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APPLICATION ARTICLE

Evaluation of six regions for their potential as DNA barcodes in epiphyllous liverworts from Thailand

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PREMISE OF THE STUDY: Studies on the diversity of epiphyllous bryophytes have been limited because of minute and incomplete specimens and a lack of taxonomic expertise. The recent development of the DNA barcoding approach has allowed taxon identification and species discovery of many obscure groups of organisms.

METHODS: With DNA extractions from 99 samples of 16 species, we compared the efficiencies of six DNA markers (*rbcL*, *matK*, *trnL-F*, *psbA*, ITS1, and ITS2) in their ability to amplify, using a standard set of primers, as well as their discriminatory power, using distance-based and tree-based approaches with nucleotide data.

RESULTS: The amplification success was relatively high (70–90%) with all of the markers, except for *matK*, which yielded no success. The barcoding gap, as calculated from the difference between inter- and intraspecific genetic distances, was the highest in ITS2, whereas the highest numbers of monophyletic groups were found with ITS2 and *rbcL*.

DISCUSSION: *rbcL* should be used as a main barcoding marker with the addition of ITS2 for epiphyllous species. The development of DNA barcoding as a tool for quantifying species diversity will provide a rapid and reliable identification tool for epiphyllous bryophytes.

KEY WORDS biodiversity; ITS region; liverworts; molecular markers; *rbcL* region.

Since its formal introduction, the concept of DNA barcoding as a tool for rapid taxon identification has continued to garner interest from the scientific community (Hebert et al., 2003; Hollingsworth et al., 2011). Although the use of molecular data to identify species is not new, remarkable successes using a single standardized region in taxon identification of animals (Hebert et al., 2003) and fungi (Schoch et al., 2014) have led to novel approaches in biodiversity inventories, as barcoding enhances the ability of taxonomists to gain more integrative insights into species delimitation (Hebert et al., 2004; Schindel and Miller, 2005; Pons et al., 2006). DNA barcoding has allowed a wide range of applications from authentication of traded plants and animals (Jiang et al., 2006; Phoolcharoen and Sukrong, 2012; Osathanunkul et al., 2015) to large-scale ecological studies without obtaining the whole organisms, or even their tissue samples (Bohmann et al., 2014).

Leaf-colonizing (epiphyllous) bryophytes offer an exciting system to test the utility of DNA barcoding. Over a thousand species of bryophytes from various taxonomic groups of mosses and liverworts can be epiphyllous. Ubiquitous in tropical ecosystems, epiphyllous bryophytes are often found on economically important plants, such as coffee and mangosteen (Roskoski, 1980; Zhu and So, 2001; Kraichak and Yaungthong, 2012). They also provide an excellent system for studying species assembly processes because each leaf represents a spatially and temporally discrete unit, and a large number of communities from host leaves can alleviate statistical power problems, which frequently hamper community assembly studies (Leibold et al., 2004; Zartman and Nascimento, 2006). Despite these features, studies on epiphyllous bryophytes have been somewhat limited. Aside from taxonomic challenges, specimens of these bryophytes are often minute and lack reproductive structures required for morphological identification. Although many bryologists have avoided working with this group, a few taxonomists who work on epiphyllous bryophytes have discovered a high level of undescribed genetic diversity (Gradstein et al., 2011; Yu et al., 2013a, 2013b) and a number of species new to science (Zhu and So, 1998; Pócs, 2011, 2012a, 2012b). The application of the DNA barcoding approach will facilitate diversity inventories of this fascinating but underappreciated group of epiphytes.

Unlike animals, bacteria, or fungi, a standardized barcoding region for land plants, including bryophytes, is far from settled. In 2009, the Consortium for the Barcode of Life (CBOL) Plant Working Group published a meta-analysis of barcoding efficiency

of individual major DNA markers and recommended two protein-coding plastid regions, *matK* and *rbcL*, as standard markers for land plants. The potentially high discriminatory power of *matK* is hindered by the need for group-specific primers, whereas *rbcL* demonstrates an impressively high success rate for amplifications across land plants, but is only mediocre in its ability to distinguish samples at the species level (CBOL Plant Working Group, 2009). Although it deviates from the original premise behind DNA barcoding, the multilocus approach with *matK* and *rbcL* was favored for the complementary potential of these two loci and eventually was approved as a standard set of barcoding regions for all land plants, with a provision that supplementary markers should also be studied. Among the proposed supplementary barcoding regions, the nuclear internal transcribed spacer (ITS hereafter; Li et al., 2011) and *trnK-psbA-trnH* (*psbA* hereafter) (Kress et al., 2005) are the most promising additions to the multilocus data set for DNA barcoding of land plants (Hollingsworth, 2011).

In bryological studies, *rbcL* and *matK* have rather limited success as barcoding regions, due to their low amplification rates and a lower variation among sequences below the rank of family (CBOL Plant Working Group, 2009). Two other regions have emerged as more promising candidates for the barcoding of bryophytes: *trnL-F* and ITS (Stech and Quandt, 2010). These regions are consistently amplified and yield high-quality sequences. The *trnL-F* spacer, in particular, has been popular among molecular ecologists, as smaller parts of the region can be amplified from highly degraded DNA obtained from herbarium specimens and environmental sampling (Taberlet et al., 2006; Hollingsworth et al., 2011). Most *trnL-F* and ITS sequences of bryophytes are the products of phylogenetic studies, so only a few studies have directly investigated their discriminatory power and reported relatively high resolution at the rank of species (Liu et al., 2010; Bell et al., 2011).

To find the best candidate loci for barcoding epiphyllous bryophytes, this study evaluated the efficiency of five candidate plant barcoding markers (*rbcL*, *matK*, *trnL-F*, *psbA*, and ITS) in distinguishing a subset of epiphyllous bryophyte species from Thailand. These markers were amplified and sequenced, using a standard set of primers for bryophytes, to assess their amplification successes. Then, the nucleotide data were subjected to distance- and tree-based analyses to determine their discriminatory power among the studied species.

