

First record and description of three new species in the land snail genus *Diplommatina* Benson, 1849 (Caenogastropoda, Diplommatinidae) from Satun Province, Thailand

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Abstract

The micro land snail genus *Diplommatina* (family Diplommatinidae) is widely distributed in Southeast Asia and includes many endemic species. Three new species of *Diplommatina* are described from Satun Province in southern Thailand. *Diplommatina bulonensis* **sp. nov.**, *D. laemsonensis* **sp. nov.** and *D. prakaiphensis* **sp. nov.** are distinguished from other species in the genus by their shell size and shape, the number of radial ribs on the penultimate whorl, the number of whorls, and features of the peristome. The agreement between phylogenetic tree based on analyses of COI and 16S sequences and comparative morphology support the delineation of these new species which, when compared to related species, belong to well-differentiated clades. The K2P distance between any of the three new species and other *Diplommatina* species included in the molecular phylogenetic analysis was at least 5.5% in COI and 3.9% in 16S. Two of the three new species (*D. prakaiphensis* **sp. nov.** and *D. bulonensis* **sp. nov.**) are apparently endemic to Prakaiph Hill and Bulon Pai Island, respectively. Additionally, we documented a new regional record for *D. naiyanetri* in Satun Province. These new species and records contribute to the knowledge of Thailand's land snail biodiversity and highlight the need of conservation protections for regional karst habitats.

Key Words

endemic, limestone, micro land snail, phylogeny, taxonomy

Introduction

Southern Thailand is located in the transition zone between two major biodiversity hotspots, the Indo-Burma and Sundaland – both of which have numerous endemic species (Myers et al. 2000). Satun Province in southern Thailand is characterized by karst features, with limestone mountains, foothills, and coastal islands. Satun's limestone hills support high biodiversity and endemism in micro land snails (Panha and Burch 2005). The taxonomy of micro land snails in Thailand's other limestone

areas has been studied comprehensively over the previous two decades, resulting in descriptions of several new species (Panha and Burch 2005). However, Satun's microsnail fauna remains largely unexplored.

Recent studies suggest that there are only five genera of micro land snails in Satun Province; namely, *Gyliotrachela* Tomlin, 1930, *Hypselostoma* Benson, 1856, *Aulacospira* Möllendorff, 1890, *Sinoennea* Kobelt, 1904 and *Diplommatina* Benson, 1849 (Panha and Burch 2005; Dumrongrojwattana and Womgkamhaeng 2013; Dumrongrojwattana and Tanmuangpak 2020). Among these

five genera, the genus *Diplommatina* is very diverse and is widely distributed in Asia; including India, Myanmar, Laos, Thailand, Malaysia, Indonesia, Japan and northern Australia (Kobelt 1902; Thiele 1929; Wenz 1939; Vermeulen 1993). The large number of species are usually diagnosed solely by shell characters (Neubert and Bouchet 2015). The shells of *Diplommatina* are generally conical in shape, with radial ribs, a lipped aperture, and an operculum (Kobelt 1902; Panha and Burch 2005; Yamazaki et al. 2015; Nurinsiyah and Hausdorf 2017). Of the more than 400 described species of *Diplommatina* (Kobelt 1902; Thiele 1929; Wenz 1939; Vermeulen 1993), 22 species that are known to occur in Thailand and about 28 additional species are known from the neighboring countries of Malaysia, Singapore and Indonesia (van Benthem Jutting 1959; Maassen 2001a; Maassen 2002; Panha and Burch 2005; Tongkerd et al. 2013; Foon et al. 2017; Inkhavilay et al. 2019; Dumrongrojwattana et al. 2020). Among the 10 species reported from southern Thailand (Maassen 2001b; Panha and Burch 2005; Tongkerd et al. 2013; Inkhavilay et al. 2019; Dumrongrojwattana et al. 2020), only a single species, *D. canaliculata*, has previously been documented in Satun province (Panha and Burch 2005).

The shell characters used to identify *Diplommatina* species (Kobelt 1902; Panha and Burch 2005; Yamazaki et al. 2015; Nurinsiyah and Hausdorf 2017) may display adaptation to environmental characteristics, including the presence of predators (Schilthuizen et al. 2006). As a consequence, morphological features may be confusing and molecular phylogenetic techniques can be powerful tools for resolving the taxonomy and additionally inform phylogenetic rela-

tionships among snail taxa (Douris et al. 1998; Chiba 1999; Thacker and Hadfield 2000; Holland and Hadfield 2002; Steinke et al. 2004; Desouky and Busais 2012). The DNA barcoding markers of cytochrome c oxidase subunit 1 mitochondrial gene (COI) and 16S ribosomal DNA (16S rDNA) have been used in discriminating land snail species, including those within Diplommatinidae, and to investigate phylogenetic relationships (Webster et al. 2012; Liew et al. 2014).

Within a larger study of the microsnails of karst areas in Saturn Province, we especially targeted the genus *Diplommatina*. After conducting an extensive field survey, we used both morphological and molecular analyses to identify known species and describe new species.

Materials and methods

Ethical statements

We followed the guidelines for animal care in the International Guiding Principles of Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences: CIOMS) including the relevant document (U1-03304-2559 to NN.).

Specimen sampling

Karst areas throughout Satun Province, Thailand (Fig. 1) were surveyed for land snails during the rainy seasons in December 2020 through January 2022. Specimens of

