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Bringing the lab to the field: a new lowland *Microparmarion* semi-slug (Gastropoda: Ariophantidae) described and DNA-barcoded in the forest

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ABSTRACT

Cybertaxonomy and portable DNA sequencing now make it possible for citizen scientists to be engaged in the discovery and description of new taxa. We here provide a proof of principle. A new semi-slug, *Microparmarion exquadratus* n. sp. (Ariophantidae) was discovered during a field course in tropical biology in Borneo for citizen scientists. The new species is the first lowland representative of its genus. It differs from other (high-elevation) *Microparmarion* species by its size, pigmentation on head and tail, and shape of dart sac and receptaculum. As part of the course programme, the participants prepared a taxonomic description and illustrations, and used the mobile genomic laboratory of the course to obtain DNA barcodes. As far as we are aware, this is the first time a new invertebrate species has been described both morphologically and genetically from the field.

INTRODUCTION

Taxonomy is currently in a state of flux, with methods and aims rapidly evolving as technological and sociological conditions change. Some of the recent developments include (1) digitization of historical literature (Gwinn & Rinaldo, 2009); (2) portable laboratories that can generate DNA sequences in the field (Menegon *et al.*, 2017) and (3) a growing involvement of citizen scientists (Schilthuizen *et al.*, 2017). Whereas, traditionally, only sampling and preliminary curation of specimens was done in the field, these new developments allow for a larger part of the taxonomic process also to take place on-site. We apply this concept in so-called 'taxon expeditions', where taxonomic experts guide untrained ecotourists in the discovery and description of new species (Schilthuizen *et al.*, 2017) during a field course set in a biodiversity hotspot. By using portable equipment and a streamlined process, the entire process can be finished within a two-week field course. Using this approach, we hereby describe a new species of the semi-slug genus *Microparmarion* Simroth, 1893.

Microparmarion is a genus of stout-bodied semi-slugs, belonging to the subfamily Ostracolethinae in the family Ariophantidae. It is characterized by a short tail and a relatively large shell. The genus is distributed across Southeast Asia and three species are known to occur on Borneo: *M. litteratus* Schepman, 1896, *M. pollonerai* Collinge & Godwin Austen, 1895 and *M. simrothi* Collinge & Godwin Austen, 1895 (Collinge & Godwin Austen, 1895; Schepman, 1896; Laidlaw, 1937; http://borneanlandsnails.myspecies.info/taxonomy/term/421). All were described in the late 19th century and, although specimens have been found that appear not to be conspecific with any of the known Bornean species (Schilthuizen & Liew, 2008; Schilthuizen, 2017), no additional species have been described from the island since then. Here, we add a fourth Bornean species that was discovered during a taxon expedition to Tawau Hills Park, a primary forest on volcanic soil in Sabah in the northeast of Borneo (Fig. 1). While all other Bornean *Microparmarion* species occur at high elevations, this new species was found in a lowland habitat.

MATERIAL AND METHODS

Tawau Hills Park is an area of chiefly lowland dipterocarp forest on volcanic soil and covers an area of 280 km^2. We sampled at night on 6 and 7 March 2018, along a 500-m long stretch of the main trail near the park headquarters (centred at 4°23.959′N, 117°53.358′E; *c.* 240 m asl) during dry weather. Slugs were encountered singly on the lower (and sometimes upper) leaf surfaces of trees and herbs along the trail (e.g. birdsnest ferns and Acanthaceae), and usually between 0.5 and 1.5 m above the ground. Several living individuals were photographed in the field

Figure 1. Map of Borneo, showing Malaysian state of Sabah and Tawau Hills Park.

and in the laboratory. In total, we found ten individuals, six of which were large; one of these was dissected and was found to possess well developed genitalia. These six are therefore considered adults. The remaining four were smaller and dissection of one of them revealed thin and poorly developed genitalia; these are considered subadults. These latter four were subsampled for DNA extraction and preserved in absolute ethanol. The adult specimens were either preserved directly in 70% ethanol, or first relaxed overnight in water and then transferred to 70% ethanol. One of these relaxed specimens was dissected. Its genitalia were fixed in pure ethanol, studied, photographed and finally cleared in clove oil, which allowed visualization of the internal structures. In describing the genitalia we use the terms 'proximal' and 'distal' in relation to the genital atrium. All specimens were deposited in the collection of Sabah Parks, Kinabalu Park, Sabah, Malaysia (SP). Images and descriptions were uploaded to http://borneanlandsnails.myspecies.info.