METHODS

Taxon sampling and morphological identification

A total of 99 samples from 16 species of epiphyllous bryophytes from Thailand were selected for DNA sequencing (Appendix 1). Because an epiphyllous habit is typical of the Lejeuneaceae, 15 species belonged to that family, while *Radula acuminata* Stephani belongs to Radulaceae. The bryophyte tissue came from the dry preserved collection of leaves from previous studies in Ranong (Kraichak and Yaungthong, 2012) and Trat provinces (Kraichak, 2015), as well as from the current study in various locations in Thailand (Appendix 1). All bryophyte specimens were identified to species according to Zhu and So (2001), through examination under a dissecting and a compound microscope, based on morphological descriptions and keys (Jovet-Ast, 1953, 1967; Tixier, 1985; Zhu and So, 2001). Voucher host plant specimens with additional bryophyte individuals were photographed for future reference. Vouchers were deposited in the herbarium at the Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand (Appendix 1). For each species, a minimum of two samples from different host leaves was acquired.

DNA isolation, amplification, and purification

Genomic DNA was isolated from dried plant material using the innuPREP Plant DNA Kit (Analytik Jena, Jena, Germany), following the manufacturer's protocol. We selected five DNA markers, including four chloroplast markers (*trnL-F*, *rbcL*, *matK*, and *trnH-psbA*) and one nuclear marker (ITS), based on their previous uses in barcoding and phylogenetic studies in bryophytes (reviewed in Stech and Quandt, 2010). Because of variable performance in past studies (Hartmann et al., 2006), we amplified and evaluated two regions of ITS: ITS1 (18S-ITS1-5.8S) and ITS2 (5.8S-ITS-26S). The chosen DNA markers were amplified with the following primers: (1) *trnL-F*: trnL/trnF-C and trnL-trnF-F (Taberlet et al., 1991); (2) *rbcL*: rbcL-640-F and rbcL-1200-R (Gradstein et al., 2006); (3) *matK*: RBGE-LIV-F1A and RBGE-LIV-R1A (Bell et al., 2011); (4) *trnH-psbA*: trnK2F and psbA576R (Forrest et al., 2006); and (5) ITS with two sets of primers: Bryo18SF–Bryo5.8R for ITS1, and Bryo5.8SF–Bryo26SR for ITS2 (Hartmann et al., 2006).

Each 25-μL reaction contained 9.5 μL of nuclease-free water, 2.5 μL of OnePCR Plus mix (GeneDireX, Las Vegas, Nevada, USA), 2.5 μL of forward and reverse primers each, and 1 μL of genomic DNA. The PCR thermocycling conditions were specific to each primer pair. For *trnL-F*, the cycle had initial denaturation at 92°C for 2 min; 30 cycles of denaturation at 92°C for 1 min, annealing at 51°C for 50 s, and elongation at 72°C for 90 s; with a final elongation at 72°C for 10 min. For *rbcL*, the cycle had initial denaturation at 94°C for 4 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 50 s, and elongation at 72°C for 90 s; with a final elongation at 72°C for 10 min. For *matK*, the cycle had initial denaturation at 94°C for 4 min; 10 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and elongation at 72°C for 1 min; 25 cycles of denaturation at 94°C for 45 s, annealing at 48°C for 45 s, and elongation at 72°C for 1 min; and a final elongation at 72°C for 10 min. For *trnH-psbA*, the cycle had initial denaturation at 94°C for 1 min; 35 cycles of denaturation at 93°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 3 min; with a final elongation at 72°C for 7 min. For ITS1, the cycle had initial denaturation at 96°C for 3 min; 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 2 min, and elongation at 72°C for 3 min; with a final elongation at 72°C for 5 min. For ITS2, the cycle had initial denaturation at 94°C for 75 s; 35 cycles of denaturation at 95°C for 35 s, annealing at 55°C for 55 s, and elongation at 72°C for 42 s; with a final elongation at 72°C for 10 min. To assess the universality of these primers, we did not optimize the PCR conditions for individual taxa.

The PCR products were visualized on a 1% ethidium bromide–free agarose gel under UV light and then purified using USB ExoSAP-IT PCR Product Cleanup (Applied Biosystems, Santa Clara, California, USA), following the manufacturer's instructions. The complementary strands were sequenced from the cleaned PCR products using the same primers as for amplifications. Sequencing reactions were performed with BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) using the provided instructions. The samples were then run on an ABI 3730 automated

sequencer at the Pritzker Laboratory for Molecular Systematics at the Field Museum (Chicago, Illinois, USA).

Sequence assembly and multiple sequence alignment

Resulting contigs and associated chromatograms were manually inspected, edited, and assembled using the program Geneious version 8.0.3 (Biomatters Ltd., Auckland, New Zealand). The identities of these sequences were examined using a "megaBLAST" search in the GenBank nucleotide database. For each marker, the verified sequences were aligned with the MUSCLE (Edgar, 2004) protocol through a Geneious plug-in. The protocol was run for a maximum of 10 iterations with the first iteration using kmer4_6 distance and the CLUSTALW sequence weighting scheme, and the subsequent iterations were run with pctid_kimura distance and the CLUSTALW sequence weighting scheme. The resulting alignments were manually examined to remove ambiguous positions and gaps and were exported as FASTA files for further analyses. The sequences were submitted to the GenBank and BOLD (Barcode of Life Data Systems) (Ratnasingham and Hebert, 2007) databases (Appendix 1).

Evaluation of barcoding efficiency

To evaluate the efficiency of the markers as barcoding regions, the following criteria were used: (1) universality, (2) information content, and (3) discriminatory power (Hollingsworth, 2011). For universality, amplification successes were counted and divided by the total number of amplification attempts. For information content, the alignment length, number of variable positions, and GC content were compared. As for the discriminatory power, distance-based and tree-based approaches were employed to evaluate the markers' ability to distinguish the species with the sequence data. First, the distance-based approach used genetic distance to determine whether the nearest neighbor was conspecific (the nearest neighbor test; Meier et al., 2006) and whether there was a sufficient gap between intraspecific and interspecific distances. The genetic distance among individual sequences was calculated using the Kimura 2-parameter (K2P) model, a standard model in barcoding studies that has been shown to be appropriate for elucidating the barcoding gap with a standard barcode region (Hebert et al., 2003; Brown et al., 2012), with the function dist.dna in the R package "ape" (Paradis et al., 2004; R Core Development Team, 2013).

The nearest neighbor test calculates the genetic distances among all the studied sequences and identifies whether sequences with the shortest distance ("nearest neighbor") are of the same species. The percentage of correct identification was calculated from the number of sequences with a conspecific nearest neighbor divided by the total number of sequences. The test was performed with the function "nearNeighbor" in the R package "spider" (Brown et al., 2012). The barcoding gap was calculated from the difference between the nearest non-conspecific and the maximum conspecific distances. These distances were determined with the functions "nonConDist" and "maxInDist" in the R package "spider" (Brown et al., 2012). The Kruskal–Wallis test was also applied to determine whether the barcoding gap was significantly different among the chosen markers. A marker with high discriminatory power should have a high percent of correct identifications from the nearest neighbor test and a positive value for the barcoding gap.

