Dorsal gills are absent. Large papillae are absent but small conical papillae are present. From eight to 12 of those papillae bear a black ‘dorsal eye’ which, when the animal is undisturbed, seems to form a slight bump. A slightly larger, central papilla bears three black ‘dorsal eyes’. In addition, the notum is finely granular throughout. When the animal is disturbed (typically, if one touches its dorsum), all papillae tend to retract but the dorsal notum remains finely granular. It may also excrete an oily mucus when disturbed, but not as frequently as *M. siongkiati*, and in a much smaller amount. When disturbed, animals do not coil up into a sphere. Crawling individuals can measure up to 33 mm, but most of them measure about 20 to 25 mm on average. Preserved specimens no longer display the distinctive colour traits of live animals. The ventral colour, in particular, is homogeneously whitish or creamish.

**Visceral cavity and pallial complex**
The kidney and the lung are slightly asymmetrical, the right part being slightly longer than the left part.
Examples of radular formulae are (Figure 18): 95 × (280–1–280) in BDMNH station 34 #1 (32 mm long), 65 × (185–1–185) in BDMNH station 34 #2 (10 mm long), 100 × (290–1–290) in ZRC.MOL.6503 #1 (33 mm long), and 90 × (260–1–260) in ZRC.MOL.6503 #2 (18 mm long). The length of the rachidian teeth is about 25 µm, significantly less than that of the lateral teeth. Along a half row, all the lateral teeth do not have exactly the same length and shape. The length of the hook of the lateral teeth gradually increases (from innermost to outermost) from about 40 to 50 µm, excluding the first few (about 5)
Figure 18. Radula, *Melayonchis annae*, Singapore (ZRC.MOL.6503, #2). (a) Median region of left half rows. (b) Rachidian and innermost lateral teeth. (c) Lateral teeth, inferior view. (d) Outermost lateral teeth. (e) Lateral teeth. The arrows indicate the lateral protuberance which is characteristic of the teeth in *Melayonchis*. Scale bars: a = 100 μm; b, d, e = 20 μm; c = 4 μm.

Figure 19. Penis, *Melayonchis annae*. (a) Singapore (ZRC.MOL.6503, #2). (b) Penis, Brunei Darussalam (BDMNH, #1). Scale bars: a = 60 μm; b = 100 μm.
innermost and outermost lateral teeth which are significantly smaller than the rest of the lateral teeth. Finally, the tip of the hook of the lateral teeth is round throughout the entire half row. The pattern of its loops is of type II (Figure 17a).

Reproductive system (Figures 17b, c, 19)
There is a small, oval, slightly bent receptaculum seminalis (caecum) along the hermaphroditic duct. The oval spermatheca (for the storage of exosperm) is large and connects to the oviduct through a very short duct (Figure 17b).

The male anterior organs consist of the penial complex (penis, penial sheath, vestibule, deferent duct, retractor muscle; Figure 17c). There is no penial accessory gland. The penial sheath is very short (about 1 mm in length) and straight. The penial sheath protects a penis which consists of a short, slightly conical papilla of about 0.4 mm long (Figure 19). There are no penial hooks. The vestibule is both longer and larger than the penial sheath. The insertion of the retractor muscle marks the separation between the penial sheath and the deferent duct. The retractor muscle is longer than the penial sheath but inserts in the anterior part of the visceral cavity. The deferent duct is highly

Figure 20. Habitats for Melayonchis aileenae. (a) Malaysia, Peninsular Malaysia, Kuala Sepatang, old forest with tall, old Rhizophora trees, high in the tidal zone with ferns (station 27). (b) Malaysia, Peninsular Malaysia, Matang, oldest and open Rhizophora forest of tallest and beautiful trees, with hard mud, many creeks, and many dead logs (station 29). (c) India, Andaman Islands, Middle Andaman, Rangat, deep mangrove with tall trees, small creeks, and plenty of dead muddy logs (station 59). (d) Vietnam, Can Gio, high intertidal, Avicennia mangrove (station 232).
convoluted with many loops, even though the deferent duct is significantly less convoluted in immature specimens.

**Distinctive field diagnostic features**
A table at the end of the introduction summarises the most important features that can help distinguish and identify *Melayonchis* species (Table 3). In the field, individuals of *M. annae* remain difficult to identify because their dorsal colour is highly variable. However, other features differ sufficiently from the other species of *Melayonchis*, such as the ventral colour (white to light grey). It secretes much less oily mucus than *M. siongkiai*. Also, unlike *M. eloisae*, animals do not coil up into a sphere. Finally, the habitat of *M. annae* seems more specific (on the dry bark of mangrove trees as well as on rocks outside mangroves) than that of the other species described here.