Tissue samples from three individuals (SP13552–SP13554) were subjected to DNA analysis with a portable field lab, as described previously (Menegon *et al.*, 2017). Briefly, DNA was extracted from a 1-mm^3 fragment of foot tissue using the DNeasy Blood & Tissue Kit (Qiagen) and then used as template in a PCR to amplify the COI barcoding region using specific primers (Folmer *et al.*, 1994), tailed at the 5' end with Oxford Nanopore adapters. After purification with AMPureXP beads (Beckman Coulter), each amplicon was indexed using the PCR-barcoding kit (Oxford Nanopore Technologies). DNA libraries were prepared from pools of 12 amplicons with the Ligation Sequencing Kit 1D (Oxford Nanopore Technologies) and sequenced on a MinION device (Oxford Nanopore Technologies) with a R9.4 flowcell, producing a minimum of 30,000 pass reads per sample. Demultiplexing and basecalling were performed by ONT Albacore Sequencing Pipeline Software (v. 2.2.2) and reads were analysed with the 'ONtoBAR' pipeline as described by Menegon *et al.* (2017). The sequences were uploaded to the Barcoding of Life Database (BOLD; www.boldsystems.com). To investigate phylogenetic relationships with other *Microparmarion* species, we obtained congeneric sequences of the cytochrome *c* oxidase subunit I (*COI*) barcoding region from BOLD and GenBank, as well as sequences of the related genus *Parmarion* Fischer, 1856, to serve as outgroup. Sequences were aligned in Geneious v. 11.1.3 using the MAFFT algorithm. The alignment was subjected to a Bayesian analysis with the GTR+gamma evolution model using MrBayes run from within Geneious (Huelsenbeck & Ronquist, 2001). We used the following MCMC settings: chain length = 1.1 million generations; number of heated chains = 4; subsampling frequency = one tree for each 200 generations, with the first 100,000 discarded as burn-in; chain temperature = 0.2.

SYSTEMATIC DESCRIPTION

Microparmarion exquadratus Schilthuizen *et al.*, new species

(Figs 2–4)

Types: Holotype: adult in 70% ethanol (SP13559). Paratypes: 1 subadult (SP13551), dissected and subsampled for DNA, in 70% ethanol; 3 subadults (SP13552–SP13554) in 100% ethanol, subsampled for DNA; 4 adults (SP13555–SP13557, SP13561) in 70% ethanol; 1 adult SP13560 dissected, in 70% ethanol. All material from vicinity of park headquarters, Tawau Hills Park, Sabah, Malaysia, 4°23.959′N, 117°53.358′ E; 240 m asl, collected at night, 6–7 March 2018; leg. taxon expedition participants.

Zoobank registration: http://zoobank.org/B64843A4-7A74-4D07-AD96-96C160E6D80B

Etymology: The name was chosen at a naming and voting session during the taxon expedition and refers to the 'squad' of participants that jointly sampled this species during night-time walks in the forest. The taxonomic authority for this species is attributed to all authors of this paper. Following Article 51 C of the Code (ICZN, 1999), the species can be referred to as *Microparmarion exquadratus* Schilthuizen *et al.*, 2018, provided the full citation of this publication appears in the bibliography or elsewhere in the referring work.

Diagnosis: Among *Microparmarion* species of Borneo, *M. exquadratus* is characterized by small size (less than half the size of *M. pollonerai* and *M. simrothi*), three dark longitudinal stripes on head (shared with *M. simrothi*, but lacking in *M. pollonerai* and *M. litteratus*), dark dorsal stripe on tail, kink in dart sac (shared with *M. pollonerai*) and reduced receptaculum.

Description: Exterior in adult, preserved specimens (Fig. 2A–D): Body length 18–21 mm. Shell 8.5–9 mm long, 5 mm wide. Body dull, greyish pink, 3 black dorsal longitudinal stripes, separated by 2 white stripes starting immediately caudal of black eye tentacles and ending ventrally of frontal edge of mantle. Lateral black stripes are half breadth of dorsal stripe. Mantle may also be dull greyish pink, with dark grey mottling. Keeled tail approximately one-third total body length (5–6 mm in adults), with 1 black dorsal stripe. Margin of foot has vertical, dark grey striated pattern. Sole of foot off-white. Tail with caudal horn, not very prominent. Shell semi-transparent, smooth, thin, pale yellow, with faint trace of spiral on right side, approximately 2 whorls. Genital opening visible on right side as short vertical slit. Dark pattern on mantle of 2, nearly continuous lines encircling shell, which are separated by pale greyish-pink ridge with about 10 dark spots.