Second, the tree-based approach used the markers to reconstruct phylogenies of the studied species. In this study, neighbor-joining (NJ) and maximum likelihood (ML) phylogenetic trees were reconstructed. The NJ trees were reconstructed using the K2P distance and the function "nj" in the R package "ape" (Paradis et al., 2004). A total of 1000 pseudo-replicates was used to calculate bootstrap support for each node. A maximum likelihood phylogenetic reconstruction for each region was performed with the program RAxML-HPC BlackBox version 8.1.11 (Stamatakis et al., 2008) on the online computing facility CIPRES (Miller et al., 2010). Following the model selection results for all of the loci from jModelTest 2 (Darriba et al., 2012), the "GTRGAMMA" model was used to perform likelihood searches to find the optimal tree and 1000 pseudo-replicates were used to calculate bootstrap support for each node. The number of monophyletic groups was counted using the function "monophyly" in the R package "spider" (Brown et al., 2012). Phylogenetic reconstruction with a small data set can often result in poorly resolved relationships among the species and is often avoided in a systematic study. However, the main focus of a tree-based test for barcoding efficiency is to determine the ability of a marker to recover monophyly among sequences of the same species, and the relationships among the studied taxa are not used as a criterion for the discriminating power of a barcoding marker (Hebert et al., 2003; Brown et al., 2012).

RESULTS

DNA extraction and amplification success

All of the studied markers, except for *matK*, were successfully amplified for 76.84% to 90.53% of the samples (Table 1). The *psbA* spacer had the highest success (90.53%), whereas the amplification of *matK* yielded no products, despite repeated attempts. From the PCR products, 304 high-quality sequences were obtained and used for the subsequent analyses. The alignment length ranged between 492 and 632 bp with 30.22% to 89.23% of positions variable and the GC content between 31.59% and 59.29% (Table 1).

TABLE 1. PCR success and characteristics of the studied markers in epiphyllous bryophytes from Thailand.

Markers	PCR success (%)	No. of sequences[a]	No. of species[b]	Alignment length (bp)	Variable site (%)	GC content (%)
matK	0	—	—	—	—	—
ITS1	85.26	46	9	526	65.21	59.29
ITS2	76.84	49	11	492	89.23	59.12
trnH-psbA	90.53	79	13	632	30.22	35.79
rbcL	87.37	56	9	522	49.62	38.82
trnL-F	86.32	74	12	430	58.84	31.59

[a]Number of high-quality sequences used in the analysis. The total number of samples included in the study was 99.
[b]The total number of species included in the study was 16.

Distance-based evaluation

The distribution of barcoding gaps showed that the interspecific distances were mostly greater than intraspecific distances in ITS2 and *rbcL* (positive barcoding gap), while the intraspecific distances were mostly greater than interspecific distances in *trnL-F* and *psbA* (negative barcoding gap; Fig. 1A). For ITS1, roughly half of the interspecific distances were greater than intraspecific distances. For ITS2 and *rbcL*, most differences between inter- and intraspecific distance were close to zero, whereas differences were more widely distributed in ITS2 (Fig. 1A). The barcoding gaps varied significantly among the studied markers (Kruskal–Wallis test, $P < 0.01$). The nearest neighbor test showed the highest percentage of conspecific nearest neighbors in ITS2 (100%) and the lowest percentage in *psbA* (72.15%) (Fig. 1B).

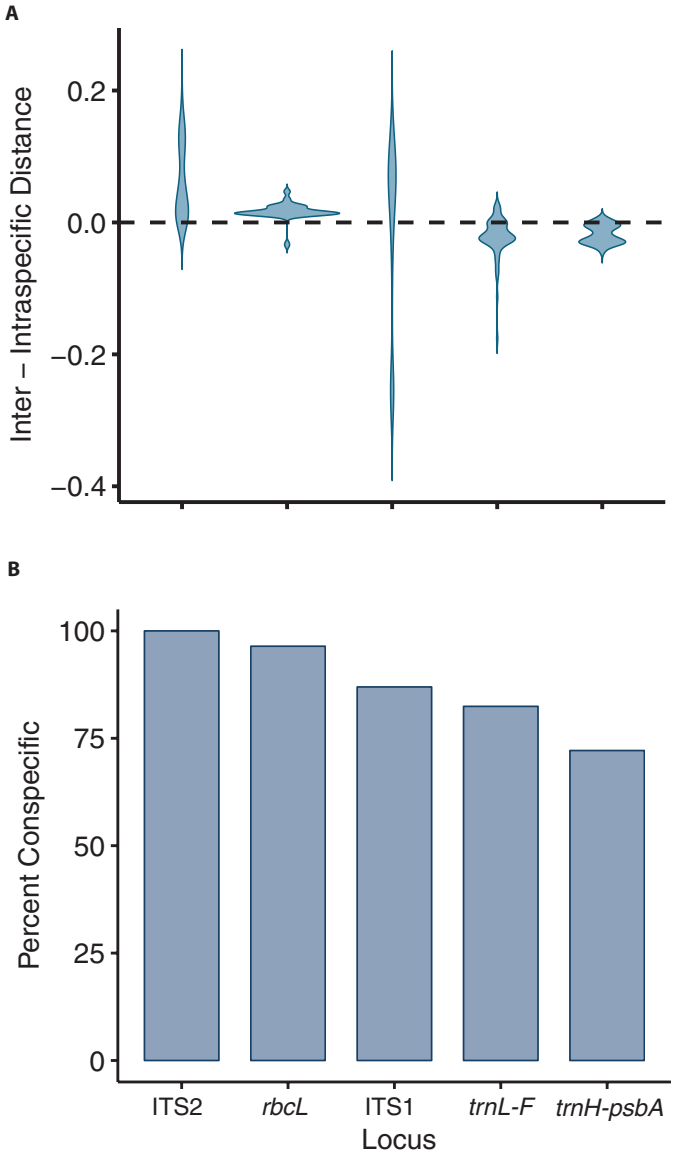

FIGURE 1. Distance-based comparison of efficiency among the studied barcoding markers for epiphyllous liverworts from Thailand. (A) Distribution of barcoding gap, as defined by the difference between the minimum non-conspecific distance and the maximum conspecific distance. (B) The percentage of correct identifications from the nearest neighbor test.

