

***Radula deflexilobula* (Radulaceae, Marchantiophyta) from Thailand, a new species based on morphological and molecular data**

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Abstract – *Radula deflexilobula* Promma, L.N.Zhang *et* R.L.Zhu is described and illustrated as a new species from Thailand. This species is distinctive in 1) the presence of dimorphic leaf lobules that are subrhombic and strongly reflexed on pendulous shoots, subquadrate and not reflexed on creeping shoots; 2) leaf lobules covering the stem ca. 1/3-1/2 of the stem-width, not auriculate at base, and 3) leaf cells thin walled with indistinct trigones and smooth cell surface. Our phylogenetic analyses based on three chloroplast regions (*trnG*, *trnL-F*, *atpB-rbcL*) confirm that this new species belongs to subg. *Radula* and is sister to *R. decurrens* Mitt. known from Fiji.

Hepaticae / liverworts / lowland rain forests / oil body / phylogeny / *Radula decurrens*

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INTRODUCTION

Marchantiophyta (liverworts) contains 369 genera (Zhu *et al.*, 2018). *Radula* Dumort. is an isolated genus belonging to family Radulaceae with 249 currently accepted species (Promma & Chantanaorrapint, 2015; Söderström *et al.*, 2016; Zhang & Zhu, 2016; Pócs, 2017; updated). *Radula* is one of the largest genera of liverworts and like other large liverwort genera has wide distribution and high species diversity in wet tropical and subtropical regions. *Radula* is easily recognized by 1) incubously inserted and orientated lateral leaves, 2) conduplicately bilobed leaves, the postical lobe forming a 'lobule', 3) absence of underleaves, 4) rhizoids restricted to the ventral surface of the leaf lobule, 5) *Radula*-type branching, 6) 1(-6) large oil bodies per leaf cell, and 7) dorsiventrally flattened perianths. Based on recent molecular phylogenetic studies, seven subgenera are currently accepted in the genus, namely subg. *Amentuloradula* Devos *et al.*, subg. *Cladoradula* Spruce, subg. *Dactyloradula* Devos *et al.*, subg. *Metaradula* R.M.Schust., subg. *Odontoradula* K.Yamada, subg. *Radula*, and subg. *Vohutoradula* Devos *et al.* (Devos *et al.*, 2011; Patiño *et al.*, 2017).

In Thailand, *Radula* was first reported by Tixier (1970), who recorded *R. acuminata* Steph. and *R. borneensis* Steph. from Ranong province. Further new records for the Thai *Radula* were published over the following four and half decades (e.g. Tixier, 1973; Kitagawa, 1979; Yamada, 1979; Chantanaorrapint *et al.*, 2004; Lai *et al.*, 2008; Sukkharak *et al.*, 2008; Chantanaorrapint & Pócs, 2014; Pócs & Podani, 2015; Promma & Chantanaorrapint, 2015), and recently Promma and Chantanaorrapint (2015) described a new epiphyllous species, *R. grandilobula* Promma *et* Chantanaorr. known only from Thailand. In the course of a revision of *Radula* in Thailand, an unusual species was collected on trees growing in limestone areas. It has a unique combination of characters not matching any described species. Here we assess the status of these plants with morphological and molecular phylogenetic evidence, both of which support the recognition of a new species.

MATERIALS AND METHODS

Morphological study. This study is based on recent collections from Thailand. Voucher specimens of the new species are deposited in BKF, HSNU and PSU herbaria. Morphological and anatomical characters were studied using stereo and compound microscopes. The distinctive characters of the species were photographed by Olympus SZX12 stereo microscope and Olympus BX51 light microscope with attached camera and illustrated with Olympus SZX12 stereo microscope with attached Olympus SZX-DA drawing tube and Nikon OPTIPHOT-2 with attached Nikon drawing tube after fully moistening samples with tap water. Mature spores, elaters, and cuticle were examined and photographed by FEI Quanta 400 scanning electron microscope. In addition, distribution and ecological data were compiled, brief descriptions, and illustrations are provided.

Taxon sampling, DNA extraction, amplification and sequencing. In order to analyze the phylogenetic placement of the new taxon, a total of 127 samples representing 104 morphological entities from all seven subgenera currently recognized of *Radula* were incorporated into a molecular data matrix. Of these

samples, 89 accessions were derived from previous studies focusing on the phylogeny of *Radula* (Devos *et al.*, 2011; Patiño *et al.*, 2017) and were included to provide the phylogenetic context for establishing the relationships of the Thai limestone plants. 30 samples belonging to *Radula* subgenus *Radula*, morphologically similar to the present taxon and previously resolved belonging to a large monophylum (including clades B–F, cf. Renner, 2014), were selected to explore their interspecific taxonomy and relationships with the new taxon. Two samples of the present taxon from Thailand and six samples of *R. javanica* Gottsche from China and Southeast Asia were newly provided. In addition, three species (*Frullania moniliata* (Reinw. *et al.*) Mont., *Jubula hutchinsiae* subsp. *pennsylvanica* (Steph.) Verd., and *Porella pinnata* L.) served as outgroups following Devos *et al.* (2011). A total of 24 sequences were newly generated in this study, and the remaining 257 sequences were downloaded from GenBank. Voucher information and GenBank accession numbers for 45 specimens all sampled in Figure 1B are presented in Appendix. The information details for the further 85 individuals were provided in the phylogeny by Devos *et al.* (2011) and Patiño *et al.* (2017).