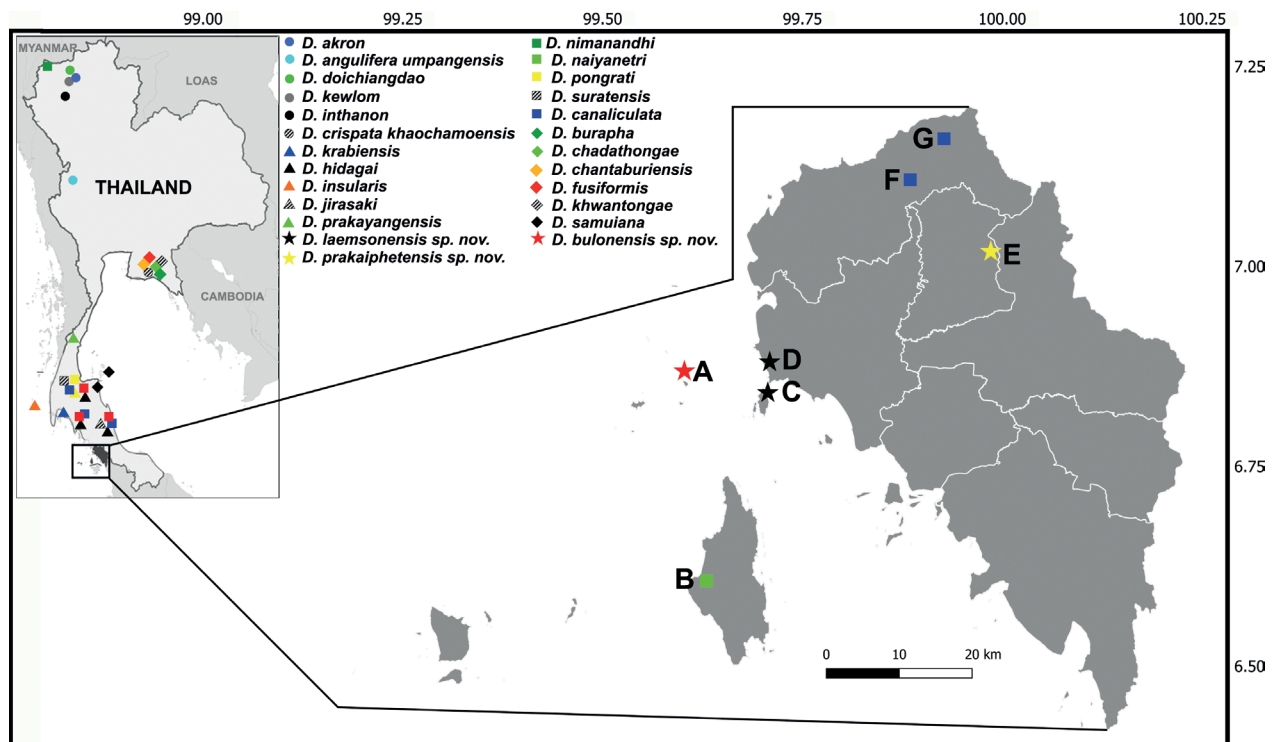


Figure 1. Localities of micro land snail sampling in Satun Province, Thailand. **A.** Bulon Pai Island; **B.** Talutao Island; **C.** Khao Yai Island; **D.** Laem Son Hill; **E.** Prakaiphet Hill; **F.** Tharn Pliew Waterfall; **G.** Phu Pha Phet Cave. The symbols without letters show previous records for different species of *Diplommatina* that are known from Thailand (Maassen 2001b; Panha and Burch 2005; Tongkerd et al. 2013; Inkhavilay et al. 2019; Dumrongrojwattana et al. 2020).

Diplommatina were collected by visual searching with one to three searchers and up to three hours of searching per site. Snails were found on soil surfaces, on limestone, stones, wood, moss and under leaf litter. Micro land snails were especially common in limestone crevices and on moist surfaces. Both live snails and empty shells were collected. All live specimens were drowned in water, preserved in 95% ethanol and processed in the Biology Department, Faculty of Science, in Chiang Mai University.

Morphological study

All *Diplommatina* specimens were identified by comparing shell morphology with type specimens and the original descriptions. Specimens of *Diplommatina* were examined using a Leica MZ16 microscope and photographs were taken using the Leica Application Suite Version 3.4.0 program. Shell morphological characteristics, including shell height and width and aperture height and width, were measured from digital images using ImageJ (Schneider et al. 2012). Shell morphological measurements and ratios among measurements (e.g., shell height to width) of each morphospecies were analyzed using ANOVA ($\alpha = 0.05$) using IBM SPSS Statistics ver. 22.0.

The outer wall of the body whorl of at least two shells of each species was removed to reveal the internal lamellar features (Vermeulen 1993; Panha and Burch 2005; Neubert and Bouchet 2015; Budha et al. 2017), which were photographed.

Shells were cleaned by using sonication prior to viewing with a scanning electron microscope (SEM). Dried shells were sputter-coated with gold and examined with a JSM 5910 LV scanning electron microscope at the Electron Microscope Research and Service, Faculty of Science, Chiang Mai University.

Using a dissecting microscope, the radulae of microsnails were extracted from the buccal cavity, cleared in 1% sodium hydroxide overnight and then washed with distilled water. Radulae were dehydrated by immersion in increasing alcohol concentrations (10%, 30%, 50%, 70%, 80% and 95%) (Nantararat et al. 2014). The dehydrated radulae were fixed onto SEM stubs with carbon tape and coated with gold (Franklin et al. 2007) and viewed on the scanning electron microscope.

Molecular study and phylogenetic analyses

This study included data from GenBank for fourteen species and three unidentified species of *Diplommatina* (totalling 20 records) (Rundell 2008; Webster et al. 2012), and twelve records of species from other genera in the family that were used as outgroups to root the tree (Rundell 2008; Webster et al. 2012). Sequences of ten individuals of *Diplommatina* spp. collected during our surveys were included in our molecular phylogenetic analysis.

The foot tissue of the ethanol-preserved snails was removed for DNA extraction. The samples were incu-

bated with 150 μ l Chelex 100 and 3 μ l Proteinase K for 1 hour at 55 °C, followed by 30 minutes at 95 °C (de Lamballerie et al. 1992). All DNA samples were stored at -20 °C for later use. The COI gene was amplified using primers LCO1490 (5'-GGTCAACAA ATCATA AAGATATTGG -3') and HCO2198 (5'-TAAACTTCAGGGT-GACCAAAA AATCA -3') (Folmer et al. 1994). PCR reactions were performed with cycle parameters of 94 °C for 3 min, followed by 36 cycles of 94 °C for 30 s, 50 °C for 60 s, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The 16S rRNA gene was amplified by using primers 16Sar (5'-CGCCTGTTTATCAAAAA-CAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGAT-CACGT-3') (Palumbi 1996). PCR amplification was performed at 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 45 °C for 1 min, and 72 °C for 2 min, and then a final 72 °C for 5 min. Primer PCR products were checked using 1% (w/v) agarose gel electrophoresis with 1X TBE buffer. Purification and sequencing were performed using Barcode-Tagged Sequencing (BTSeq) Services. After sequencing, the sequences were proofread on chromatograms, and the coding was aligned using Clustal W (Thompson et al. 1994) and then manually edited with MEGA X (Kumar et al. 2018). A total of 42 sequences were used in this study. New sequences have been deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank>) and are shown in Table 2. Phylogenetic trees were constructed using Maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood analyses were performed based on the TIM3+F+I+G4 model for the COI gene and GTR+F+I+G4 model for the 16S gene by using IQ-TREE on XSEDE and CIPRES (Miller et al. 2010; Nguyen et al. 2015) with 1000 bootstrap repeats (Hoang et al. 2018). The Bayesian Inference analysis was performed using MrBayes (Ronquist et al. 2012) version 3.2.7. Parameter settings were 4 nchains and 4 nruns in a Markov Chain Monte Carlo algorithm (MCMC). The BI analysis was performed for 10,000,000 generations, with a temperature parameter for heating = 0.8, sampled every 100 generations, and setting burn-in at 25% of the run (Ronquist and Huelsenbeck 2003).

Morphological abbreviations

AH	aperture height
AH/AW	aperture height/ aperture width
AW	aperture width
cd	columellar denticle
col	columellaris
con	constriction
N1	ribs/0.5 mm on the penultimate whorl
N2	total ribs on the penultimate whorl
prt	parietalis
SH	shell height
SH/SW	shell height/ shell width
SW	shell width
tp	transversal palatalis
W	number of whorls.

Results

Land snails from 41 survey sites in Satun Province were identified based on morphological characters combined with molecular analysis. Five species of *Diplommatina* were found. One species, *D. canaliculata*, had previously been reported from Satun Province and a second species, *D. naiyanetri*, is a new provincial record and was found on Tarutao Island. The three remaining species are new species and are described below.

Systematics

Family Diplommatinidae Pfeiffer, 1856

Genus *Diplommatina* Benson, 1849

Type species. *Diplommatina folliculus* Pfeiffer, 1846.

Diplommatina bulonensis Boonmachai & Nantarat, sp. nov.

<https://zoobank.org/94CA70AE-54F1-4B9D-8604-29766AE5FB80>
Figs 2A, 3A–C

Type material. *Holotype* CMUZ 9050001 (Fig. 2A); Shell measurements: SH = 2.72 mm, SW = 1.27 mm, AH = 0.94 mm, AW = 0.91 mm, W = 6 1/2. *Paratypes* CMUZ 9050002–9050006 (5 shells); Shell measurements: SH = 2.58–2.82 mm, SW = 1.26–1.34 mm, AH = 0.97–1.04 mm, AW = 0.92–1.03 mm, W = 6 1/2–7 1/2.

Type locality. Thailand, Satun Province, La-ngu District, Bulon Pai Island on limestone rocks and under leaves, 6°49'53.3"N, 99°35'19.5"E, 22 December 2020, coll. T. Boonmachai.

Other material examined. Thailand, Satun Province; La-ngu District, Bulon Pai Island, 6°49'53.3"N, 99°35'19.5"E, 22 December 2020: CMUZ 9050007–9050016 (10 shells); La-ngu District, Bulon Pai Island, 6°49'53.3"N, 99°35'19.5"E, 15 February 2022: CMUZ 9050017–9050018 (2 shells).