Tree-based evaluation

ITS2 recovered the highest percentage of monophyletic groups in both NJ and ML reconstructions at 100% and 90%, respectively. The *rbcL* phylogeny recovered 77.78% and 88.89% of the monophyletic groups in NJ and ML reconstructions, respectively. The rest of the markers recovered less than half of the monophyletic groups. The *psbA* spacer yielded the lowest number of monophyletic groups at 7.69% in the ML reconstruction, whereas *trnL-F* recovered the lowest number of monophyletic groups at 16.67% in the NJ reconstruction (Fig. 2; Appendices S1, S2).

DISCUSSION

The current study evaluated the efficiencies of six barcoding markers in distinguishing epiphyllous bryophyte species from Thailand. The amplification success was similar among most of the markers, with the notable exception of *matK*, suggesting that these markers can be successfully amplified equally well with a proper protocol and set of primers. However, the discriminatory power varied substantially, with *rbcL* and ITS2 showing the highest discriminatory power. These markers have also been proposed as part of the standard barcoding set for land plants. However, for epiphyllous bryophytes, *rbcL* and ITS2 displayed different strengths and weaknesses in their barcoding applications.

In the proposal for the standardized barcoding regions for land plants, *rbcL* was proposed, along with *matK*, as a core barcoding region (Kress and Erickson, 2007; CBOL Plant Working Group, 2009). Some of the key attributes for *rbcL* are its universality across the plant kingdom and the ease of alignment. Nevertheless,

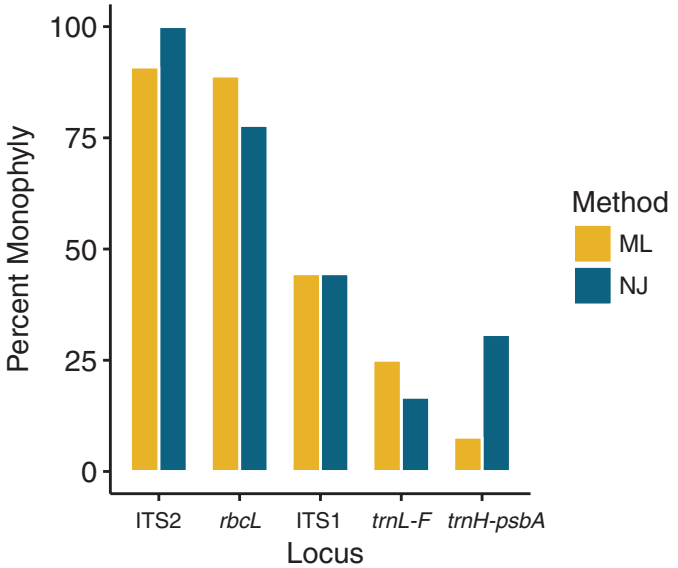

FIGURE 2. Tree-based comparison of efficiency among the studied barcoding markers for epiphyllous liverworts from Thailand, using the percentage of monophyletic groups recovered from the neighbor-joining (NJ = dark blue) and the maximum likelihood (ML = yellow) phylogenetic reconstructions.

it suffers from moderate discriminatory power in many groups and has somewhat lower universality in bryophytes (CBOL Plant Working Group, 2009; Hollingsworth et al., 2009). In our study, *rbcL* was similar to ITS2 in its high discriminatory power among the epiphyllous species, even though the barcoding gap was close to zero. Such a small gap is the direct result of the conserved nature of protein-coding genes, such as *rbcL*, which makes them easy to align and simultaneously less suitable for providing resolution at the species rank (Stech and Quandt, 2010; Hassel et al., 2013). In our case, this small barcoding gap reduced the number of successes in the distance-based approaches. Moreover, we obtained *rbcL* sequences from fewer species, suggesting a potential issue of universality of primers for this region. Existing *rbcL* data for bryophytes in databases are uneven across the group because it is not a typical marker for systematic studies and has only been thoroughly sampled in specific groups (Stech and Quandt, 2010). This uneven distribution of data has made it difficult to test and develop universal primers for bryophytes to date, but the gradually increasing amount of data for *rbcL*, both from single-locus and genomic studies, will allow us to see the full potential of *rbcL* as a barcoding marker for bryophytes in the future (Forrest et al., 2006; Hassel et al., 2013; Myszczyński et al., 2017).

ITS has been widely used in plant systematics and has only recently begun to gain traction as a barcoding region for land plants. In an early attempt to standardize barcoding regions, ITS was proposed as the most promising marker from a relatively small data set from flowering plants (Kress et al., 2005). However, issues of multiple copies and fungal contamination led to a decline in the use of ITS as a barcoding region (Hollingsworth, 2011; Cheng et al., 2016). However, ITS has since reemerged as a barcoding region with the separate consideration of two regions (ITS1 and ITS2), along with studies with a broader taxon sampling (Hollingsworth et al., 2009; Liu et al., 2010; Li et al., 2011). Many recent studies have included ITS2 in plant barcoding and even support ITS2 as the best candidate for plant barcoding (e.g., Yao et al., 2010; Li et al., 2011; Feng et al., 2015). Our study similarly demonstrated that ITS2 had the highest discriminatory power among the tested regions for epiphyllous bryophytes, although the sequences were difficult to align and often of low quality due to the low specificity of primers. Although we observed large barcoding gaps, we also observed a large variation in the inter- and intraspecific distances, a problem that can potentially be worsened with broader taxon sampling. Despite these difficulties, the use of ITS2 can be beneficial for advancing barcoding studies in bryophytes, as a relatively large amount of data already exist in global databases from phylogenetic studies (Stech and Quandt, 2010). The recent development of universal plant-specific markers for ITS (Cheng et al., 2016) will most likely enhance our ability to produce data from ITS2 and increase its use as a barcoding region.