Plant tissue was isolated from the distal portions of gametophyte shoots from the herbarium specimens, and dried in silica gel. Total genomic DNA was extracted with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), then kept at -20°C for polymerase chain reaction (PCR) amplification. Three chloroplast DNA markers and their primers for PCR were employed according to Renner's series of studies on Australian *Radula* (Renner *et al.*, 2013a, b, c; Renner, 2014): *trnG* G2 intron, *trnL*-F region, and *atpB-rbcL* spacer. The PCR were carried out using the following protocols: an initial denaturation at 95°C for 5 min, followed by 33 cycles of 1 min denaturation at 95°C, 1 min annealing at 53°C for *trnG* and *trnL*-F regions or at 50°C for *atpB-rbcL* region, and 1 min elongation at 72°C, finally with an ending of elongation at 72°C for 10 min. The PCR products were checked on 1% TAE agarose gels by electrophoresis, and the successful amplifications were sent to Shanghai Majorbio Bio-Pharm Technology Co. Ltd., China (<http://www.majorbio.com>) for bidirectional sequencing using the same primers.

Sequence alignment and analysis. The sequences newly generated were edited using PhyDE[®]0.997 (Müller *et al.*, 2010), and a consensus sequence was assembled by comparison of the forward and reverse sequence of each individual. Alignment for the consensus sequences and published sequence data retrieved from GenBank was initially performed in MEGA v.6.0 (Tamura *et al.*, 2013), and then checked by visual inspection and manually adjusted in PhyDE[®]0.997. Ambiguously aligned sites and gap-rich columns were excluded from phylogenetic analyses. Lacking parts of sequences and missing nucleotides were coded as missing.

The phylogenetic trees of individual and combined datasets were constructed using Bayesian inference (BI) and maximum likelihood (ML) analysis. The three individual data sets were first analysed separately to check for incongruence between them. As shown in previous phylogenetic researches of *Radula* (Devos *et al.*, 2011; Renner *et al.*, 2013a, b, c; Renner, 2014; etc.), no topological conflicts were identified among significantly supported (≥ 0.95 Bayesian posterior probability/80% ML bootstrap) clades, and well-supported topologies (especially at the subgeneric and interspecific levels) were obtained from the combined plastid datasets. Thus we concatenated the three markers into a single matrix.

Bayesian inference analysis was performed using MrBayes v.3.2 (Ronquist *et al.*, 2012). The optimal nucleotide substitution model for each DNA region was tested in MrModeltest v.2.3 (Nylander, 2004) by the Akaike information criterion (AIC), as implemented in PAUP* v.4.0b10 (Swofford, 2003). The nucleotide models

selected were as follows: GTR+I+G for *trnG* and *trnL-F*, GTR+I for *atpB-rbcL*. The dataset then was analysed using Markov chain Monte Carlo (MCMC) heuristic searches, performing four independent runs with four chains of 3.5 million generations, and trees were sampled every 1000 generations. A burn-in of 10% of the trees based on Tracer v.1.5 (Rambaut & Drummond, 2009) was discarded before inferring a single majority-rule consensus phylogeny with Bayesian posterior probability (PP) confidence values. Maximum likelihood analysis was performed with RAxML v.8.2 (Stamatakis, 2014). A rapid bootstrap analysis was conducted using the GTRGAMMAI substitution model with 1000 rapid bootstrapping replicates. Clades were considered supported if Bayesian posterior probability (PP) ≥ 0.90 and maximum likelihood bootstrap percentages (BS) $\geq 70\%$, sufficiently supported when PP values ≥ 0.98 and BS values $\geq 80\%$.

RESULTS

In this study, the concatenated dataset with three plastid markers (*trnG*, *trnL-F* and *atpB-rbcL*) contains 1678 characters (625, 466 and 587 characters, respectively). Of the total characters, 1060 were constant, 432 parsimony informative, 186 variable but parsimony uninformative. Phylogenetic analyses produced with BI and ML analyses resulted in congruent topologies and differed only in unsupported nodes. The Bayesian consensus trees from the combined dataset were presented as Figure 1 (1B enlarged from a group specified on 1A), with posterior probabilities (PP ≥ 0.90) and maximum likelihood bootstrap (MLBS ≥ 70) values plotted on the nodes.

In the tree topology (Fig. 1A), *Radula* was divided into seven fully supported major lineages corresponding to the seven subgenera of *Radula* circumscribed by Devos *et al.* (2011). Within subgenus *Radula* lineage, a large group (Fig. 1B) with 45 terminals contained the new taxon and 19 morphological entities, which were resolved on a polytomy containing some supported subclades. The present taxon, *R. deflexilobula*, just fell into an unsupported clade that was consistent with Renner (2014)'s clade B (highlighted on Fig. 1B). This clade was divided into two subclades, one strongly supported (PP = 1; MLBS = 97) including nine terminals, comprising *R. oreopsis* and three morphological entities; the other containing the new taxon with two individuals strongly supported (PP = 1; MLBS = 100) and as sister to *R. decurrens*, but not supported at the base of the subclade.

Radula javanica was resolved into a monophyletic clade with high support (PP = 1; MLBS = 86). The groups in it roughly corresponded to their geographical distributions in the Indo-China Peninsula / Brunei, Australasia-Pacific, and East Asia, implying isolation by distance.