Etymology. The specific epithet *bulonensis* is an adjective referring to the type locality (Bulon Pai Island in Satun Province, Thailand).

Differential diagnosis. Among approximately 400 species of *Diplommatina*, the new species is most similar to *D. conditioria* Maassen, 2007, *D. baliana* Fulton, 1899, *D. maduana* Laidlaw, 1949, *D. gomantongensis* Smith, 1894 and *D. antheae* Vermeulen, 1993 (Table 1). Typical characteristics shared by *D. bulonensis* with all these species are shell dextral with a slender fusiform shape with rather evenly rounded sides, radial ribs not sinuous, constriction situated halfway on the parietal side of the peristome and aperture round. However, *D. bulonensis* sp. nov. differs from *D. gomantongensis* and *D. antheae* by the presence of a transverse palatalis and an inconspicuous columellar denticle in the aperture (Fig. 4A). *D. bulonensis* sp. nov. dif-

fers in the combination of shell height, shell width, aperture height and aperture width from *D. maduana* and *D. antheae* (Table 1). *D. bulonensis* sp. nov. differs from *D. conditioria* and *D. maduana* in the number of whorls (Table 1) and differs from *D. maduana*, *D. gomantongensis*, *D. antheae* and *D. baliana* in the number of radial ribs/0.5 mm on the penultimate whorl (Table 1). *D. bulonensis* sp. nov. has fewer radial ribs on body whorls above the aperture than *D. conditioria* (12–13 vs. 16 ribs/1 mm) (Maassen 2007). Moreover, it differs from *D. baliana* in having a columella fold.

Description. Shell dextral, fusiform, convex and red rushes or red-orange in color (Figs 2A, 3A). Penultimate whorl slightly wider than body whorl, side flat. Spire conical with slightly convex sides. Suture impressed. Constriction level with the middle parietal side of the peristome, with three lamellae: one parietalis very distinct, one distinct and long transversal palatalis and one distinct columellaris. (Fig. 4A, B). Protoconch smooth with dimples and about 2 1/2 whorls (Fig. 3B). Radial ribs straight, inconspicuous but distinct, moderately spaced about 6 1/2–7 ribs/0.5 mm on the penultimate whorl (Table 1). The aperture slightly tilted against the coiling axis, columellar denticle slightly weak in the aperture. Peristome double, rather expanding, palatal side not sinuous, basal side and columella side weakly sinuous. Outer peristome expanded less than inner peristome, inner peristome expanded at parietalis side, with a palatal lip. Umbilicus closed. Operculum multispiral, flat, corneous, transparent, slightly concave, outer surface smooth, inner surface smooth with raised peripheral circular margin (Fig. 3C). The radula of the taenioglossate type (Fig. 5B). The central tooth round and flat with seven cusps of similar size. The basal plate of the central tooth, cusped and small. The lateral teeth with five cusps. The marginal teeth with inner and outer teeth, the inner teeth with seven small cusps and the outer teeth with five cusps and larger than the inner teeth (Fig. 5B).

Diplommatina laemsonensis Boonmachai & Nantarat, sp. nov.

<https://zoobank.org/A1F06C5D-F1EC-41FC-8345-D9BA7693D8E2>
Figs 2B, 3D–F

Type material. *Holotype* CMUZ 9050019 (Fig. 2B); Shell measurements: SH = 2.26 mm, SW = 1.17 mm, AH = 0.77 mm, AW = 0.83 mm, W = 7. *Paratypes* CMUZ 9050020–9050024 (5 shells); Shell measurements: SH = 2.14–2.49 mm, SW = 1.08–1.21 mm, AH = 0.76–0.91 mm, AW = 0.79–0.86 mm, W = 6 1/2–7.

Type locality. Thailand, Satun Province, La-ngu District, Limestone Hill, 6°54'43.7"N, 99°41'59.0"E, 25 December 2020, coll. T. Boonmachai.

Other material examined. Thailand, Satun Province; La-ngu District, Limestone Hill, 6°54'43.7"N, 99°41'59.0"E, 25 December 2020: CMUZ 9050025–9050047 (23 shells); La-ngu District, Khoa Yai Island, 6°83'08.6"N, 99°69'70.1"E, 21 December 2022: CMUZ 9050048–9050065 (18 shells).

Etymology. The specific epithet *laemsonensis* is an adjective referring to the type locality (Laem Son subdistrict, Lan-gu district, Satun Province, Thailand).

Differential diagnosis. Among the sinistral species of *Diplommatina*, *Diplommatina laemsonensis* sp. nov. is most similar to *D. diminuta* Möllendorff, 1891, *D. sinistra* Tomlin, 1938 and *D. acme* Laidlaw, 1949 (Table 1). All these species share a moderately slender fusiform shape, rounded periphery, shell height range and number of whorls (Table 1). However, *Diplommatina laemsonensis* differs from *D. acme*, *D. diminuta* and *D. sinistra* in the number of radial ribs in the penultimate whorl (Table 1). It differs from *D. diminuta* in shell width and the ratio of AH/AW (Table 1). Moreover, it differs from *D. diminuta* by the absence of teeth on the basal margin of the aperture and differs from *D. sinistra* in having a distinct columellar denticle in the aperture.

Description. Shell sinistral, fusiform, convex and honey brown in color (Figs 2B, 3D). The penultimate whorl widest. Spire conical with slightly convex sides. Suture impressed. Constriction level with the edge between the parietal and columellar side of the peristome, with three lamellae: one distinct parietalis which starts at the constriction, one transversal palatalis, one distinct columellaris which continues into the tuba and visible in the aperture (Fig. 4C, D). Protoconch sculpture smooth with fine pitting and about 1 whorl (Fig. 3E). Radial ribs straight, distinct, rather dense on top of the teleoconch, suddenly changing to a moderately spaced about 8–9 ribs/0.5 mm on the penultimate whorl (Table 1). The aperture tilted up to 30° in relation to the coiling axis, columellar denticle visible in the aperture, deflected downwards. Peristome double, expanding, palatal side rounded with slightly edge, basal side with edge slightly protruding. Outer peristome expands beyond the inner peristome with about four layers, inner peristome with distinct a palatal, basal and columellar lips. Umbilicus closed. Operculum multispiral, flat, corneous, transparent, slightly concave, outer surface smooth with small pits distributed over whole surface, inner surface smooth with raised peripheral circular margin (Fig. 3F). The radula of the taenioglossate type (Fig. 5A). Central tooth strong with a large central cusp, with 2 pairs of developed lateral cusps. Basal plate of central tooth prominent with 2 small cusps. The lateral teeth with six cusps, the third cusp longest. In the marginal teeth, the inner teeth larger than the outer, with six cusps, the third cusp longest. The outer tooth with four cusps (Fig. 5A).

***Diplommatina prakaiphetensis* Boonmachai & Nantarat, sp. nov.**

<https://zoobank.org/C468B471-1728-4C9C-ABC3-4C1530C644D8>

Figs 2C, 3G–I

Type material. *Holotype* CMUZ 9050066 (Fig. 2C); Shell measurements: SH = 1.76 mm, SW = 0.89 mm, AH = 0.66 mm, AW = 0.64 mm, W = 6 1/2. *Paratypes* CMUZ 9050067–9050071 (5 shells); Shell measurements:

SH = 1.62–1.75 mm, SW = 0.83–0.90 mm, AH = 0.59–0.69 mm, AW = 0.61–0.72 mm, W = 6–7.

Type locality. Thailand, Satun Province, Thung Wa District, Prakaiphet Hill, 7°00'00.1"N, 99°46'08.7"E, 19 January 2022, coll. T. Boonmachai.