For the other markers, their subpar performance in bryophytes was not entirely surprising. For *matK*, data have been extremely difficult to obtain in bryophytes and ferns (CBOL Plant Working Group, 2009) due to secondary structures at the priming sites of this marker (Wicke and Quandt, 2009). Even with attempts to design specific *matK* primers for bryophytes, success has been limited to only a few groups (Wicke and Quandt, 2009; Bell et al., 2011), as reflected in a small amount of existing data of *matK* for bryophytes in global nucleotide databases. In our study, the amplification success for *matK* was zero with every tested primer set. Combinations of PCR conditions were also attempted for *matK* on our genomic DNA that could be amplified for other markers, suggesting the ongoing problem with priming sites for this region. Therefore, at this point, it is not clear whether the discriminatory power of *matK* in flowering plants will extend to bryophytes. The other two markers, *psbA* and *trnL-F*, amplified well for epiphyllous bryophytes but showed only limited success in distinguishing species. Although *psbA* has been used in phylogenetic studies of various groups of bryophytes (Shaw et al., 2003; Forrest et al., 2006) and has a high discriminatory power within flowering plants, it is considered unsuitable as a standalone barcoding region, especially for pleurocarpous mosses (Stech and Quandt, 2010). We provided yet another example of how the conserved nature of *psbA* sequences among bryophyte species renders this marker less optimal for barcoding purposes. Finally, *trnL-F* is one of the most popular phylogenetic markers and was expected to be a prime candidate for barcoding in bryophytes (Taberlet et al., 2006; Stech and Quandt, 2010). However, owing to its short length (the shortest alignment in our study), it can only offer a limited amount of information for species identification (Liu et al., 2010; Stech and Quandt, 2010; Bell et al., 2011).

From our results, ITS2 and *rcbL* exhibited the greatest potential for discriminating epiphyllous liverwort species with a DNA barcoding approach, owing to their primer universality, sequencing success, and high discriminatory power. However, these two markers still had some limitations: *rbcL* showed small differences between intra- and interspecific genetic distances, whereas the ITS2 sequences showed problems with low sequence quality and resulted in numerous gaps in the alignment, making it difficult to unambiguously use the data. In future work, a broader selection of species will validate the efficiency of these markers as barcoding regions for bryophytes in Thailand and elsewhere.

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DATA ACCESSIBILITY

The sequence data are deposited in and accessible from the U.S. National Center for Biotechnology Information's GenBank database (https://www.ncbi.nlm.nih.gov; accession no. MH579787–MH580157).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Single-locus neighbor-joining trees of five studied markers for studied epiphyllous bryophyte species: ITS1 (A), ITS2 (B), *psbA* (C), *rbcL* (D), and *trnL-F* (E). Black circles at the nodes indicate nodes with bootstrap support greater than 70.

APPENDIX S2. Single-locus maximum likelihood trees of five studied markers for studied epiphyllous bryophyte species: ITS1 (A), ITS2 (B), *psbA* (C), *rbcL* (D), and *trnL-F* (E). Black circles at the nodes indicate nodes with bootstrap support greater than 70.

LITERATURE CITED

Bell, D., D. G. Long, A. D. Forrest, M. L. Hollingsworth, H. H. Blom, and P. M. Hollingsworth. 2011. DNA barcoding of European *Herbertus* (Marchantiopsida, Herbertaceae) and the discovery and description of a new species. *Molecular Ecology Resources* 12: 36–47.

Bohmann, K., A. Evans, M. T. P. Gilbert, G. R. Carvalho, S. Creer, M. Knapp, D. W. Yu, and M. de Bruyn. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution* 29: 358–367.

Brown, S. D. J., R. A. Collins, S. Boyer, M.-C. Lefort, J. Malumbres-Olarte, C. J. Vink, and R. H. Cruickshank. 2012. Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources* 12: 562–565.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences USA* 106: 12794–12797.

Cheng, T., C. Xu, L. Lei, C. Li, Y. Zhang, and S. Zhou. 2016. Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources* 16: 138–149.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Edgar, R. C. 2004. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 113.

Feng, S., Y. Jiang, S. Wang, M. Jiang, Z. Chen, Q. Ying, and H. Wang. 2015. Molecular identification of *Dendrobium* species (Orchidaceae) based on the DNA barcode ITS2 region and its application for phylogenetic study. *International Journal of Molecular Sciences* 16: 21975–21988.

Forrest, L. L., E. C. Davis, D. G. Long, B. J. Crandall-Stotler, A. Clark, and M. L. Hollingsworth. 2006. Unraveling the evolutionary history of the liverworts (Marchantiophyta): Multiple taxa, genomes and analyses. *Bryologist* 109: 303–334.

Gradstein, S. R., R. Wilson, A. L. Ilkiu-Borges, and J. Heinrichs. 2006. Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Botanical Journal of the Linnean Society* 151: 293–308.

Gradstein, S. R., A. L. Iilkiu-Borges, and A. Vanderpoorten. 2011. Habitat specialization triggers the evolution of unusual morphologies: The case of *Cololejeunea stotleriana* sp. nov. from Ecuador. *Bryologist* 114: 9–22.

Hartmann, F. A., R. Wilson, S. R. Gradstein, H. Schneider, and J. Heinrichs. 2006. Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *International Journal of Plant Sciences* 167: 1205–1214.

Hassel, K., R. Segreto, and T. Ekrem. 2013. Restricted variation in plant barcoding markers limits identification in closely related bryophyte species. *Molecular Ecology Resources* 13: 1047–1057.

Hebert, P. D. N., S. Ratnasingham, and J. R. de Waard. 2003. Barcoding animal life: Cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B. Biological Sciences* 270 Suppl: S96–S99.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences USA* 101: 14812–14817.

Hollingsworth, M. L., A. Andra Clark, L. L. Forrest, J. Richardson, R. T. Pennington, D. G. Long, R. Cowan, et al. 2009. Selecting barcoding loci for plants: Evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Molecular Ecology Resources* 9: 439–457.

Hollingsworth, P. M. 2011. Refining the DNA barcode for land plants. *Proceedings of the National Academy of Sciences USA* 108: 19451–19452.

Hollingsworth, P. M., S. W. Graham, and D. P. Little. 2011. Choosing and using a plant DNA barcode. *PLoS ONE* 6: e19254.

Jiang, H., Z. Xie, H. J. Koo, S. P. McLaughlin, B. N. Timmermann, and D. R. Gang. 2006. Metabolic profiling and phylogenetic analysis of medicinal *Zingiber* species: Tools for authentication of ginger (*Zingiber officinale* Rosc.). *Phytochemistry* 67: 1673–1685.

Jovet-Ast, S. 1953. Le genre *Colura*: Hépatiques: Lejeuneaceae, Diplasiae. *Revue Bryologique et Lichénologique* 22: 206–312.