Live specimens (Fig. 2E–J): Colour dull orange with deep brown pattern. Mantle lobes meet along curved suture, bulging to left, lying left of midline of body. Shell lobes with slight granulation. Frontal part of mantle with slight longitudinal ridges. Caudal horn

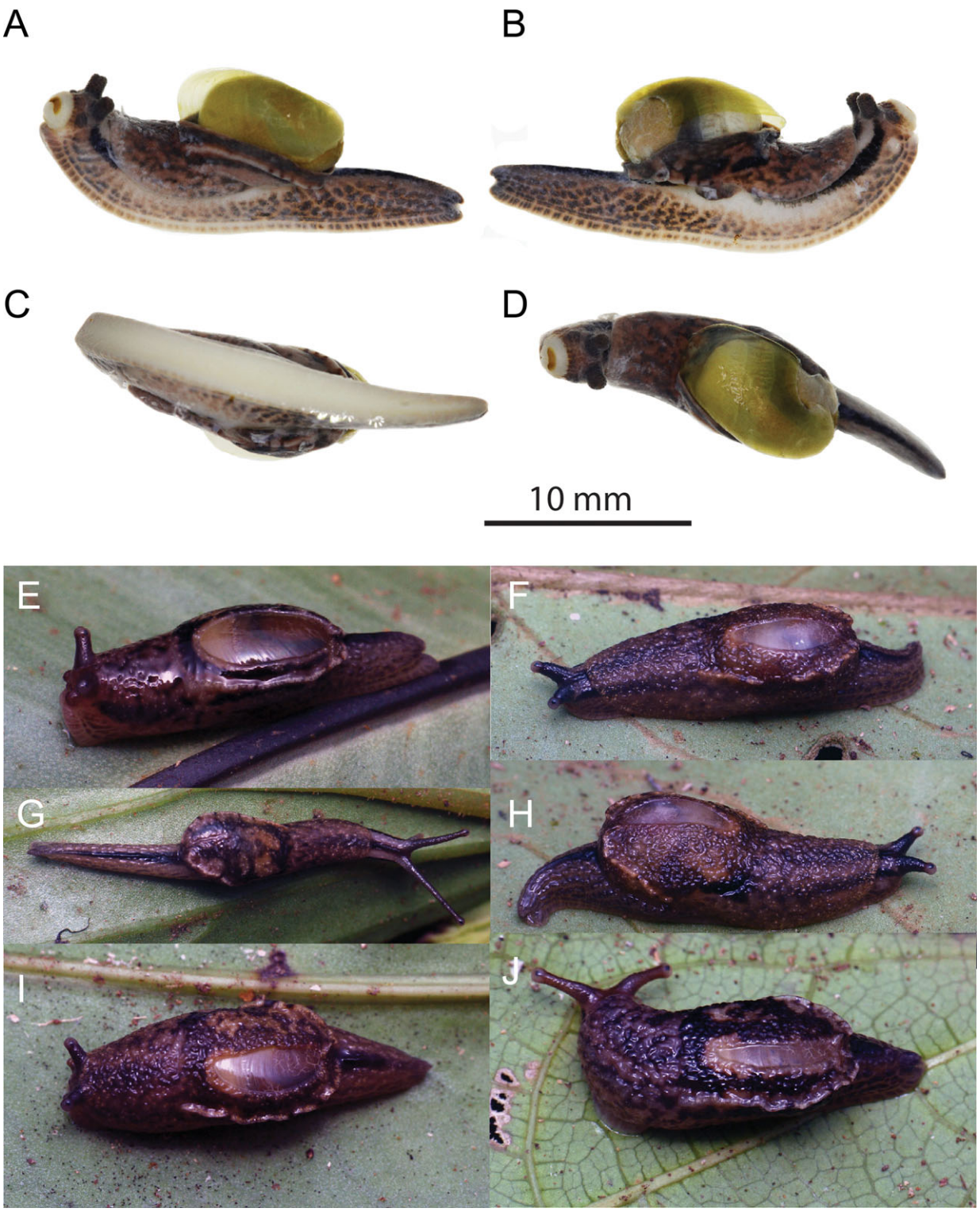

Figure 2. External morphology of (presumably) adult *Microparmarion exquadratus* n. sp. A preserved individual (syntype SP.13561) showing left lateral (**A**), right lateral (**B**), ventral (**C**) and dorsal (**D**) views. Six of the syntypes (**E–J**) in living state as photographed in the field in Tawau Hills Park, Sabah.

triangular, slightly longer than wide, pointing caudally. Eye tentacles, when fully extended, as long as shell.