Other material examined. Thailand, Satun Province, Thung Wa District, Prakaiphet Hill, 7°00'00.1"N, 99°46'08.7"E, 19 January 2022: CMUZ 9050072–9050088 (17 shells).

Etymology. The specific epithet *prakaiphetensis* is an adjective referring to the type locality (Prakaiphet Hill in Na Thon subdistrict, Thung Wa District, Satun Province, Thailand).

Differential diagnosis. Among sinistral diplommatinids, *Diplommatina prakaiphetensis* sp. nov. most closely resembles *D. krabiensis* Panha & J. B. Burch, 1998 from Thailand, and *D. karoensis* Maassen, 2002 from Sumatra, Indonesia as all these species share a minute size, an ovate shell shape with rather flat whorls, radial ribs being distinct and comparatively widely spaced. However, *D. prakaiphetensis* sp. nov. differs from both other species in the number of whorls (Table 1). It also differs from *D. karoensis* in the number of radial ribs/0.5 mm on the penultimate whorl and ratio of SH/SW (Table 1). Moreover, the absence of radial ribs on the body whorl on the parietal side and slightly wavy radial ribs distinguishes it from *D. krabiensis*.

Description. Shell sinistral, fusiform, thin, convex, color Aztec gold, (Figs 2C, 3G). Penultimate whorl width slightly smaller than the body whorl, suture impressed. Constriction level with the middle parietal side of the peristome, with three lamellae: one parietalis, one short transverse palatalis and one prominent columellaris that continues into the tuba and is visible in the aperture (Fig. 4E, F). Protoconch smooth, with very fine pits, about 1 1/4 whorl (Fig. 3H). Radial ribs are very fine and closely spaced on top of the teleoconch; the following ones are more widely spaced, and become prominent on third to last whorl and are wavy, inconspicuous or almost absent on the body whorl on the parietal side of peristome with about 3–4 ribs/0.5 mm on the penultimate whorl (Table 1). Aperture rounded, columellar denticle distinct in the aperture. Peristome double and expanding. The outer peristome expanding beyond the inner, with palatal and columellar side protruding, basal side not protruding, inner peristome expanding with a weak parietal lip. Umbilicus closed. Operculum multispiral, flat, corneous, transparent, slightly concave, outer surface smooth, inner surface smooth with a large ridge and raised peripheral circular edge (Fig. 3I). The radula of the taenioglossate type (Fig. 5C). The central tooth strong and pointed cusps with a large central cusp, two pairs of developed lateral cusps. The basal plate of the central tooth prominent with two small cusps. The lateral teeth with five cusps; the longest is the third cusp. In the marginal teeth, the inner teeth larger than the outer, with four cusps, and the longest is the second cusp. The outer marginal teeth with three cusps (Fig. 5C).

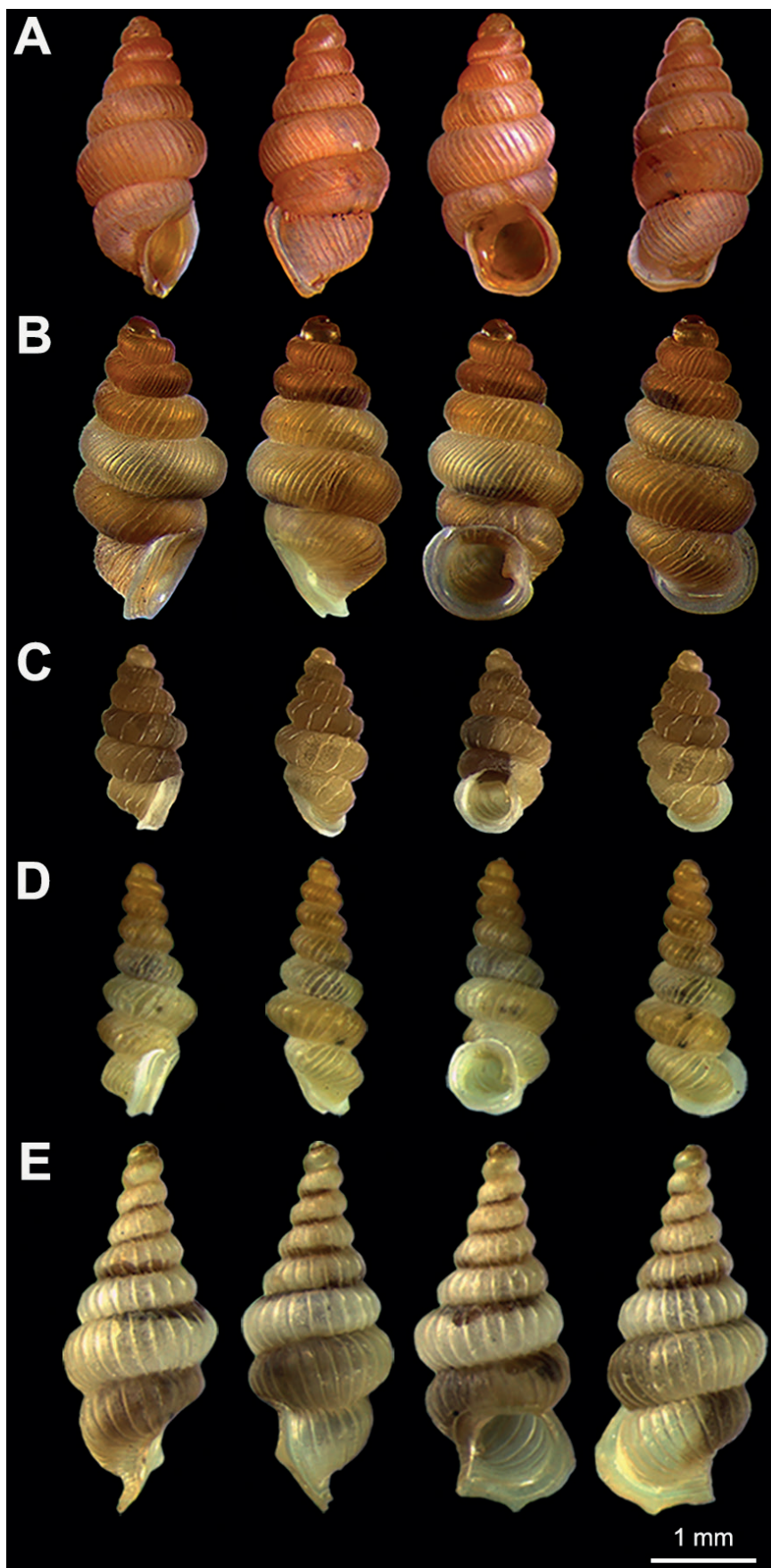


Figure 2. Shell morphology of *Diplommatina* from Satun Province. **A.** *D. bulonensis* sp. nov. (Holotype CMUZ 9050001; Bulon Pai Island); **B.** *D. laemsonensis* sp. nov. (Holotype CMUZ 9050019; Laem Son Hill); **C.** *D. prakaiphetensis* sp. nov. (Holotype CMUZ 9050066; Prakaiphet Hill); **D.** *D. naiyanetri* (CMUZ 905090; Tarutao Island); **E.** *D. canaliculata* (CMUZ 9050158; Tharn Pliew Waterfall).