Jovet-Ast, S. 1967. *Colura* récoltes du Pakistan aux Philippines par Pierre Tixier. *Revue Bryologique et Lichénologique* 35: 138–142.

Kraichak, E. 2015. Relationships between moisture and community structure of epiphyllous bryophytes. Ninth Botanical Conference of Thailand BCT–Stepping toward Global Forum, Bangkok, Thailand.

Kraichak, E., and K. Yaungthong. 2012. Ecology and diversity of epiphyllous bryphytes in the Naka Wildlife Sanctuary, Ranong Province: A preliminary study. Report to Department of National Parks, Wildlife, and Plant Conservation No. 0907.1/13812, Bangkok, Thailand.

Kress, W. J., and D. L. Erickson. 2007. A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS ONE* 2: e508.

Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weigt, and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences USA* 102: 8369–8374.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, et al. 2004. The metacommunity concept: A framework for multi-scale community ecology. *Ecology Letters* 7: 601–613.

Li, D.-Z., L.-M. Gao, H.-T. Li, H. Wang, X. J. Ge, J.-Q. Liu, Z.-D. Chen, et al. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences USA* 108: 19641–19646.

Liu, Y., H. F. Yan, T. Cao, and X. J. Ge. 2010. Evaluation of 10 plant barcodes in Bryophyta (Mosses). *Journal of Systematics and Evolution* 48: 36–46.

Meier, R., K. Shiyang, G. Vaidya, and P. K. L. Ng. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana, USA.

Myszczyński, K., A. Bączkiewicz, K. Buczkowska, M. Ślipiko, M. Szczecińska, and J. Sawicki. 2017. The extraordinary variation of the organellar genomes of the *Aneura pinguis* revealed advanced cryptic speciation of the early land plants. *Scientific Reports* 7: 1–12.

Osathanunkul, M., P. Madesis, and H. de Boer. 2015. Bar-HRM for authentication of plant-based medicines: Evaluation of three medicinal products derived from Acanthaceae species. *PLoS ONE* 10: e0128476.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics (Oxford, England)* 20: 289–290.

Phoolcharoen, W., and S. Sukrong. 2012. Molecular analysis of *Vitex* species using candidate DNA barcoding and PCR-RFLP of the *matK* gene for authentication of *Vitex glabrata*. *Natural Product Communications* 8: 125–128.

Pócs, T. 2011. New or little known epiphyllous liverworts, XIV. The genus *Colura* (Lejeuneaceae) in São Tomé Island, with the description of *Colura thomeensis* sp. nov. *Bryologist* 114: 362–366.

Pócs, T. 2012a. Bryophytes from the Fiji Islands, VI. The genus *Cololejeunea* Raddi (Jungermanniopsida), with the description of seven new species. *Acta Botanica Hungarica* 54: 145–188.

Pócs, T. 2012b. New or little known epiphyllous liverworts, XVI. A small collection from Laos. *Acta Biologica Plantarum Agriensis* 2: 5–10.

Pons, J., T. Barraclough, J. Gomez-Zurita, A. Cardoso, D. Duran, S. Hazell, S. Kamoun, et al. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595–609.

R Core Development Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org [accessed 30 July 2018].

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364.

Roskoski, J. P. 1980. N_2 fixation (C_2H_2 reduction) by epiphylls on coffee, *Coffea arabica*. *Microbial Ecology* 6: 349–355.

Schindel, D. E., and S. E. Miller. 2005. DNA barcoding a useful tool for taxonomists. *Nature* 435: 17.

Schoch, C. L., B. Robbertse, V. Robert, D. Vu, G. Cardinali, L. Irinyi, W. Meyer, et al. 2014. Finding needles in haystacks: Linking scientific names, reference specimens and molecular data for Fungi. *Database* 2014: 1–21.

Shaw, A. J., C. J. Cox, and S. B. Boles. 2003. Polarity of peatmoss (*Sphagnum*) evolution: Who says bryophytes have no roots? *American Journal of Botany* 90: 1777–1787.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.

Stech, M., and D. Quandt. 2010. 20,000 species and five key markers: The status of molecular bryophyte phylogenetics. *Phytotaxa* 9: 196.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Taberlet, P., E. Coissac, F. Pompanon, L. Gielly, C. Miquel, A. Valentini, T. Vermat, et al. 2006. Power and limitations of the chloroplast *trnL* (UAA) intron for plant DNA barcoding. *Nucleic Acids Research* 35: e14.

Tixier, P. 1985. Contribution à la connaissance des Cololejeunoideae. Bryophytorum Bibliotheca, vol. 27. J. Cramer, Vaduz, Liechtenstein.

Wicke, S., and D. Quandt. 2009. Universal primers for the amplification of the plastid *trnK*/*matK* region in land plants. *Anales del Jardín Botánico de Madrid* 66: 285–288.

Yao, H., J. Song, C. Liu, K. Luo, J. Han, Y. Li, X. Pang, et al. 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS ONE* 5: e13102.

Yu, Y., J. Heinrichs, R.-L. Zhu, and H. Schneider. 2013a. Empirical evidence supporting frequent cryptic speciation in epiphyllous liverworts: A case study of the *Cololejeunea lanciloba* complex. *PLoS ONE* 8: e84124.

Yu, Y., T. Pócs, A. Schäfer-Verwimp, J. Heinrichs, R.-L. Zhu, and H. Schneider. 2013b. Evidence for rampant homoplasy in the phylogeny of the epiphyllous liverwort genus *Cololejeunea* (Lejeuneaceae). *Systematic Botany* 38: 553–563.

Zartman, C. E., and H. E. M. Nascimento. 2006. Are habitat-tracking metacommunities dispersal limited? Inferences from abundance-occupancy patterns of epiphylls in Amazonian forest fragments. *Biological Conservation* 127: 46–54.

Zhu, R. L., and M. L. So. 1998. Two epiphyllous liverworts, *Cololejeunea dozyana* (Sande Lac.) Schiffn. and *Cololejeunea sigmoidea* Jovet-Ast & Tixier (Hepaticae, Lejeuneaceae), new to Taiwan. *Botanical Bulletin of Academia Sinica* 39: 125–130.

Zhu, R.-L., and M. L. So. 2001. Epiphyllous liverworts of China. Nova Hedwigia Beiheft, vol. 121. J. Cramer, Vaduz, Liechtenstein.

APPENDIX 1. List of specimens, their locality, and GenBank accession numbers for the sequences used in the study. Vouchers were deposited in the herbarium at the Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand.