*Melayonchis aileenae* Dayrat and Goulding sp. nov.
(Figures 20–26)

**Type locality**
Malaysia, Peninsular Malaysia, Kuala Sepatang, 04°50.434 N, 100°38.176 E, 18 July 2011 [station 27, old forest with tall, old *Rhizophora* trees, high in the tidal zone (ferns), following boardwalk in educational preserve, reached a creek lower in the tidal zone, with mud] (USMMC 00018).

**Type material**
Holotype, designated here: one specimen 20/10 [DNA 970] mm, in 95% alcohol, leg. B. Dayrat and T. Goulding (USMMC 00018). Note that other specimens (not part of the type material) were collected from the same locality and are mentioned below in the additional material examined.

**Additional material examined**
**Malaysia**, Peninsular Malaysia, Kuala Sepatang, 04°50.434 N, 100°38.176 E, 18 July 2011, 8 specimens (25/16 [#1] to 15/8 mm; 20/12 [#2]), leg. B. Dayrat and T. Goulding [station 27, old forest with old, tall *Rhizophora* trees, high in the tidal zone (ferns), following boardwalk in educational preserve, reached a creek lower in the tidal zone, with mud] (USMMC 00019); Malaysia, Peninsular Malaysia, Matang, off Kuala Sepatang, Crocodile River, Sungai Babi Manpus, 04°49.097 N, 100°37.370 E, 19 July 2011, 1 specimen (18/14 [DNA 968] mm), leg. B. Dayrat and T. Goulding [station 28, old and open *Rhizophora* forest with tall trees, hard mud, creeks, and many dead logs] (USMMC 00020); Malaysia, Peninsular Malaysia, Matang, close to the jetty, facing the fisherman’s village on the other side of the river, 04°50.154’ N, 100°36.368’ E, 20 July 2011, 1 specimen (25/10 mm), leg. B. Dayrat and T. Goulding [station 29, oldest and open *Rhizophora* forest of tallest and beautiful trees, with hard mud, many creeks, and many dead logs] (USMMC 00021); **Brunei Darussalam**, Mentiri, Jalan Batu Marang, 04°59.131’ N, 115°01.820’ E, 29 July 2011, 1 specimen (13/8 [DNA 1047] mm), leg. B. Dayrat, T. Goulding and S. Calloway [station 34, old mangrove with tall *Rhizophora* trees with high roots and *Thalassina* mounds]
Vietnam, Can Gio, 10°24.108’ N, 106°53.019’ E, 13 July 2015, 2 specimens (19/12 [DNA 5606] and 15/12 mm), leg. T. and J. Goulding [station 224, mangrove with big Rhizophora trees mixed with Avicennia] (ITBZC IM 00005); Vietnam, Can Gio, 10°27.803’ N, 106°53.288’ E, 16 July 2015, 3 specimens (25/11 to 14/10 mm), leg. T. and J. Goulding [station 228, high intertidal, open forest of tall Rhizophora trees, hard mud, near a small river] (ITBZC IM 00006); Vietnam, Can Gio, 10°27.620’ N, 106°53.316’ E, 17 July 2015, 1 specimen (20/10 [DNA 5631] mm), leg. T. and J. Goulding [station 231, open mangrove with large Avicennia trees, soft mud, some dead logs] (ITBZC IM 00007); Vietnam, Can Gio, 10°27.692’ N, 106°53.308’ E, 18 July 2015, 5 specimens (32/20 to 20/14 mm), leg. T. and J. Goulding [station 232, high intertidal, Avicennia mangrove separated from tall Rhizophora trees (station 228) by a road] (ITBZC IM 00008). India, Andaman Islands, Middle Andaman, Rangat, Shyamkund, 12°28.953’ N, 092°50.638’ E, 11 January 2011, 1 specimen 14/8 mm, leg. B. Dayrat and V. Bhave [station 59, by a large river, deep mangrove with tall trees, small creeks, and plenty of dead muddy logs, next to a road and a small cemented bridge above a creek] (BNHS 90).