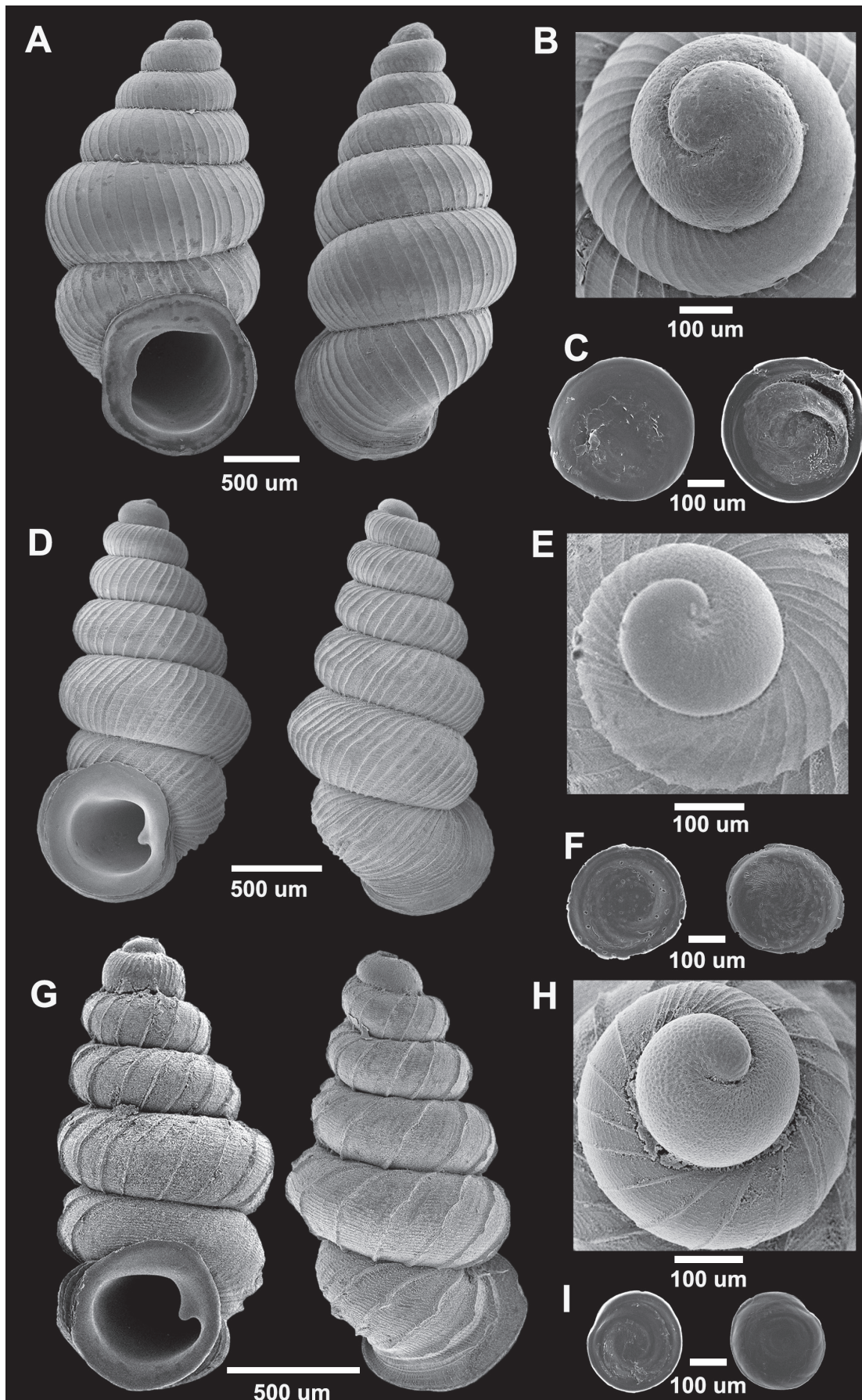


Figure 3. Scanning electron microscope of shell morphology, protoconch and operculum of *Diplomatina* new species. **A–C.** *D. bulonensis* sp. nov. (Bulon Pai Island); **D–F.** *D. laemsonensis* sp. nov. (Laem Son Hill); **G–I.** *D. prakaiphensis* sp. nov. (Prakaiphet Hill).

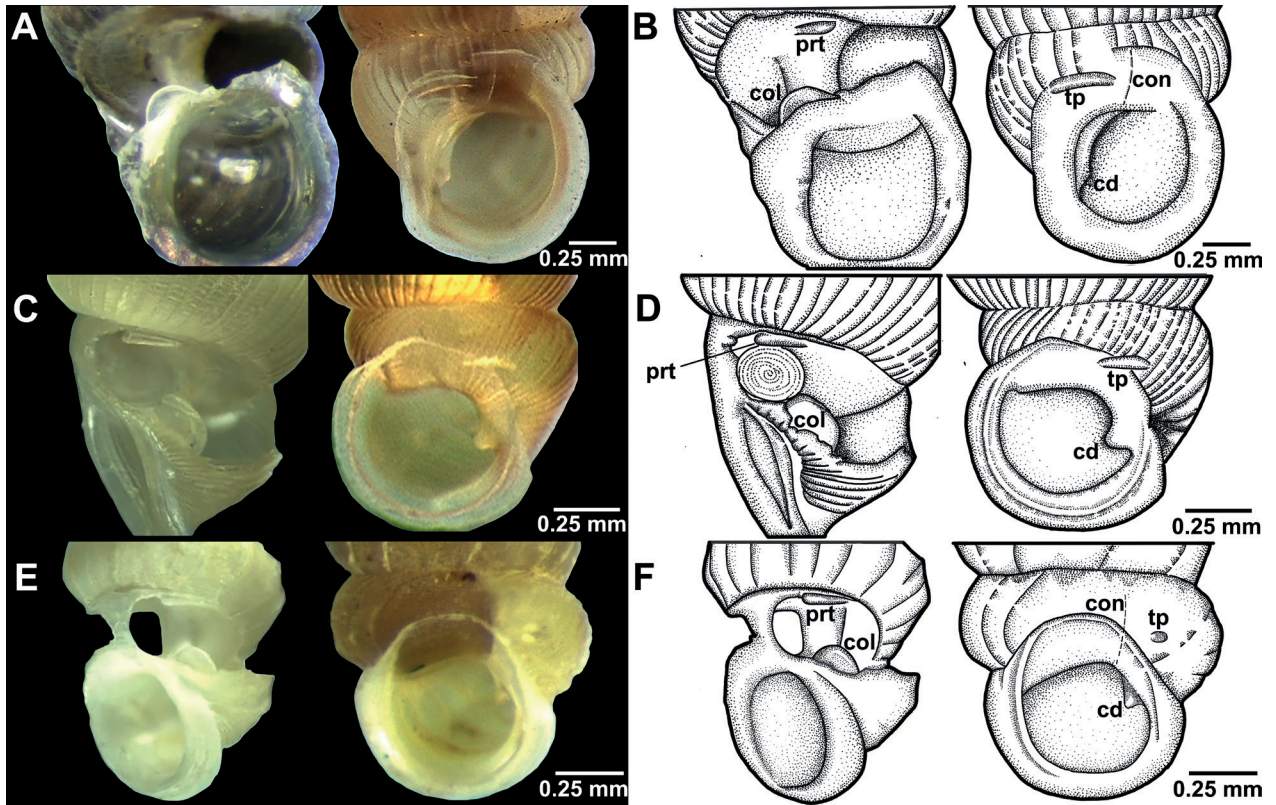


Figure 4. Shell internal terminology of new species genus *Diplommatina* from Satun Province under the stereo microscope and the drawing. **A, B.** *D. bulonensis* sp. nov. (Bulon Pai Island); **C, D.** *D. laemsonensis* sp. nov. (Laem Son Hill); **E, F.** *D. prakaiphensis* sp. nov. (Prakaiph Hill).