							GenBank accession no.				
Specimen	Species	Province	Country	Latitude	Longitude	DNA no.	ITS1	ITS2	*psbA*	*rbcL*	*trnL-F*
EK1720	*Leptolejeunea elliptica*	Chumpon	Thailand	10°45′30″N	99°3′34″E	1	MH579856	MH579787	—	—	MH579920
EK1720A	*Cololejeunea tenella*	Chumpon	Thailand	10°45′30″N	99°3′34″E	2	MH579857	MH579788	—	—	MH579921
EK1707	*Colura ornata*	Chumpon	Thailand	10°45′30″N	99°3′34″E	3	—	MH579789	—	—	MH579922
EK1712	*Leptolejeunea epiphylla*	Chumpon	Thailand	10°45′30″N	99°3′34″E	4	MH579858	MH579790	—	—	MH579923
EK1719	*Caudalejeunea reniloba*	Chumpon	Thailand	10°45′30″N	99°3′34″E	5	MH579859	MH579791	MH580002	MH580131	MH579924
EK1723	*Cololejeunea lanciloba*	Uthaithani	Thailand	15°36′31″N	99°19′15″E	11	MH579860	—	—	MH580083	MH579925
EK893_1	*Radula acuminata*	Ranong	Thailand	9°22′31″N	98°23′53″E	13	—	—	—	—	MH579926
EK883_2	*Caudalejeunea reniloba*	Ranong	Thailand	9°22′31″N	98°23′53″E	15	—	MH579792	—	—	MH579927
EK1726	*Leptolejeunea elliptica*	Uthaithani	Thailand	15°36′31″N	99°19′15″E	16	—	—	—	MH580084	MH579928
EK1724	*Leptolejeunea elliptica*	Uthaithani	Thailand	15°36′31″N	99°19′15″E	17	—	—	—	—	MH579929
EKEP001	*Radula acuminata*	Pang-Nga	Thailand	9°2′32″N	98°26′55″E	29	—	MH579793	—	—	—
EKEP003	*Cololejeunea lanciloba*	Pang-Nga	Thailand	9°2′32″N	98°26′55″E	30	—	—	—	—	MH579930
EKEP002	*Radula acuminata*	Pang-Nga	Thailand	9°2′32″N	98°26′55″E	31	—	MH579794	—	—	MH579931
SRS071	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	32	MH579861	—	MH580003	MH580085	MH579932
SRS078	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	33	—	—	MH580004	MH580087	MH579933
SRS067	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	34	MH579862	—	MH580005	MH580088	MH579934
SRS070	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	36	—	—	MH580006	MH580089	—
SRS038	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	37	MH579863	—	MH580007	MH580093	MH579935

(*continues*)

APPENDIX 1. (continued)

Specimen	Species	Province	Country	Latitude	Longitude	DNA no.	GenBank accession no.				
							ITS1	ITS2	*psbA*	*rbcL*	*trnL-F*
SRS037	*Colura inflata*	Trat	Thailand	12°22′55″N	102°39′21″E	38	MH579864	—	MH580008	MH580094	MH579936
SRS046	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	39	MH579865	MH579795	MH580009	MH580090	MH579937
SRS034	*Colura inflata*	Trat	Thailand	12°22′55″N	102°39′21″E	40	—	MH579796	MH580010	MH580132	MH579938
SRS083	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	41	—	—	MH580011	MH580095	MH579939
SRS035	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	42	MH579866	MH579797	MH580012	MH580096	MH579940
SRS045	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	44	MH579867	MH579798	MH580013	MH580097	MH579941
SRS043	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	46	MH579868	—	MH580014	MH580133	MH579942
SRS095	*Colura ornata*	Trat	Thailand	12°22′55″N	102°39′21″E	47	MH579869	—	MH580015	MH580134	MH579943
JW002	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	48	—	MH579799	—	—	MH579944
SRS088	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	49	—	—	—	MH580098	—
EKE002	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	50	MH579870	—	MH580016	MH580099	MH579945
EKE001	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	51	—	MH579800	MH580017	MH580135	MH579946
SRS039	*Cololejeunea denticulata*	Trat	Thailand	12°22′55″N	102°39′21″E	52	MH579871	MH579801	MH580018	MH580091	MH579947
SRS087	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	53	MH579872	MH579802	MH580019	MH580100	MH579948
SRS027	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	55	—	—	MH580020	—	MH579949
SRS044	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	56	MH579873	MH579803	MH580021	MH580103	MH579950
SRS051	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	57	MH579874	MH579804	MH580022	MH580101	MH579951
JW001	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	58	MH579875	MH579805	MH580023	MH580136	MH579952
SRS011	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	60	MH579876	MH579806	MH580024	MH580086	MH579953
SRS012	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	61	—	MH579807	MH580025	MH580104	MH579954
SRS042	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	62	—	MH579808	MH580026	MH580105	MH579955
SRS026	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	63	—	MH579809	MH580027	MH580106	MH579956
SRS021	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	65	MH579877	MH579810	MH580028	MH580137	MH579957
SRS040	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	66	MH579878	—	MH580029	MH580138	MH579958
SRS016	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	67	—	MH579811	MH580030	MH580092	MH579959
SRS018	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	68	MH579879	MH579812	MH580031	MH580139	MH579960
SRS108	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	69	MH579880	—	MH580032	MH580140	MH579961
SRS079	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	70	—	—	MH580033	MH580107	MH579962
SRS097	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	71	—	MH579813	MH580034	MH580108	MH579963
SRS009A	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	73	MH579881	—	MH580035	MH580142	MH579964
SRS102	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	75	MH579882	MH579814	MH580036	MH580109	MH579965
SRS104	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	76	MH579883	MH579815	MH580037	MH580143	MH579966

(continues)

APPENDIX 1. (continued)