TAXONOMIC TREATMENT

Radula deflexilobula Promma, L.N.Zhang *et* R.L.Zhu *sp. nov.*

Figs 2-43

Radula deflexilobula is similar to *R. decurrens* Mitt., but differs in having dimorphic leaf lobules, strongly reflexed on pendulous shoots and somewhat flattened

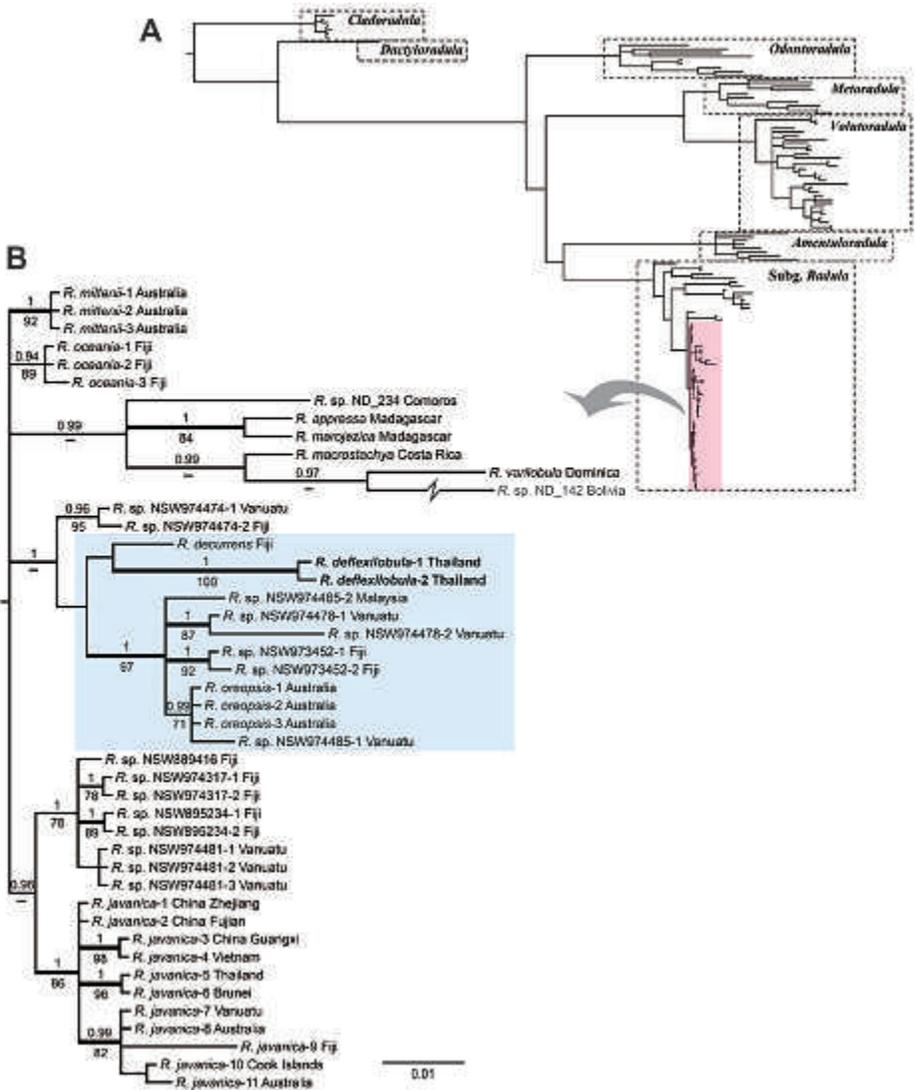


Fig. 1. Majority-rule consensus tree based on Bayesian analyses of the combined dataset of *trnG*, *trnL-F* and *atpB-rbcL*. Bayesian posterior probability values (PP) are indicated above the branches, while the ML bootstrap percentage values (MLBS) are presented under the branches. Branches in bold are well supported (PP \geq 0.98; MLBS \geq 80). Support values of PP < 0.9 and MLBS < 70 are not shown or indicated by a dash (-).

on creeping shoots; both forms of leaf lobules being remote, base covers ca. 1/3-1/2 of the stem-width and not auriculate at base; leaf cells thin walled with indistinct trigones and smooth cuticle.

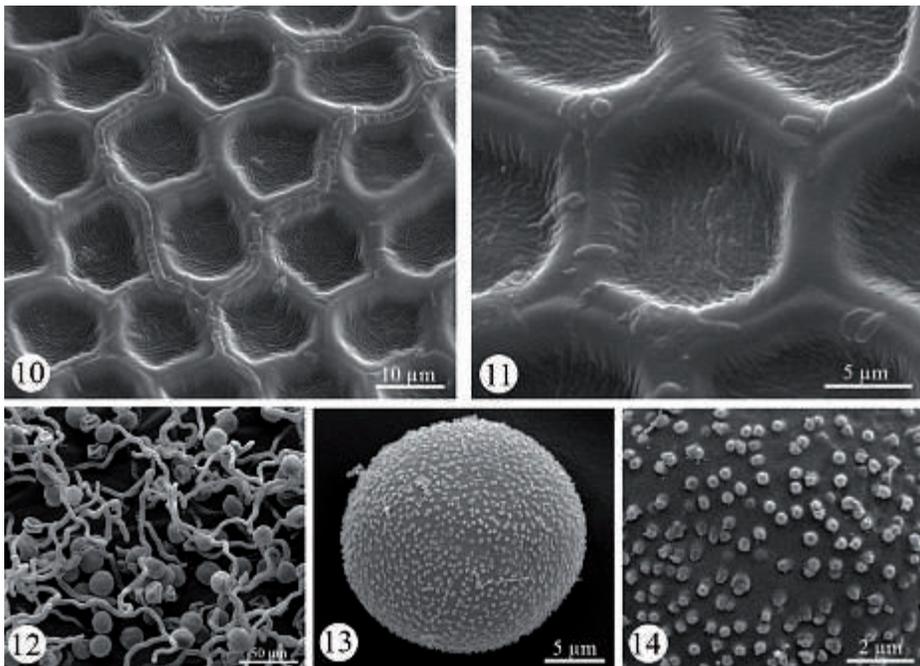
Type: Thailand. Trang, Palian, Chao Pa Waterfall, 7°14'21.90" N, 99°50'39.18" E, 75 m, epiphytic on branches along stream of limestone rapids in