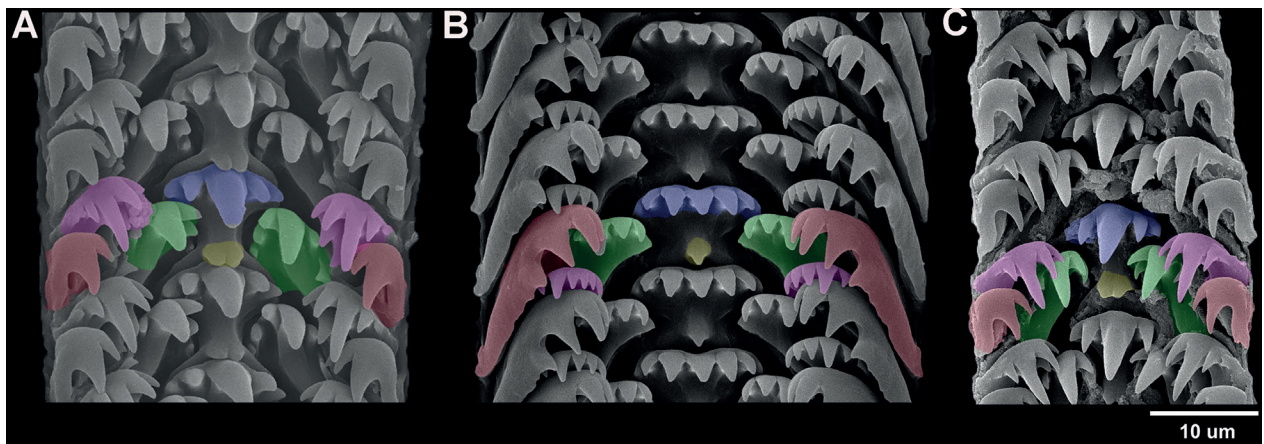


Figure 5. Radular morphology of *Diplommatina*. **A.** *Diplommatina laemsonensis* sp. nov. (Laem Son Hill); **B.** *Diplommatina bulonensis* sp. nov. (Bulon Pai Island); **C.** *Diplommatina prakaiphensis* sp. nov. (Prakaiph Hill). Colours show the position of the teeth of the radula; blue: central tooth, yellow: the basal plate of the central tooth, green: lateral teeth, pink: inner marginal teeth, and red: outer marginal teeth.

Molecular phylogenetic analyses

Sequences from a total of 30 individuals of *Diplommatina* spp. and 12 individuals from four additional diplommatinid genera (*Hungerfordia* Beddome, 1889, *Palaina* Semper, 1865, *Opisthoporus* Manter, 1947 and *Opisthostoma* W. T. Blanford & H. F. Blanford, 1860; the out groups) were used for phylogenetic reconstruction (Table 2). The concatenated alignment of COI and

16S sequences had a total length of 996 base pairs (COI = 594, 16S = 402). The phylogenetic tree based on the analyses of the concatenated COI and 16S sequences supported *Diplommatina* as a monophyletic group. Within *Diplommatina*, the phylogenetic tree was divided into three main clades. Clade A was strongly supported and included all dextral species from Boneo, Peninsular Malaysia, and southern Thailand (Fig. 6). Within this clade, *D. bulonensis* sp. nov. formed the sister group contain-

Table 1. Comparison of shell morphological characters of closely related *Diplommatina* species with new species. Shell morphological characters were tested using one-way ANOVA ($P < 0.05$).

Species	Characters (mm)								
	(SH)	(SW)	(AH)	(AW)	SH/SW	AH/AW	W	N1	N2
<i>D. bulonensis</i> sp. nov.	2.58–2.82 (2.71±0.08)*	1.26–1.34 (1.31±0.04)*	0.94–1.04 (1.00±0.03)*	0.91–1.03 (0.97±0.04)*	1.93–2.15 (2.07±0.07)	0.96–1.10 (1.03±0.05)*	6 1/2–7 (6.95±0.27)	6–7 (6.33±0.52)*	44–51 (47.33±3.51)*
<i>D. laemsonensis</i> sp. nov.	2.14–2.49 (2.34±0.10)*	1.08–1.21 (1.15±0.04)*	0.76–0.91 (0.82±0.05)*	0.79–0.86 (0.81±0.02)*	1.94–2.14 (2.04 ±0.08)*	0.93–1.09 (1.00±0.05)	6–7 (6.86±0.31)	8–9 (8.50±0.55)*	36–44 (39.33±4.16)*
<i>D. prakaiphetensis</i> sp. nov.	1.62–1.76 (1.69±0.04)*	0.83–0.90 (0.86±0.02)*	0.59–0.69 (0.64±0.03)*	0.61–0.72 (0.66±0.03)*	1.92–2.06 (1.97±0.04)*	0.89–1.05 (0.97±0.06)*	6–7 (6.88±0.30)	3–4 (3.50±0.58)*	15–17 (16.00±1.00)*
<i>D. karoensis</i>	1.60–1.90 (1.75±0.21)*	0.90–1.10 (1.00±0.14)*	–	–	1.73–1.78 (1.76±0.04)*	–	5–5 1/2 (5.20±3.54)*	2*	–
<i>D. krabiensis</i>	1.60–1.70 (1.65±0.71)*	0.90*	–	–	1.89–2.39 (2.14±0.35)	–	5.75*	–	–
<i>D. canaliculata</i>	3.20–4.95 (3.93±0.81)*	1.30–2.07 (1.62±0.33)*	0.60–1.48 (0.97±0.40)*	0.50–1.67 (0.97±0.51)*	2.23–2.63 (2.43±0.17)*	0.89–1.20 (1.05±0.13)*	7–9 1/8 (8.03±0.96)*	3–5 (4.25±0.96)*	–
<i>D. hidagai</i>	2.20–2.70 (2.45±0.35)*	1.20–1.40 (1.30±0.14)*	–	–	1.83–1.93 (1.88±0.07)*	–	8*	–	–
<i>D. naiyanetri</i>	1.90–2.63 (2.41±0.31)*	1.00–1.13 (1.10±0.06)*	0.70–0.75 (0.73±0.03)*	0.70–0.80 (0.76±0.06)	1.90–2.35 (2.18±0.19)*	0.94–1.00 (0.96±0.03)	6–8 1/2 (7.88±1.25)*	6–7 (6.33±0.58)*	29–36 (32.67±3.51)*
<i>D. acme</i>	2.20*	1.00*	–	–	2.20*	–	7 ½	–	26*
<i>D. diminuta</i>	2.00*	0.90*	–	–	2.22*	–	7 ½	–	24*
<i>D. sinistra</i>	1.90*	1.00*	–	–	1.91*	–	7 ½	–	24*
<i>D. conditoria</i>	2.50*	1.20*	–	–	2.08	–	6*	7*	–
<i>D. maduana</i>	1.3–1.7 (1.50±0.28)*	0.70–0.90 (0.80±0.14)*	0.30–0.40 (0.35±0.07)*	0.30–0.40 (0.35±0.07)*	1.86–1.89 (1.88±0.02)*	1.00	5–6 1/2 (5.75±1.06)*	7–12 (9.50±3.54)*	–
<i>D. gomantongensis</i>	2.50–3.50 (3.00±0.71)*	1.20–1.60 (1.40±0.28)*	0.50–0.60 (0.55±0.07)*	0.50–0.60 (0.55±0.07)*	2.08–2.19 (2.14±0.08)	1.00	6 1/4–7 1/2 (6.88±0.88)	3–5 (4.00±1.41)*	–
<i>D. antheae</i>	1.60–2.10 (1.85±0.35)*	0.80–1.00 (0.90±0.14)*	0.30–0.40 (0.35±0.07)*	0.30–0.40 (0.35±0.07)*	2.00–2.20 (2.10±0.14)	1.00	5 3/4–6 8/5 (6.68±1.31)	7–13 (10.00±4.24)*	–
<i>D. baliana</i>	2.20–2.85 (2.52±0.33)*	1.05–1.50 (1.27±0.23)*	–	–	1.90–2.10 (2.00±1.00)	–	5 3/4–6 3/4 (6.33±0.52)	12–13 (12.50±0.71)*	36–60 (48.00±16.97)*

* Significant difference in shell morphology between *Diplommatina* species and new species at $P < 0.05$.

ing *D. gomantongensis*, *Diplommatina* sp. from Borneo and *D. canaliculata* (Fig. 6). Clade B consisted of most *Diplommatina* species from Borneo, such as *D. centralis*, *D. rubra*, *D. rubicunda*, *D. electa* and two *Diplommatina* spp., plus *D. laidlawi* from Peninsular Malaysia. Clade C contained the sister pair of *D. prakaiphetensis* sp. nov. and *D. laemsonensis* sp. nov. The pair’s sister group was *D. naiyanetri* (Fig. 6).