Specimen	Species	Province	Country	Latitude	Longitude	DNA no.	GenBank accession no.				
							ITS1	ITS2	*psbA*	*rbcL*	*trnL-F*
SRS106	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	77	MH579884	MH579816	MH580038	MH580144	MH579967
SRS006	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	78	MH579885	MH579817	MH580039	MH580145	MH579968
SRS081	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	79	—	—	MH580040	MH580111	MH579969
SRS105	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	80	MH579886	MH579818	MH580041	MH580146	MH579970
SRS107	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	81	MH579887	MH579819	MH580042	MH580147	MH579971
SRS004	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	82	MH579888	—	MH580043	MH580148	MH579972
SRS007	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	83	MH579889	MH579820	MH580044	MH580150	MH579973
SRS025	*Cololejeunea gottschei*	Trat	Thailand	12°22′55″N	102°39′21″E	84	MH579890	MH579821	MH580045	MH580151	MH579974
SRS025	*Cololejeunea tenella*	Trat	Thailand	12°22′55″N	102°39′21″E	85	—	—	MH580046	MH580112	MH579975
SRS002	*Cololejeunea denticulata*	Trat	Thailand	12°22′55″N	102°39′21″E	86	MH579891	—	MH580047	MH580152	—
SRS003	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	87	MH579892	—	MH580048	MH580113	MH579976
SRS033	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	88	MH579893	—	MH580049	MH580153	MH579977
SRS010	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	89	MH579894	—	MH580050	MH580149	MH579978
SRS013	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	90	MH579895	MH579822	MH580051	MH580154	MH579979
SRS009B	*Cololejeunea lanciloba*	Trat	Thailand	12°22′55″N	102°39′21″E	91	MH579896	—	MH580052	MH580141	MH579980
SRS014	*Caudalejeunea reniloba*	Trat	Thailand	12°22′55″N	102°39′21″E	92	MH579897	MH579823	MH580053	MH580155	MH579981
SRS023	*Cololejeunea indosinica*	Trat	Thailand	12°22′55″N	102°39′21″E	93	MH579898	MH579824	MH580054	MH580114	MH579982
SRS024	*Cololejeunea indosinica*	Trat	Thailand	12°22′55″N	102°39′21″E	94	—	—	MH580055	—	MH579983
EKE005	*Leptolejeunea epiphylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	95	MH579899	MH579825	MH580056	MH580156	—
EKE006	*Leptolejeunea epiphylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	96	MH579900	MH579826	MH580057	MH580115	MH579984
EKE007	*Leptolejeunea epiphylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	97	MH579901	MH579827	MH580058	MH580116	MH579985
EKE008	*Cololejeunea tenella*	Ranong	Thailand	9°22′31″N	98°23′53″E	98	—	MH579828	MH580059	—	MH579986
EKE009	*Cololejeunea tenella*	Ranong	Thailand	9°22′31″N	98°23′53″E	99	MH579902	MH579829	MH580060	MH580117	MH579987
EKE010	*Cololejeunea tenella*	Ranong	Thailand	9°22′31″N	98°23′53″E	100	—	MH579830	MH580061	—	—
EKE011	*Leptolejeunea epiphylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	101	MH579903	MH579831	MH580062	MH580118	MH579988
EKE012	*Cololejeunea tenella*	Ranong	Thailand	9°22′31″N	98°23′53″E	102	—	MH579832	MH580063	—	MH579989
EKE013	*Cololejeunea goebelii*	Ranong	Thailand	9°22′31″N	98°23′53″E	103	MH579904	MH579833	MH580064	MH580119	MH579990
EKE014	*Cololejeunea goebelii*	Ranong	Thailand	9°22′31″N	98°23′53″E	104	MH579905	MH579834	MH580065	MH580120	MH579991
EKE015	*Cololejeunea goebelii*	Ranong	Thailand	9°22′31″N	98°23′53″E	105	—	MH579835	MH580066	MH580121	MH579992
EKE016	*Cololejeunea goebelii*	Ranong	Thailand	9°22′31″N	98°23′53″E	106	MH579906	MH579836	MH580067	MH580122	—

(continues)

APPENDIX 1. (continued)

Specimen	Species	Province	Country	Latitude	Longitude	DNA no.	GenBank accession no.				
							ITS1	ITS2	*psbA*	*rbcL*	*trnL-F*
EKE017	*Leptolejeunea maculata*	Ranong	Thailand	9°22′31″N	98°23′53″E	107	MH579907	MH579837	MH580068	MH580123	MH579993
EKE018	*Leptolejeunea maculata*	Ranong	Thailand	9°22′31″N	98°23′53″E	108	MH579908	MH579838	MH580069	MH580124	MH579994
EKE019	*Leptolejeunea maculata*	Ranong	Thailand	9°22′31″N	98°23′53″E	109	MH579909	MH579839	MH580070	MH580125	MH579995
EKE020	*Leptolejeunea maculata*	Ranong	Thailand	9°22′31″N	98°23′53″E	110	—	MH579840	MH580071	MH580126	—
EKE021	*Lejeunea anisophylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	111	—	MH579841	MH580072	—	—
EKE022	*Lejeunea anisophylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	112	MH579910	MH579842	MH580073	MH580127	—
EKE023	*Lejeunea anisophylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	113	MH579911	MH579843	MH580074	MH580128	MH579996
EKE024	*Lejeunea anisophylla*	Ranong	Thailand	9°22′31″N	98°23′53″E	114	MH579912	MH579844	—	—	—
EKE025	*Cololejeunea lanciloba*	Ranong	Thailand	9°22′31″N	98°23′53″E	115	MH579913	MH579845	MH580075	MH580110	MH579997
EKE026	*Cololejeunea lanciloba*	Ranong	Thailand	9°22′31″N	98°23′53″E	116	MH579914	MH579846	MH580076	—	MH579998
EKE028	*Cololejeunea lanciloba*	Ranong	Thailand	9°22′31″N	98°23′53″E	118	MH579915	MH579847	MH580077	MH580102	MH579999
JW003	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	119	—	MH579848	—	—	—
JW002	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	120	MH579916	MH579849	MH580078	—	MH580000
JW004	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	121	MH579917	MH579850	MH580079	MH580129	—
JW005	*Diplasiolejeunea cavifolia*	Ranong	Thailand	10°30′49″N	98°54′26″E	122	—	MH579851	—	—	—
SRS091A	*Cololejeunea* sp.	Trat	Thailand	12°22′55″N	102°39′21″E	123	MH579918	MH579852	MH580080	MH580130	MH580001
SRS091B	*Cololejeunea* sp.	Trat	Thailand	12°22′55″N	102°39′21″E	124	—	MH579853	MH580081	MH580157	—
SRS091C	*Cololejeunea* sp.	Trat	Thailand	12°22′55″N	102°39′21″E	125	—	MH579854	—	—	—
SRS091D	*Cololejeunea* sp.	Trat	Thailand	12°22′55″N	102°39′21″E	126	MH579919	MH579855	MH580082	—	—