Figs 2-9. *Radula deflexilobula* Promma, L.N.Zhang *et* R.L.Zhu. 2. Plant habit. 3-5. Portions of sterile plant, ventral view, 4. Creeping shoot, 5. Pendulous shoot. 6-7. Median cells of leaf lobe, showing smooth surface. 8. Cells of leaf lobe with oil bodies. 9. Cells of leaf lobule with oil bodies. All from *Chantanaorrapint & Promma 3933* (holotype, PSU).

lowland evergreen forest, 24 Nov. 2014, *Chantanaorrapint & Promma 3933* (holotype: PSU!, isotypes: BKF!, HSNU!).

Plants bright green when fresh, yellowish brown in dry condition, up to 20 mm long, 1.6-2.6 mm wide, usually irregularly bipinnate; dimorphic with creeping stem or lower portions and pendulous shoot or upper portions. **Stem** in cross-section 153-205 μm in diameter, 10-13 cells thick, epidermal cells somewhat smaller than the medullary cells, both cells brown, thick-walled, with medium-sized to large trigones. **Rhizoids** brown, rhizoid-initial area slightly convex. **Leaves** loosely to moderately imbricate on creeping shoots, remote to contiguous on pendulous shoots, widely spreading; leaf-lobes oblong-ovate to oblong, 0.88-1.33 mm long, 0.6-0.83 mm wide, apex rounded, margin entire, dorsal base arched and covering the stem ca. 4/5 of the stem-width to completely; leaf lobe cells thin-walled with minute trigones, without intermediate thickenings, at margin quadrate to rectangular, 11.05-20.5 \times 11.05-18.95 μm , in the middle round to round-oblong, 15.8-28.5 \times 15.8-25.3 μm , near base similar to median cells in shape, but slightly larger, 20.5-38 \times 18.95-31.6 μm ; cuticle smooth; oil bodies grayish brown, ovoid or ellipsoidal, in leaf-lobe 1 per cell, coarsely botryoidal, in leaf-lobule 2-(3) per cell, coarsely botryoidal with 1-3 large spherical hyaline inclusions; leaf-lobules on creeping shoots subquadrate, ca. 1/3-2/5 of the lobe-length, 0.35-0.58 mm long, 0.32-0.42 mm, apex obtuse, not reflexed, abaxial free margin straight or slightly sinuate, adaxial free margin straight



Figs 10-14. SEM photos of *Radula deflexilobula* Promma, L.N.Zhang *et* R.L.Zhu. **10-11.** Cells with smooth surface. **12.** Spores associated with elaters. **13-14.** Spore, **13.** Whole spore showing papillate ornamentation, **14.** High magnification of the surface showing papillate. 10-11 from *Chantanaorrapint & Promma 3933* (holotype, PSU), 12-14 from *Chantanaorrapint & Promma 1832* (PSU).