Genitalia (Figs 3, 4): Atrium wide. Penis doubly folded into Z-shape, proximally narrow (one-quarter width of atrium), gradually doubling in width, then narrowing to original width distally. Penis sharply folding back on itself as a very narrow tube until half length of proximal part of penis, then folding, broadening, eventually tapering into vas deferens. When following the vas deferens from where it departs from penis, it remains detached from free oviduct until level with middle of dart sac. Vagina short, receptaculum reduced, externally invisible, only discernible after clearing in clove oil. Free oviduct irregularly looped and coiled. Dart sac long, equivalent to combined length of vagina and free oviduct, doubly folded into a Z-shape. Basal portion of dart sac is

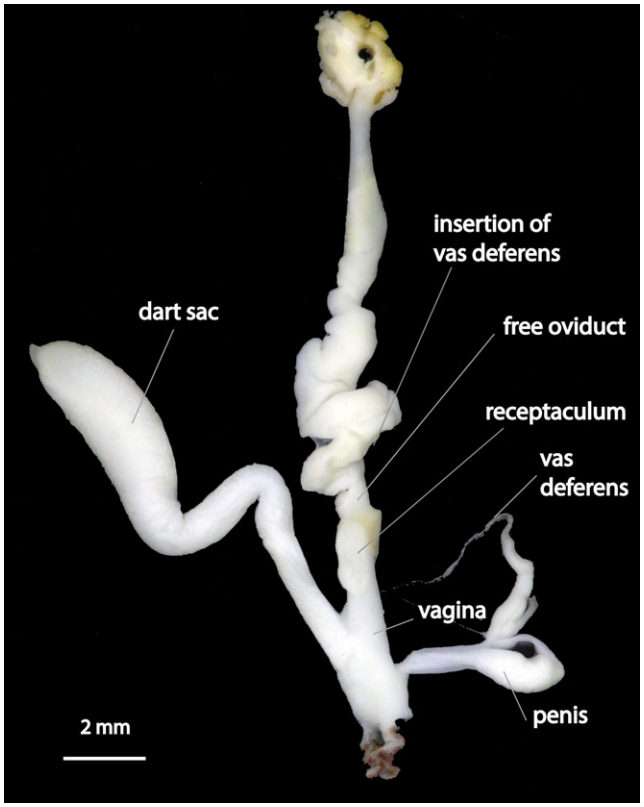

Figure 3. Genital system of *Microparmarion exquadratus* n. sp. (syntype SP13560).

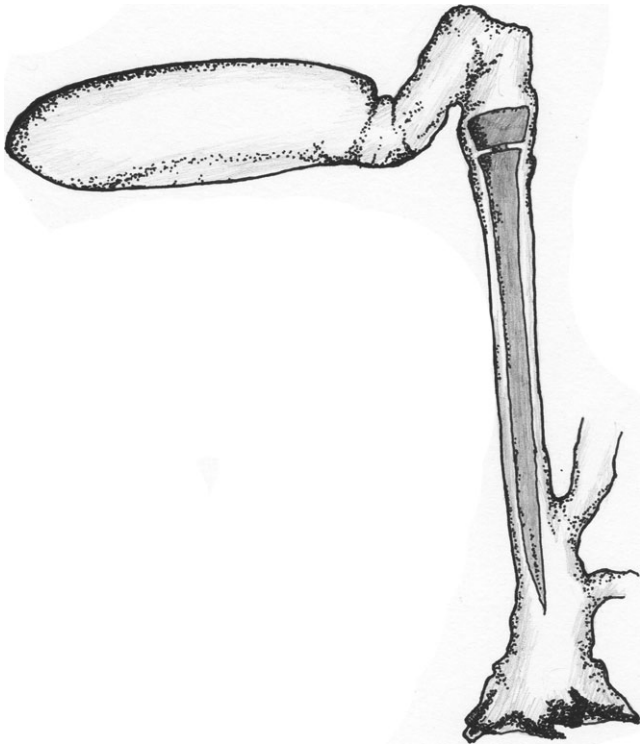

Figure 4. Dart sac of *Microparmarion exquadratus* n. sp. (syntype SP13560), cleared with clove oil and showing dart (shaded) in position.

narrow, containing the dart; middle portion half as long as basal portion, externally somewhat granulate; distal portion inflated, sausage-shaped, 3 times as long as wide, flattened on one side. Dart straight, with gently tapering point, possibly with several low, longitudinal flanges; crown twice as wide as dart.