**Distribution**
Malaysia (type locality), India (Andaman Islands), Brunei Darussalam and Vietnam.

*Figure 21.* Live specimens, *Melayonchis aileenae*. (a) Holotype, dorsal view, 20 mm long [DNA 970], Malaysia (USMMC 00018). (b) Dorsal view, 22 mm long, Malaysia (USMMC 00019). (c) Dorsal view, 23 mm long, Malaysia (USMMC 00019). (d) Dorsal view, 20 mm long, Malaysia (USMMC 00019). (e) Ventral view, same as (b). (f) Ventral view, same as (d).
**Etymology**

*Melayonchis aileenae* is dedicated to our dear friend Dr Tan Shau Hwai (Aileen), professor at the Universiti Sains Malaysia, Penang, whose generous help was critical for us to conduct field work in Malaysia and other parts of South East Asia.

**Habitat (Figure 20)**

*Melayonchis aileenae* is found in the high intertidal and it especially favours old *Rhizophora* forests. It lives on trunks and roots of mangrove trees, often not muddy but covered with algae instead. It can also be found on dead logs. In Vietnam, a few specimens were found under rotting bark on mangrove tree roots. Occasionally, it can also be found on cemented walls at the margin of a mangrove. It is not found directly on mud.

**Figure 22.** Live specimens, *Melayonchis aileenae*. (a) Dorsal view, 13 mm long [DNA 1047], Brunei Darussalam (BDMNH). (b) Dorsal view, 14 mm long, Andaman Islands (BNHS 90). (c) Dorsal view, 23 mm long, Vietnam (ITBZC IM 00006). (d) Dorsal view, 20 mm long [DNA 5631], Vietnam (ITBZC IM 00007). (e) Dorsal view, 32 mm long, Vietnam (ITBZC IM 00008). (f) Ventral view, same as (e).
Abundance

*Melayonchis aileenae* is a rare species. It was found at only a few of the dozens of mangrove sites that we visited in the region, and we found only a few specimens. It is not particularly cryptic so we believe that it actually is uncommon.

Colour and morphology of live animals (Figures 21, 22)

Live animals are usually not covered dorsally with a thin layer of muddy mucus and the colour of their dorsal notum can readily be seen. Occasionally, live animals are covered with a thin layer of mud which first needs to be removed to see the dorsal colour. The background of the dorsal notum varies from light brown to black. The notum bears additional markings (white dots and inconspicuous darker, irregular, longitudinal lines) which vary among individuals. The colour of the hyponotum is very light and varies from cream to light grey. Because it is lightly coloured, its margin is not marked by a significantly lighter ring. However, there is a ring of white dots around the margin of the hyponotum that could correspond to gland openings. The foot is cream to pale yellow (Figures 21e, f, 22f). The brown ocular tentacles are short and extend for only a few millimetres beyond the notum margin when the animal crawls undisturbed. The head is small and remains covered by the dorsal notum as the animal crawls.

The body is not flattened. The dorsal notum is elongated, oval. The dorsal notum is not particularly thick. Its surface is remarkably smooth (occasionally very finely granular). Dorsal gills are absent. Large or small papillae are absent. ‘Dorsal eyes’ are present and at the tip of very tiny tubercles which can be barely noticeable on the notum surface. There are about 20 isolated ‘dorsal eyes’ throughout the dorsal notum. In the centre, there are three ‘dorsal eyes’ clustered together. When disturbed, animals do not coil up into a sphere. Crawling individuals can measure up to 32 mm, but most of them measure about 20 mm on average.

External morphology

Some of the distinct colour traits of live animals are lost in preservation (and this clearly worsens with time). The dorsal colour of preserved animals varies from homogeneously cream to black with occasional, irregular, longitudinal lines. The preserved foot tends to be slightly yellow while the hyponotum is white. The width of the hyponotum relative to the width of the pedal sole varies among individuals but represents on average about 1/3 of the total width (Figures 21e, f, 22f).

Digestive system (Figures 23a, 24, 25)