Pairwise K2P genetic distances between *Diplommatina* species ranged from 3.9–21.8% for COI and 3.2–21.3% for 16S. Intraspecific K2P distances ranged from 0–1.9% and 0 to 1.2% for COI and 16S, respectively. The new species were separated from other species by K2P distances of at least 5.5% in COI and 3.9% in 16S. The intraspecific K2P distances were minuscule (Suppl. material 1).

Discussion

Both morphological and molecular evidence support the recognition of the three new species *Diplommatina laemsonensis* sp. nov., *Diplommatina bulonensis* sp. nov. and *Diplommatina prakaiphetensis* sp. nov. Two of the species are presumed to be endemic to the region: *D. bulonensis* sp. nov. has been found only on Bulon Pai Island and *D. prakaiphetensis* sp. nov. has been found only on Prakaiphet Hill on the mainland. In addition, we found an additional regional record of *D. naiyanetri* (Figs 1–3).

One species, *D. canaliculata*, had previously been reported from Satun Province. This species was also found in other areas of Thailand, such as Trang, Nakhon Si Thammarat and Krabi Provinces (Panha and Burch 2005), as well as in Malaysia (Peninsular and Borneo) and Indonesia (Sumatra, Java and Borneo) (Laidlaw 1949; Vermeulen 1993; Maassen 2001a, 2002). A second species, *D. naiyanetri*, is a new Satun Province record and was found on Tarutao Island. Previously, this species had been reported in three other provinces in Thailand; namely, Trang, Phatthalung and Nakhon Si Thammarat (Panha and Burch 2005). All of these provinces share a similar origin of Paleozoic limestones on the continental Shan-Thai fragment, which may explain the observed present-day pattern of distribution (Agematsu et al. 2006a, b).

Several shell morphological characters can be used to distinguish the new species from other *Diplommatina* species; these include shell height, shell width, aperture height, aperture width, the ratio of SH/SW, the ratio of AH/AW, the internal lamellar system, radial ribs on the penultimate whorl, the number of whorls and the structure of the peristome. Moreover, scanning electron microscopy (SEM) showed additional details in the sculpturing and number of whorls of the protoconch in the three new species (Fig. 3). These protoconch features have previously been used as criteria for identification in *Diplommatina* species (Panha and Burch 2001; Maassen 2002; Dumrongrojwattana et al. 2020). Furthermore,

Table 2. List of sampling from GenBank for use in phylogenetic analysis.

Species	Voucher codes	Locality	Accession number		Reference
			COI	16S	
<i>Diplommatina ventriculus</i> Möllendorff, 1891	MOL119823	Malaysia, Perak	HM753324	HM753495	Webster et al. 2012
<i>Diplommatina centralis</i> Vermeulen, 1993	MOL119785	Malaysia, Borneo	HM753339	HM753505	Webster et al. 2012
<i>Diplommatina electa</i> Fulton, 1905	MOL119815	Malaysia, Borneo	HM753359	HM753503	Webster et al. 2012
	MOL119816	Malaysia, Borneo	HM753360	HM753517	Webster et al. 2012
<i>Diplommatina plecta</i> Fulton, 1901	MOL119818	Malaysia, Borneo	HM753362	HM753519	Webster et al. 2012
<i>Diplommatina rubicunda</i> Martens, 1864	MOL119819	Malaysia, Borneo	HM753363	HM753520	Webster et al. 2012
<i>Diplommatina rubra</i> Godwin-Austen, 1889	MOL119797	Malaysia, Borneo	HM753346	HM753514	Webster et al. 2012
	MOL119814	Malaysia, Borneo	HM753358	HM753516	Webster et al. 2012
<i>Diplommatina laidlawi</i> Sykes, 1903	MOL119821	Malaysia, Kelanta	HM753364	HM753522	Webster et al. 2012
<i>Diplommatina canaliculata</i> Möllendorff, 1887	MOL119783	Malaysia, Pahang	HM753338	HM753504	Webster et al. 2012
<i>Diplommatina demorgani</i> Laidlaw, 1949	MOL119787	Malaysia, Pahang	HM753340	HM753507	Webster et al. 2012
<i>Diplommatina suratensis</i> Panha & Burch, 1998	MOL119827	Thailand, Krabi	HM753328	HM753499	Webster et al. 2012
<i>Diplommatina hidagai</i> Panha, 1998	MOL119791	Thailand, Trang	HM753327	HM753498	Webster et al. 2012
<i>Diplommatina hidagai</i> Panha, 1998	MOL119826	Thailand, Trang	HM753343	HM753510	Webster et al. 2012
<i>Diplommatina naiyanetri</i> Panha, 1997	MOL119794	Thailand, Trang	HM753344	HM753512	Webster et al. 2012
<i>Diplommatina prava</i> Pilsbry & Hirase, 1905	MOL119796	Taiwan	HM753345	HM753513	Webster et al. 2012
<i>Diplommatina gomantongensis</i> Smith, 1894	MOL119800	Malaysia, Borneo	HM753342	HM753509	Webster et al. 2012
<i>Diplommatina</i> sp.	MOL119822	Malaysia, Borneo	HM753365	HM753523	Webster et al. 2012
	MOL119780	Malaysia, Borneo	HM753337	HM753501	Webster et al. 2012
	MOL119813	Malaysia, Borneo	HM753357	HM753515	Webster et al. 2012
<i>Diplommatina laemsonensis</i> sp. nov.	MCMU 0401	Thailand, Satun	ON568751	OP857482	This study
	MCMU 0409	Thailand, Satun	OP825126	OP857486	This study
<i>Diplommatina canaliculata</i> Möllendorff, 1887	MCMU 0402	Thailand, Satun	ON568749	OP857480	This study
<i>Diplommatina bulonensis</i> sp. nov.	MCMU 0403	Thailand, Satun	ON568752	OP857483	This study
	MCMU 0410	Thailand, Satun	OP825127	OP857487	This study
	MCMU 0411	Thailand, Satun	OP825128	OP857488	This study
<i>Diplommatina naiyanetri</i> Panha, 1997	MCMU 0406	Thailand, Satun	ON568750	OP857481	This study
	MCMU 0408	Thailand, Satun	OP825125	OP857485	This study
<i>Diplommatina prakaiphetensis</i> sp. nov.	MCMU 0407	Thailand, Satun	ON568753	OP857484	This study
	MCMU 0412	Thailand, Satun	OP825129	OP857489	This study
<i>Hungerfordia</i> sp.	FMNH310545	Belau	EU742109	EU742028	Rundell 2008
	FMNH310544	Belau	EU742107	EU742026	Rundell 2008
	–	Belau	HM753351	HM753526	Webster et al. 2012
	FMNH310550	Belau	EU742113	EU742032	Rundell 2008
<i>Palaina moussoni</i> Crosse, 1866	FMNH310574	Belau	EU742089	EU742008	Rundell 2008
	MOL 119811	Belau	HM753355	HM753532	Webster et al. 2012
<i>Palaina rubella</i> Beddome, 1889	FMNH310572	Belau	EU742090	EU742009	Rundell 2008
	FMNH310573	Belau	EU742091	EU742010	Rundell 2008
<i>Palaina striolata</i> Crosse, 1866	MOL 119812	Belau	HM753356	HM753533	Webster et al. 2012
<i>Opisthostoma fraternum</i> Smith, 1905	MOL 119824	Malaysia, Borneo	HM753325	HM753496	Webster et al. 2012
<i>Opisthostoma mirabile</i> Smith, 1893	MOL 119807	Malaysia, Borneo	HM753353	HM753529	Webster et al. 2012
<i>Opisthoporus birostris</i> Pfeiffer, 1854	MOL.119772	Malaysia, Borneo	HM753333	HM753488	Webster et al. 2012

this is the first study of radular morphology of *Diplommatina*. Differences in the shape of basal plate, and the shape and number of cusps on central tooth were observed in the three new species (Fig. 3), indicating that radular characters are useful for identification within this genus. In contrast, the opercula are very similar, being paucispiral, flat, transparent, with a smooth outer and inner surface with a raised peripheral circular margin. The operculum is infrequently used in taxonomy among diplommatinids because it has few characters and is very similar among species (Neubert and Bouchet 2015).