to slightly arched, not reflexed, base rounded, covering the stem ca. 1/2 of the stem-width, line of insertions longitudinal with the stem, nearly straight or slightly arched, carinal region slightly inflated, keel straight to slightly sinuate, extending at angles of 45-60° with the stem, sinus very wide; leaf-lobules on pendulous shoots, ca. 1/3 of the lobe-length, 0.33-0.5 mm long, 0.27-0.5 mm, apex narrowly obtuse, strongly reflexed from adaxial free margin to apex or 1/3(-2/3) of abaxial free margin, along a line angled at 45 degrees outward from the stem, abaxial free margin straight or slightly sinuate, adaxial free margin straight to slightly arched, strongly reflexed, base rounded, covering the stem ca. 1/3 of the stem-width, insertion line longitudinal with the stem, nearly straight, carinal region not inflated, keel strongly sinuate, extending at angles of 30-45° with the stem, sinus very wide; slime papillae usually 2, one borne on the lobule-apex, clavate or cylindrical, the others smaller, near the base of the free interior margin of leaf lobule. **Asexual reproduction** by bulbils and caducous leaves (rarely observed). **Diocous**. **Androecia** terminal or intercalary on stems and branches, with 3-28 pairs of bracts, moderately to densely imbricate; bract-lobes 0.56-1.03 mm long, 0.3-0.42 mm wide, oblong-obovate, apex rounded, margin entire; bract-lobules ca. 1/2-3/4 of bract-lobe, ovate, apex obtuse to subacute, often reflexed, base free 1/2 of the length, somewhat extended, rounded, margin entire, keel strongly arched, carinal region strongly inflated. **Gynoeceia** terminal on stems and branches, with 1-2 subfloral innovations; bracts 1 pair, subequal in size, bract-lobe obovate, 1.08-1.25 mm long, 0.66-0.7 mm wide, apex narrowly rounded or rounded, margin entire, bract-lobules ca. 1/2 of bract-lobe, oblong or narrowly obovate, apex nearly rounded or obtuse, keel sinuate; perianths usually flat-cylindrical, 2.25-3.08 mm long, 0.62-0.95 mm wide at middle, mouth 0.67-1.0 mm wide, repand to irregularly weakly crenate; perianth walls unistratose above, with bistratose bands extending from half way up perianth, increasing in width and becoming confluent toward the perianth base, perianth walls progressively increasing in thickness toward base where 2-3 stratose; low stem perigynium present, 5-7 stratose, external cell wall heavily thickened and brown-pigmented, internal walls unthickened and unpigmented, perianth-calyptra fusion elevated above female bracts; calyptal perigynium present, 2-4 stratose at base, strata progressively lost, unistratose above, unfertilized archegonia elevated on surface of calyptra. **Capsules** long-ellipsoidal to cylindrical, 0.95-1.38 mm long, 0.42-0.57 mm wide at middle. **Elaters** linear, 100-300 µm long. **Spores** medium-sized, 20-24 µm in diameter, subspherical to spherical, papillate.

Etymology: The specific epithet “*deflexilobula*” refers to the reflexed leaf lobules.

Habitat and ecology: *Radula deflexilobula* is generally found on tree trunks and branches in riparian vegetation alongside waterfalls flowing over limestone in lowland evergreen forests, at altitudes between 50 and 550 m. It may grow associated with other bryophytes such as *Calymperes* spp., *Pinnatella* spp., *Plagiochila bantamensis* (Reinw. et al.) Mont., and *Radula vrieseana* Sande Lac.

Distribution: The new species is widely distributed in western and southern Thailand, and seems to be specific with the limestone waterfalls. It may occur in other areas of Myanmar with similar habitat conditions.

Additional specimens examined: Thailand. Tak, Umphang, Umphang Wildlife Sanctuary, Thi Lo Su Waterfall, 560 m, 13 Aug. 2013, *Chantanaorrapint* & *Promma* 2766 (PSU). Phangnga, Mueang, Sa Nang Manora Forest Park, Sa Nang Manora Waterfall, 50 m, 20 Jul. 2013, *Chantanaorrapint* & *Promma* 2698, 2701, 2702 (PSU). Nakhon Si Thammarat, Noppitam, Khao Luang National Park, Krung Ching Waterfall, 225 m, 9 Dec. 2012, *Chantanaorrapint* & *Promma* 1809 (PSU); Sichon, Sikiet Waterfall National Park, 45 m, 19



Fig 43. Limestone rapids in lowland evergreen forests in Thailand, type locality.

Jan. 2014, *Chantanaorrapint & Promma 3461, 3464, 3466, 3472* (PSU). Trang, Palian, Chao Pa Waterfall, 75-260 m, 11 Jan. 2012, *Chantanaorrapint et al. 781, 783A, 785, 794B, 796B* (PSU), 2 Oct. 2012, *Chantanaorrapint & Promma 1656, 1661C, 1662A, 1663* (PSU), 24 Nov. 2014, *Chantanaorrapint & Promma 3933, 3934* (PSU); Thung Khai, Peninsular Botanic Garden (Thung Khai), 47 m, 12 Dec. 2014, *Chantanaorrapint & Promma 3946* (PSU). Satun, Thung Wa, Than Plew Waterfall, 140 m, 12 Jan. 2013, *Chantanaorrapint & Promma 1829C, 1830, 1832, 1835, 1842* (PSU), 145 m, 16 Dec. 2011, *Zhu et al. 20111216-8, 20111216-21* (HSNU). Songkhla, Hat Yai, Ko Hong Hill, 65 m, 11 May 2014, *Chantanaorrapint & Promma 3827* (PSU); Ton Nga Chang Wildlife Sanctuary, Pha Dam Waterfall, 132m, 14 Dec. 2011, *Zhu et al. 20111214-16B* (HSNU), 135 m, 11 Oct. 2013, *Chantanaorrapint et al. 2983, 2988A* (PSU).

DISCUSSION

The new species exhibits dimorphic shoot systems: creeping and pendulous shoots having different leaf-lobes and leaf-lobules. Leaf-lobes are loosely to moderately imbricate on creeping shoots, but remote to contiguous on pendulous shoots. Leaf-lobules of pendulous shoots are subrhombic to subquadrate with narrowly obtuse apex and strongly reflexed or reflexed on adaxial free margin only and base of adaxial free margin cover the stem ca. 1/3 of the stem-width. Whereas leaf-lobules of creeping shoots are subquadrate, apex not reflexed, and base of adaxial free margin cover the stem ca. 1/2 of the stem-width. Polymorphism in shoot morphology is known in some other *Radula* species, for example different individuals of *R. splendida* M.A.M. Renner *et Devos* express different shoot morphology (Renner *et al.*, 2010). *Radula deflexifolia* is somewhat unusual in that polymorphism in shoot

morphology occurs within individuals. Among Thai species, the new species is similar to *R. javanica* Gottsche group, but stands out on strongly reflexed lobule on pendulous shoot.