Examples of radular formulae are (Figures 24, 25): 100 × (290−1−290) in USMMC 00019 #1 (25 mm long), 140 × (380−1−380) in USMMC 00019 #2 (20 mm long), 95 × (250−1−250) in USMMC 00021 (25 mm long), and 95 × (250−1−250) in ITBZC IM 00007 (20 mm long). Along a half row, all the lateral teeth do not have exactly the same length and shape. The length of the hook of the lateral teeth gradually increases (from innermost to outermost) from about 25–35 µm to about 40–50 µm, excluding the first few (about 5) innermost and outermost lateral teeth which are significantly smaller than the rest of the lateral teeth. Finally, the tip of the hook of the lateral teeth is quite variable and can be pointed, tapered or round. The pattern of its loops is intermediary between types II and III (Figure 23a).
Figure 23. *Melayonchis aileenae*, Malaysia (USMMC 00019, #1). (a) Digestive system. (b) Posterior, hermaphroditic (female) reproductive system. (c) Anterior, male apparatus. Abbreviations: dd, deferent duct; ddg, dorsal lobe of digestive gland; fgm, female gland mass; hd, hermaphroditic duct; hg, hermaphroditic gland; i, intestine; ldg, lateral lobe of the digestive gland; ov, oviduct; pdg, posterior lobe of the digestive gland; ps, penial sheath; rg, rectal gland; rm, retractor muscle; rs, receptaculum seminis; sp, spermatheca; spv, spermoviduct; st, stomach. Scale bar: a = 3 mm; b = 2.6 mm; c = 1.6 mm.
Reproductive system (Figures 23b, c, 26)

There is a small, pear-shaped, slightly bent receptaculum seminalis (caecum) along the hermaphroditic duct. The oval spermatheca (for the storage of exosperm) is very large and connects to the oviduct through a very short duct (Figure 23b).

The male anterior organs consist of the penial complex (penis, penial sheath, vestibule, deferent duct, retractor muscle) (Figure 23c). There is no penial accessory gland. The penial sheath is short (less than 2 mm in length), straight and of similar width to the distal vestibule. The penial sheath protects a penis which consists of a short, slightly conical papilla of about 0.5 mm long (Figure 26). The penial papilla is slightly longer and more elongated (0.8 mm) in Vietnam (Figure 26a). There are no penial hooks. The vestibule is continuous with and similar to the penial sheath. The insertion of the retractor muscle

Figure 24. Radula, *Melayonchis aileenae*, Malaysia (USMMC 00019, #2). (a) Median region of left half rows. (b) Rachidian and innermost teeth. (c) Rachidian and innermost teeth. (d) Outermost lateral teeth. (e) Lateral teeth. The arrows indicate the lateral protuberance which is characteristic of the teeth in *Melayonchis*. Scale bars: a = 40 μm; b, e = 10 μm; c, d = 5 μm.
marks the separation between the penial sheath and the deferent duct. The retractor muscle is short (as long as the penial sheath) and inserts in the anterior part of the visceral cavity. The deferent duct is highly convoluted with many loops, even though it is significantly less convoluted in immature specimens (Figure 23c).

Figure 25. Radula, *Melayonchis aileenae*, Malaysia. (a) Innermost lateral and lateral teeth (USMMC 00019, #1). (b) Rachidian tooth (USMMC 00019, #1). (c) Lateral teeth, inferior view (USMMC 00019, #1). (d) Outermost lateral teeth (USMMC 00021). The arrows indicate the lateral protuberance which is characteristic of the teeth in *Melayonchis*. Sclae bars: a, d = 20 μm; b, c = 5 μm.

Figure 26. Penis, *Melayonchis aileenae*. (a) Vietnam (ITBZC IM 00007). (b) Malaysia (USMMC 00021). – (c) Malaysia (USMMC 00019, #1). Scale bars: 100 μm.
Distinctive field diagnostic features
A table at the end of the introduction summarises the most important features that can help distinguish and identify *Melayonchis* species (Table 3). In the field, *M. aileenae* is easy to identify because no other species looks like it. Its dorsal colour (brown background with lighter and darker markings), although variable, combined with its exceptionally smooth notum, make it unique and unmistakable.

Discussion

Geographic distribution

Given the available data, the geographic distribution of *Melayonchis* ranges from the Andaman Sea (Andaman Islands) to the South China Sea (southern Vietnam and eastern Borneo) through the Strait of Malacca. We have not encountered this new genus in the other places where we have done field work, from South Africa to Australia, nor did we find any museum specimens that could be identified as *Melayonchis* (within or outside its range of distribution). Also, no existing species described outside the known geographic range of *Melayonchis* deserves to be classified within this genus. The current range of distribution of *Melayonchis* is similar to that of the genus *Onchidium* (Dayrat et al. 2016), although *Onchidium* is also found in Bengal (India) and China.