Molecular phylogenetic techniques were used to verify taxonomy and indicate classification, and are especially useful where morphological characters alone do not provide clear delineations (Douris et al. 1998; Chiba 1999; Thacker and Hadfield 2000; Holland and Hadfield 2002; Steinke et al. 2004; Desouky and Busais 2012). The phylogenetic tree revealed three main clades with-

in *Diplommatina* and species within these clades shared the same sinistral or dextral shell spire and had similar distribution patterns. Clade A was a strongly supported clade consisting of dextral species from Borneo and Peninsular Malaysia, southern Thailand and Taiwan (Fig. 6). *D. bulonensis* sp. nov. had high support as a sister group of *D. gomantongensis* and *D. canaliculata* and was also placed in the same clade as *D. hidagai* from Thailand (Fig. 6). The phylogenetic tree was consistent with the pattern of difference in shell morphology of *D. bulonensis* sp. nov. versus *D. canaliculata* and *D. hidagai* in the ratio of SH/SW and the number of whorls, and the difference from *D. gomantongensis* in the radial ribs/0.5 mm on the penultimate whorl (Table 1).

All sinistral species were placed in Clade B and Clade C, which were geographically distinct. All species in Clade B were from Borneo, Malaysia and all species in Clade C were from Peninsular Malaysia and southern

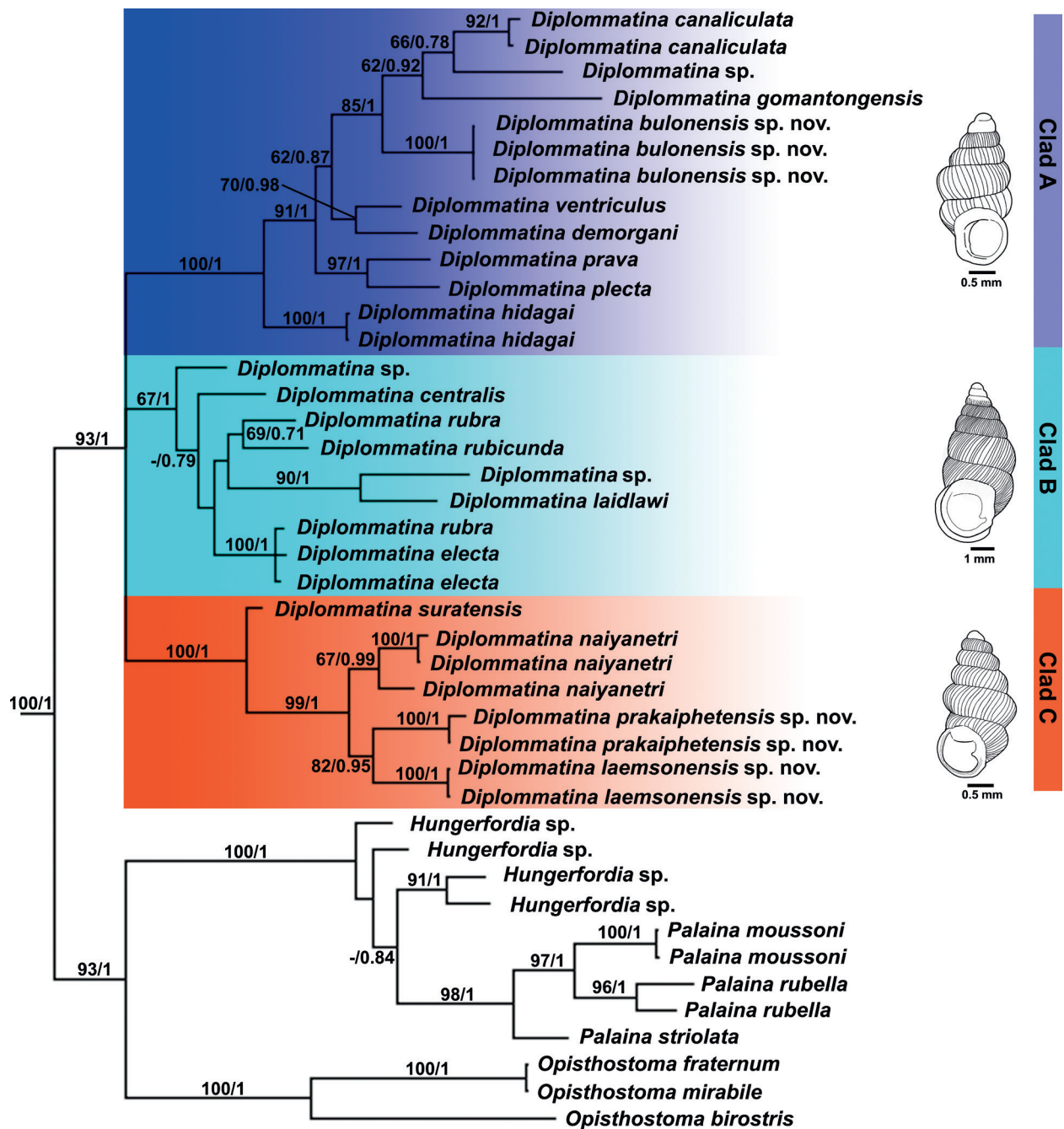


Figure 6. Phylogenetic tree of *Diplommatina* species based on COI and 16S genes. The number on each clade are statistic supports based on ML and BI methods, respectively. The ML exceed less than 60% of the Boorstrap support and the posterior probability of BI is greater than 0.70.

Thailand. The close placement of *D. laemsonensis* sp. nov. and *D. prakaiphetensis* sp. nov. with their sister species *D. naiyanetri* in Clade C was strongly supported (Fig. 6). The phylogenetic tree agreed with the significant differences in shell morphology of *D. laemsonensis* sp. nov. and *D. prakaiphetensis* sp. nov. versus *D. naiyanetri* in the ratio of SH/SW, the number of whorls, and the number of radial ribs/0.5 mm on the penultimate whorl (Table 1). In addition, the genetic K2P distance based on COI and 16S genes demonstrated that the distances of the three new species were greater than the overall mean of the species in this genus. Thus, the morphological char-

acters and molecular analysis both indicate that the three described taxa of *Diplommatina* in this study are, indeed, new species.

Many tropic and subtropic karst regions support high biodiversity and endemic species of land snails, especially microsnails (Panha and Burch 2005). A variety of human activities impact limestone outcrop habitats in Southeast Asia (Sodhi et al. 2010; Hughes 2017). The destruction of limestone outcrop habitats leads to the extinction of species, especially endemic species (Sodhi et al. 2010). Hence, it is necessary to understand unique aspects of biodiversity for these small ‘hotspots’

for planning and implementing conservation strategies, especially for threatened endemic species, including land snails.

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Supplementary material 1

Linked data table for primary biodiversity data of *Diplommatina*

Authors: Tuangthong Boonmachai, Elizabeth A. Bergey, Nattawadee Nantarat

Data type: occurrences and phylogenetic (word document)

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