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Radula deflexilobula is morphologically similar to *R. decurrens* Mitt., and our molecular analyses also revealed they have a very close phylogenetic relationship (Fig. 1B). The two species share the similarities in reflexed leaf lobules. *Radula decurrens* has been recorded from Samoa, Carolines, New Ireland, Solomons, New Hebrides, Society Island, Hawaii, Macronesia, and Pacific Islands (Castle, 1965; Yamada, 1979; So, 2006) and has been excluded from Asia by Yamada (1979). *Radula decurrens* is distinguished from *R. deflexilobula* by the densely implicate, subquadrate leaf lobules with widely obtuse apex, strongly reflexed from the upper 2/3 of adaxial free margin to 1/3 of abaxial free margin, a zig-zag pattern across the stem of lines of reflexion, auriculate base fully covering the stem, and the large to nodulose trigones of leaf cells. *Radula reflexa* Nees *et* Mont. also resembles *R. deflexilobula* but can be separated by having the leaf lobules with reflexed from the upper 2/3 of adaxial free margin to apex or 1/3 of abaxial free margin, reflexed perpendicular to the stem, slightly auriculate base covering the stem 3/4 of the stem-width to fully. *Radula reflexa* is very similar to *R. decurrens* in general appearance and So (2006) suggested that *R. decurrens* might be a robust form of *R. reflexa*. *Radula reflexa* was known from Asia (Indonesia, Borneo), New Guinea, and Hawaii (Yamada, 1979; So, 2005). The specimen determined as *R. reflexa* in the molecular phylogeny by Devos *et al.* (2011) had been misidentified by Renner, and was subsequently treated by him as *R. javanica* (Renner 2014; i.e. *R. javanica*-9 in Fig. 1B). He also proposed that many Australian *Radula* samples attributed to *R. reflexa* were mis-identifications of *R. javanica* and excluded it from Australia. No individuals actually correspondence with the types of *R. reflexa* have been included in molecular phylogenetic studies. *Radula deflexilobula* also resembles *R. javanica* Morph 1, known from Australia (Renner, 2014), the lobules of this form often have the whole free portion reflexed. In the phylogenetic tree of this study, we added six specimens of *R. javanica* from Asia to the samples representing three forms from Australasia-Pacific as described by Renner (2014). The results showed that *R. javanica* is a separate species with apparently different geographical patterns (Fig. 1B).

Though the whole enlarged group (Fig. 1B) contains 20 morphological entities including *Radula deflexilobula*, the new species is easily distinguished from most by the outstanding feature of reflexed lobules. In addition to *R. deflexilobula*, reflexed lobules occur in *R. decurrens*, *R. reflexa* and *R. javanica*.

Characters of lobules are critical to species circumscription within *Radula*, and distinctive lobule characters have been used as diagnostic features of many species (Yamada, 1979; Renner, 2014). There are some species that have been named for lobule characters, especially in reflexed lobule species including *R. decurrens* and *R. reflexa*. The similarities of these species are discussed as above. The new species have several outstanding features providing further morphological differences between these, see Table 1. Variation in ecology and morphology has been observed in other *Radula* species such as *R. javanica* (Renner, 2014), but this correlation between ecology and morphology manifests among individuals. Rheophytic plants of *R. javanica* have two outstanding features, reflexed lobule

Table 1. Morphological comparison of *Radula deflexilobula*, *R. decurrens* and other closely related *Radula*.

Characters	<i>Radula deflexilobula</i>	<i>R. decurrens</i>	<i>R. oreopsis</i>	<i>R. mittenii</i>	<i>R. javanica</i>	<i>R. reflexa</i>
	<i>pendulous shoot</i>	<i>creeping shoot</i>				
Dimorphism	dimorphism associated with substrate; creeping shoot and pendulous shoot	no	no	no	no	no
Shoot systems	bipinnate branching	pinnate branching	pinnate branching	pinnate branching (or bipinnate in older sectors)	pinnate branching (or become irregularly bipinnate in older sectors)	pinnate branching
Leaf lobes	remote to contiguous	loosely to moderately implicate	moderately implicate	moderately to densely imbricate	remote to implicate	moderately implicate
Leaf lobe shape	oblong-ovate to oblong, ±falcate	oblong-falcate	ovate-falcate	ovate to oblong, somewhat falcate	oblong-ovate, ±falcate	oblong-ovate, ±falcate
Leaf lobe cells	thin wall with indistinct trigones	thin wall with large to nodulose trigones	thin wall with large to triangular trigones	thin wall with concave thin-walled with minute but distinct to moderately trigones	thin wall with small to triangular trigones	thin wall with medium to large trigones
Leaf lobe surface	smooth	rugose ornamentation	ruminate ornamentation, bearing ridges with irregular profile and size that impart an irregular, eroded honeycomb appearance to the surface	distinctly rugose	weakly bulging, bearing punctulate ornamentation, or smooth	smooth
Leaf lobules	remote	densely implicate	remote to contiguous	slightly remote or contiguous, or imbricate at the upper portion of plant	remote	remote or nearly contiguous to loosely implicate
Leaf lobe shape	subrhombic or subquadrate	subquadrate	broadly and asymmetrically rhombic	more or less quadrate	subquadrate to subrhombic	subquadrate or subrhombic