Nomenclature of existing species

A review of all the existing species names with type localities within the range of distribution of *Melayonchis* reveals that no names are available for the taxa being described here (for a complete checklist of species-group and genus-group names of Onchidiidae, see Dayrat 2009). Because the supra-specific classification of onchidiids has remained extremely confusing (most species were arbitrarily classified in *Onchidium* by default), all existing names from the geographic range of *Melayonchis* are commented on here. Comments are based on the examination of all types available and a careful study of the original descriptions, as well as a comparison with our new, fresh specimens (including specimens from most type localities).

Three species names refer to *Peronia* species, which are characterised by dorsal gills, among other features, and thus cannot be part of *Melayonchis*: *Quoyella indica* Labbé, 1934, type locality in the Indian Ocean (unspecified); *Paraperonia gondwanae* Labbé, 1934, type locality in Mumbai, India; *Scaphis lata* Labbé, 1934, type locality in Vietnam. Two species names refer to *Onchidium* species (Dayrat et al. 2016), which are characterised by large dorsal conical papillae as well as penial hooks, among other features, and thus cannot be part of *Melayonchis*: *Onchidium typhae* Buchannan, 1800, type locality in Bengal, India; *Labella ajuthiae* (Labbé 1935), type locality in Thailand. Six species names refer to *Platevindex* species, which are characterised by a very narrow foot and a very flattened body, among other features, and thus cannot be part of *Melayonchis*: *Onchidium tigrinum* Stoliczka, 1869, type locality in the Ganges Delta, India; *Platevindex inspectabilis* (von Plate, 1893), type locality in Burma; *Platevindex coriaceum* (Semper 1885), type localities in Singapore and Malaysia (and Philippines and Australia); *Platevindex luteum* (Semper 1885), type locality in Singapore; *Platevindex martensi* (Semper 1885), type locality in Singapore (or Thailand); *Onchidella*
condoriana Rochebrune, 1882, type locality in Vietnam. Two species names refer to Peronina species, which are characterised by the anus located on the margin of the notum, among other features, and thus cannot be part of Melayonchis: Peronina alta von Plate, 1893 type locality in Chennai, India; Onchidium tenerum Stoliczka, 1869, type locality in the Ganges Delta, India. A few names are synonyms of Onchidium vaigiense Quoy and Gaimard 1824 (see, Dayrat 2010b), which differs from Melayonchis species by a male apparatus with penial hooks, among other things: Onchidium steenstrupii Semper, 1885, type locality in Nicobar Islands (and Micronesia and New Guinea); Onchidium ambiguum Semper, 1885, type locality in Singapore (and Palau). The names Onchidium tumidum Semper, 1885, type locality in Singapore, and Onchidium nangkauriense von Plate, 1893, type locality in Nicobar Islands, apply to species with no rectal gland, which, thus, cannot be part of Melayonchis. The last species name that needs to be commented on is Onchidium simrothi von Plate, 1893, type locality in Nicobar Islands. The two syntypes (ZMB 45,658) are empty, very poorly preserved body walls. No internal organs are left. No illustration accompanied the original description, which is not particularly informative because the colour of live animals is not described. von Plate did not describe the radular formula but he indicated that the pattern of intestinal loops is of type I, which is incompatible with the species described here. O. simrothi likely is a synonym of O. nangkauriense (with a type locality also in the Nicobar Islands; see above) and its status will be discussed in a future paper.

Three names are regarded here as nomina dubia: the type locality of Onchidium aberrans Semper, 1885 is mentioned as Singapore with a question mark and the type material is likely lost; Onchidella griseo-fusca Tapparone-Canefri, 1874, type locality in Singapore, cannot be linked to any of the 11 onchidiid species we found in Singapore because the original description is too brief and uninformative and the type material is likely lost; Onchidium harmandianum Rochebrune, 1882, type locality from Vietnam, cannot be linked to any of the nine onchidiid species we found in Vietnam because the original description is too brief and uninformative and the type specimen is likely not an onchidiid (it is a small piece of unidentified tissue).