DNA barcode: We obtained 3 sequences for the *COI* DNA-barcoding region. These sequences were identical, with the following exceptions when compared with the generated consensus (i.e. the most frequent nucleotide at each position among the three sequences). The sequence for SP13554 lacked a TA dinucleotide at the 5' end, which might be an artefact; we have replaced these nucleotides by unknown bases in the BOLD sequence. The sequence for SP13553 had an A instead of G at position 268, a T instead of C at position 628, and a C instead of T at position 637. The three sequences form a well-supported clade in the tree (Fig. 5), which is clearly separated (by >10% sequence divergence) from all other *Microparmarion COI* sequences available (all from Sabah as well). Sequences for *M. litteratus* were not available. *Microparmarion exquadratus* has been assigned BIN ADM3588.

Distribution: So far only known from lowland dipterocarp forest around the headquarters of Tawau Hills Park (*c.* 240 m asl). It is surprising to find a *Microparmarion* at such a low elevation, since the genus is normally found in montane forest—it is therefore also not sympatric with any other known *Microparmarion* species. There is a slight possibility that the population is nonindigenous to the lowlands, since the park's botanical garden (stocked with plants from elsewhere in the park, including the highlands) is nearby. The semi-slug may thus have been introduced from a higher-elevation habitat in the surrounding area. However, we failed to find the species on nearby Mount Lucia (1,300 m asl) and we also did not find it in the botanic garden itself, so we tentatively conclude that it is indigenous and possibly widespread throughout Tawau Hills Park. To confirm this, nocturnal sampling will have to be carried out in other parts of the park.

Remarks: In colour pattern, *M. exquadratus* is somewhat similar to a *Microparmarion* species found at an elevation of 1,200 m asl in Long Pa Sia, southwestern Sabah, and also to a species photographed (but not collected) at 1,000 m asl in Penrissen, Sarawak (Schilthuizen, 2017). However, it is unlikely to be conspecific with either, because it differs in colour pattern (the Penrissen specimen has two, rather than three, head stripes, and the Long Pa Sia specimen lacks the black markings on the mantle lobes) and is larger in size. For the time being, therefore, we consider the *M. exquadratus* to be restricted to Tawau Hills Park. Our phylogenetic reconstruction (Fig. 5), although primarily intended to assess the genetic distinctness of the new species, also suggests that the genus *Microparmarion* consists of multiple clades that are not fully congruent with named species. We therefore recommend that the genus be subjected to a formal revision.

We must also stress that our work was conducted under several limitations and constraints. It was part of a full 10-day field course schedule, carried out by untrained citizen scientists in a field station with limited equipment and erratic electricity supply. Specimens could not be exported. Consequently, the extent of our work is less than is customary for descriptions of new semi-slug species: we sequenced only three individuals and dissected only one. Also, all individuals were obtained from a very small geographic area. Nonetheless, the genetic and morphological features are sufficient to recognize the species unambiguously.

DISCUSSION

This paper shows that it is possible to carry out small, but meaningful, primary-taxonomic descriptive projects in the context of a field course for citizen scientists. In this particular case, the new