<i>Characters</i>	<i>Radula deflexilobula</i>	<i>R. oreopsis</i>	<i>R. mitteni</i>	<i>R. javanica</i>	<i>R. reflexa</i>
	<i>pendulous shoot</i>	<i>creeping shoot</i>			
Leaf lobule apex	narrowly obtuse	obtuse	obtuse	obtuse	narrowly obtuse
Free margin of leaf lobule	strongly reflexed from adaxial free margin to apex or 1/3(-2/3) of abaxial free margin, along a line angled at 45 degrees outward from the stem	wildly obtuse strongly reflexed from the upper 2/3 of adaxial free margin to 1/3 of abaxial free margin, lines of reflexion form a zig-zag pattern across the stem	not reflexed	±reflexed on adaxial margin **in <i>R. javanica</i> Morph. 1 (Renner, 2014) reflexed along a line at 90 degrees to the stem, and on old shoot perpendicular to the sectors reflexed across stem the whole lobule between the lobule-lobule junction and the top of the stem insertion	reflexed from the upper 2/3 of adaxial free margin to apex or 1/3 of abaxial free margin, reflexed at 90 degrees to the stem, and on old shoot perpendicular to the sectors reflexed across stem
Abaxial margin of leaf lobule	straight or slightly sinuate, reflexed	straight or slightly sinuate, reflexed	straight, more or less parallel to stem	straight or slightly sinuate	straight, more or less decurrent to the lobe
Adaxial margin of leaf lobule	straight to slightly arched, strongly reflexed	arched, strongly reflexed	arched toward the base straight or nearly straight, more or less perpendicular	arched, ±reflexed	arched
Leaf lobule base	not auriculate, cover the stem ca. 1/2 of the stem-width	not auriculate, amplate, fully covering the stem	not auriculate, weakly amplate, fully covering the stem ca. 1/4-3/4 of the stem-width	not auriculate, covering the stem ca. 1/3-2/3 of the stem-width	slightly auriculate, covering the stem 3/4 of the stem-width to fully
Leaf lobule keel	strongly sinuate	more or less sinuate	shallowly arched	straight to shallowly arched	more or less sinuate
Insertion line of leaf lobule	longitudinal with the stem, nearly straight	longitudinal with the stem to nearly transverse	longitudinal with the stem, nearly arched at acroscopic end	line of insertions longitudinal with the stem, nearly straight or slightly arched	obliquely longitudinal with the stem, nearly straight or the stem slightly arched

apices and copious caducous leaf production, while epiphytic plants may possess neither.

Since the molecular phylogenetic study of Devos *et al.* (2011) seven subgenera have been accepted in *Radula*. Following this subgeneric classification and the updated world checklist of hornworts and liverworts (Söderström *et al.*, 2016), six subgenera are recognized in Thailand, namely subg. *Amentuloradula* Devos *et al.*, subg. *Cladoradula* Spruce, subg. *Metaradula* R.M.Schust., subg. *Odontoradula* K.Yamada, subg. *Radula*, and subg. *Volutoradula* Devos. The species having no special or diagnostic characters and cannot be placed in any other subgenus, were placed within subg. *Radula* (Yamada, 1979; Schuster, 1980; Devos *et al.*, 2011). After a new subgeneric classification based on molecular phylogenetic studies was proposed, many species were removed to another subgenus, particularly to the new subg. *Volutoradula* (Devos *et al.*, 2011). The species of subg. *Radula* and subg. *Volutoradula* may be morphologically similar, despite their degree of phylogenetic separation (Devos *et al.*, 2011). Species of subg. *Radula* do not bear microphyllous branches, have rounded-obtuse and entire leaf lobes, longitudinal lobule insertion, gynoecia on main axis or long branches, with innovations, and presence of perigynium. *Radula* subg. *Volutoradula* has high species diversity in the Neotropics, and few species are known in Asia. Even though it has smooth cell surfaces, *R. deflexilobula* belongs to subg. *Radula* and the demonstrated polymorphism in this character system further complicates the attribution of species to subg. *Radula* and subg. *Volutoradula* on the basis of morphological data alone. Morphological characters circumscribing these two lineages need to be sought in the sporophyte generation.

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Appendix. List of specimens sampled in Figure 1B of this study. Voucher information and GenBank accession numbers are given as follows: taxon name, country, collector(s) and collection number followed by herbarium, GenBank accession numbers (*trnG*, *trnL-F* and *atpB-rbcL*). An asterisk (*) indicates newly generated sequences in this study, a dash (-) denotes missing data.