Finally, there are two more species names which, even though it is still unclear what species they refer to, do not refer to any of the Melayonchis species described here. Onchidium pallidum Stoliczka, 1869, type locality in the Ganges Delta, India, is only known from the original description and no type material could be located. Even though we collected several onchidiids in the Ganges Delta, we do not seem to have found any species matching Onchidium pallidum. Its male copulatory apparatus includes both a penis and an accessory penial gland so it could only be a name for M. siongkiati (the accessory penial gland is absent in the three other species described here). However, there are many striking differences between M. siongkiati and O. pallidum. The distinctive colour of live animals of O. pallidum (pale yellowish white dorsum with a longitudinal black, median line and transparent foot) was never observed in any of the specimens of M. siongkiati; the thick penis of about 20 mm long in O. pallidum is incompatible with the 4 mm long penial sheath protecting a 1 mm long penial papilla found in M. siongkiati. Finally, M. siongkiati was found in the Strait of Malacca but was found neither in the Andaman Islands nor in Bengal, where it would be expected to be if O. pallidum and M. siongkiati referred to the same species. Even though O. pallidum is not thought to refer to any of the species described here, however, it could belong to Melayonchis because the radular formula – $150 \times (250-1-250)$ – is compatible with
Melayonchis but not with Onchidium (Dayrat et al. 2016). Also, the fact that O. pallidum lacks the distinctive dorsal conical papillae that characterise the genus Onchidium strongly suggests that it does not belong to that genus.

Onchidium pallidipes Tapparone-Canefri, 1889, type locality in Moulmein, Burma, is only known from the type locality and three syntypes (which we examined). The written description, not accompanied by any illustration and based on preserved material, is hardly informative. One syntype, 10/5 mm (ZMB 47,190), is completely destroyed (it dried at some point). Another syntype, 12/9 mm (ZMH 27,467/1) is a completely immature specimen with no reproductive system (male or female). The third syntype, 15/12 mm (NMNH 127,328), not well preserved externally, was opened for the present study (to check). Its male apparatus includes an accessory penial gland with a hollow spine that is much longer (3 mm) than the hollow spine found in M. siongkiati (maximum observed 0.9 mm). Also, the pattern of the intestinal loops of O. pallidipes (intermediary type between types II and III, as in M. eloisa) is different from the pattern of intestinal loops of M. siongkiati (type III). Future fresh material from Burma may help determine what O. pallidipes really is. We cannot exclude the possibility that it belongs to Melayonchis, and it could even be a synonym of O. pallidum (both species were named after their white foot) because the notum of the syntype from the NMNH (poorly preserved but lacking the dorsal papillae found in Onchidium) indicates that it does not belong to Onchidium.

Individual variation of important morphological features

The present contribution also is an opportunity to review the variation of morphological characters within species and among closely related species. A table at the end of the introduction summarises the most important features that can help distinguish and identify Melayonchis species (Table 3). Colour variation is very high within each Melayonchis species. However, the patterns of dorsal colour do not overlap between species and, therefore, species can still be identified, in spite of their high intraspecific colour variation. For instance, M. aileenae and M. eloisa can easily be identified, even though their intraspecific dorsal colour is highly variable. It is worth pointing out that there are other genera of onchidiids, such as Peronia and Platevindex, in which species can hardly be identified externally because the intraspecific colour variation overlaps between species.

In the digestive system, the shape of the radular teeth is very similar among all four Melayonchis species, and the radular teeth of Melayonchis differ from the teeth of all the other onchidiids we have examined thus far. Indeed, the protuberance on the inner lateral margin of each lateral tooth is a distinctive feature of Melayonchis. As a result, based on the radula alone, it would be possible to identify an onchidiid as a Melayonchis, but it would not be possible to identify it as a particular species within Melayonchis. As expected, the number of teeth per half row varies greatly within each species, mostly depending on the animal length. Also, the number of teeth per half row is extremely high, especially in M. aileenae, with up to nearly 400 teeth per half row. For comparison, in Onchidium, the number of teeth per half row remains under 100 (Dayrat et al. 2016). In Melayonchis, the pattern of intestinal loops does not vary intraspecifically but three types of intestinal loops are found in the genus: types II and III, and a type intermediate
between types II and III (which von Plate and Labbé did not encounter). It is very similar to what was observed in Onchidiunm (Dayrat et al. 2016). A rectal gland is present in all the individuals of all four Melayonchis species, which was also observed in Onchidiunm (Dayrat et al. 2016).

In the reproductive system, the hermaphroditic (mostly female) posterior parts vary greatly intraspecifically and look quite similar between species. However, the shape of the receptaculum seminis and the shape of the spermatheca seem to differ between species. In the male parts, an accessory penial gland can be present or absent between species (M. siongkiati is the only species with an accessory penial gland) but it does not vary intraspecifically (an accessory penial gland is found in all individuals of M. siongkiati and is absent in all the individuals of the three other species). Finally, not every species could be identified based on the penis alone. The penis of M. eloisae is quite distinctive, but those of M. annae and M. aileenae are quite similar.

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