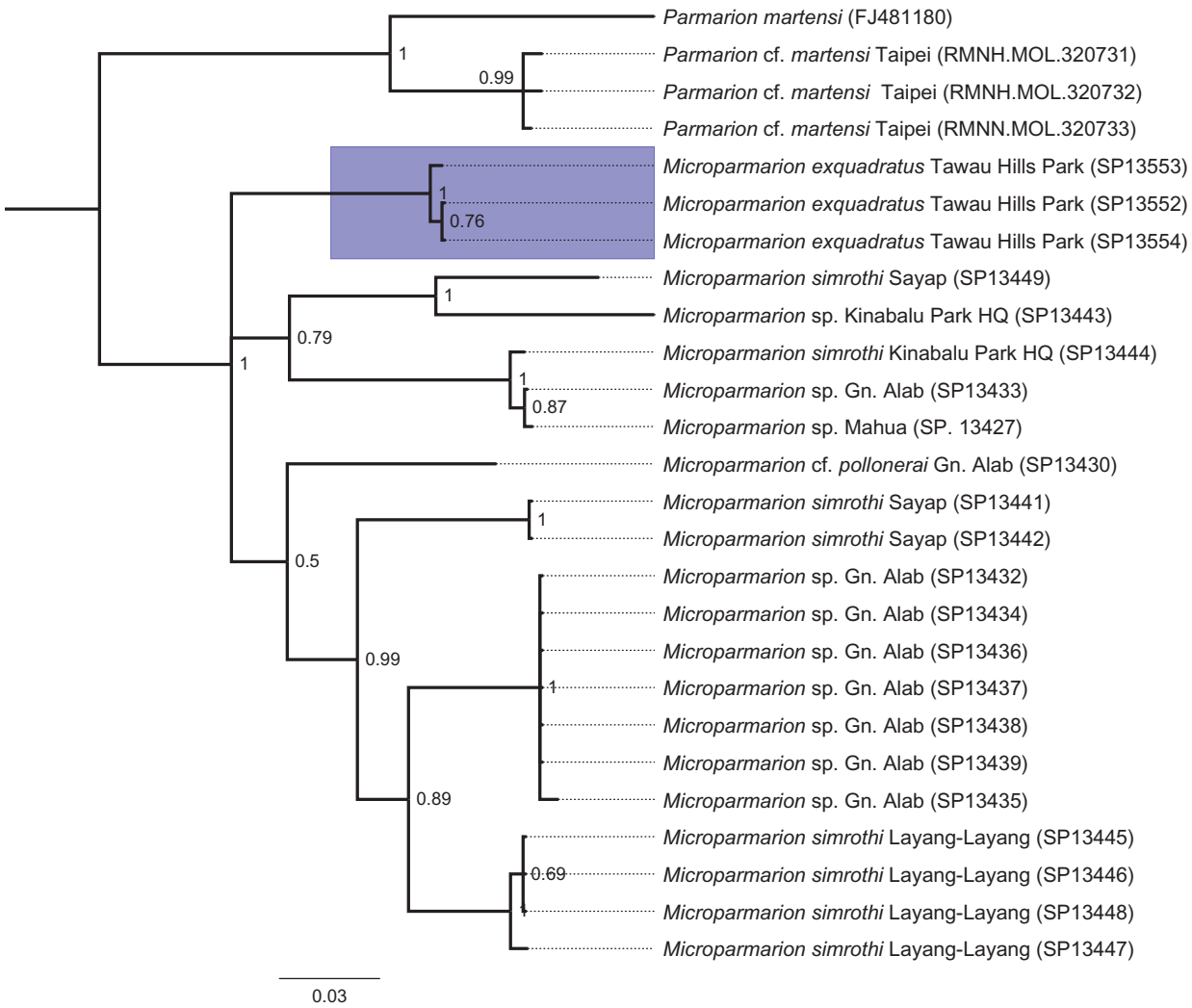

Figure 5. Phylogenetic reconstruction for Bornean *Microparmarion* based on *COI* barcode locus, with *Parmarion* as outgroup. Numbers on nodes are Bayesian posterior probabilities. Registration numbers in parentheses: RMNH, Naturalis Biodiversity Center, Leiden, The Netherlands; SP, Sabah Parks collection; FJ481180 is a GenBank accession number. Scale bar indicates substitutions per site.

species was, in fact, a surprise discovery by one of the untrained participants during a night walk. As digital copies of the relevant publications and images of reference specimens were present in the field laboratory, the discovery could be confirmed almost straightaway as a new species by the specialist. By a concerted effort, the group of participants was able to collect sufficient specimens for the new species to be described reliably. This way of working is possible because taxonomic experts can now draw on a range of different resources (digital libraries, field microscopes, portable macro-photography equipment and mobile genomic laboratories), while still in the field. Several other recent papers have used a similar approach. For example, all participants on a field course for MSc students in Borneo jointly described a new species of the spider genus *Crassignatha* (Miller *et al.*, 2014) as well as the male of the spider *Opadometa sarawakensis* (Miller *et al.*, 2018). Several participants in a citizen-scientist taxon expedition described three new Coleoptera species from the Maliau Basin of Sabah (Schilthuizen *et al.*, 2017). The present paper is, however, the first also to include in-the-field DNA sequencing. Recent studies using small, portable DNA sequencers (Menegon *et al.*, 2017; Parker *et al.*, 2017) indicate that on-site DNA sequencing will soon become a standard component of any field expedition, allowing rapid documentation of species and excluding the necessity of exporting material from the country of origin. We therefore expect that the coming years will see an increase of on-site DNA barcoding and species discovery in the context of field courses.

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