Radula appressa Mitt., Madagascar, *Pócs 90113/AH* (EGR), HM992386, HM992465, -; *R. decurrens* Mitt., Fiji, *Söderström 2011/56* (NSW978127), KM220061, KM220127, KM220197; *R. deflexilobula* Promma, L.N.Zhang *et al.* R.L.Zhu -1, Thailand, *Chantanaorrapint & Promma 1842* (PSU), MH938633*, MH938641*, MH938649*; *R. deflexilobula* Promma, L.N.Zhang *et al.* R.L.Zhu -2, Thailand, *Zhu et al. 20111216-21* (HSNU), MH938634*, MH938642*, MH938650*; *R. javanica* Gottsche -1, China, Zhejiang, *Cheng et al. 20150424-4* (HSNU), MH938627*, MH938635*, MH938643*; *R. javanica* Gottsche -2, China, Fujian, *Zhang et al. 20150831-137A* (HSNU), MH938628*, MH938636*, MH938644*; *R. javanica* Gottsche -3, China, Guangxi, *Wei et al. 20140423-119* (HSNU), MH938629*, MH938637*, MH938645*; *R. javanica* Gottsche -4, Vietnam, *Zhu et al. 20131024-54* (HSNU), MH938630*, MH938638*, MH938646*; *R. javanica* Gottsche -5, Thailand, *Zhu et al. 20111214-32* (HSNU), MH938631*, MH938639*, MH938647*; *R. javanica* Gottsche -6, Brunei, *Zhu et al. 20151215-7* (HSNU), MH938632*, MH938640*, MH938648*; *R. javanica* Gottsche -7, Vanuatu, *Brown s.n.* (NSW974490), KM220085, KM220153, KM220222; *R. javanica* Gottsche -8, Australia, *Renner 6546* (NSW978121), KM220081, KM220149, KM220218; *R. javanica* Gottsche -9, Fiji, *Pócs s.n.* (EGR), HM992381, HM992460, -; *R. javanica* Gottsche -10, Cook Islands, *de Lange CK191* (AK323599), KM220070, KM220137, KM220207; *R. javanica* Gottsche -11, Australia, *Renner 6317* (NSW896712), KM220080, KM220148, KM220217; *R. macrostachya* Lindenb. *et* Gottsche, Costa Rica, *Gradstein & Dauphin DB12894* (GOET), HM992318, HM992404, -; *R. marojezica* E.W.Jones, Madagascar, *Pócs 90103/AE* (EGR), HM992388, HM992467, -; *R. mittenii* Steph.-1, Australia, *Renner 6288* (NSW896672), KF432389, KF440471, KF432303; *R. mittenii* Steph.-2, Australia, *Renner 6486* (NSW897201), KF432391, KF440473, KF432305; *R. mittenii* Steph.-3, Australia, *Renner 6497* (NSW909664), KF432392, KF440474, KF432306; *R. oceania* Castle -1, Fiji, *Renner 5732* (NSW974315), KM220078, KM220146, KM220215; *R. oceania* Castle -2, Fiji, *Renner 5741* (NSW974316), KM220048, KM220111, KM220181; *R. oceania* Castle -3, Fiji, *Renner 5412* (NSW889516_2), KM220079, KM220147, KM220216; *R. oreopsis* M.A.M.Renner -1, Australia, *Renner 6581* (NSW970377), KM220042, KM220104, KM220174; *R. oreopsis* M.A.M.Renner -2, Australia, *Renner 6543* (NSW899756), KM220045, KM220107, KM220177; *R. oreopsis* M.A.M.Renner -3, Australia, *Renner 6572* (NSW970425), KM220046, KM220108, KM220178; *R.*

sp. ND_142, Bolivia, *Churchill et al.* (MO22187), HM992365, HM992448, –; **R. sp.** ND_234, Comoros, *Pócs et al. 9288/R* (EGR), HM992389, HM992468, –; **R. sp.** **NSW889416**, Fiji, *Renner 5373* (NSW889416), KM220092, KM220161, KM220230; **R. sp.** **NSW895234 -1**, Fiji, *Renner 5583* (NSW895234), KM220073, KM220141, KM220210; **R. sp.** **NSW895234 -2**, Fiji, *Renner 5577* (NSW895228), KM220074, KM220142, KM220211; **R. sp.** **NSW973452 -1**, Fiji, *Renner 5732* (NSW973452), KM220066, KM220132, KM220202; **R. sp.** **NSW973452 -2**, Fiji, *Renner 5782* (NSW978103), KM220035, KM220097, KM220167; **R. sp.** **NSW974317 -1**, Fiji, *Renner 5717* (NSW974317), KM220077, KM220145, KM220214; **R. sp.** **NSW974317 -2**, Fiji, *Renner 5691*, (NSW978178), –, KM220114, KM220184; **R. sp.** **NSW974474 -1**, Vanuatu, *Brown s.n.* (NSW974474), –, KM220159, KM220228; **R. sp.** **NSW974474 -2**, Fiji, *Renner 5522* (NSW890202), KM220036, KM220098, KM220168; **R. sp.** **NSW974478 -1**, Vanuatu, *Brown s.n.* (NSW974478), KM220090, KM220158, KM220227; **R. sp.** **NSW974478 -2**, Vanuatu, *Brown s.n.* (NSW974489), KM220091, KM220160, KM220229; **R. sp.** **NSW974481 -1**, Vanuatu, *Brown s.n.* (NSW974481), KM220084, KM220152, KM220221; **R. sp.** **NSW974481 -2**, Vanuatu, *Brown s.n.* (NSW974480), KM220083, KM220151, KM220220; **R. sp.** **NSW974481 -3**, Vanuatu, *Brown s.n.* (NSW974483), KM220088, KM220156, KM220225; **R. sp.** **NSW974485 -1**, Vanuatu, *Brown s.n.* (NSW974485), KM220086, KM220154, KM220223; **R. sp.** **NSW974485 -2**, Malaysia, *Kilgour 939* (NSW978177), KM220072, KM220139, KM220209; **R. varilobula** Castle, Dominica, *Hill NY21274* (NY), HM992374, HM992454, –.