MOLECULAR PHYLOGENY OF *MACROSOLEN* (BLUME) RCHB. (LORANTHACEAE) FROM VIETNAM BASED ON MOLECULAR DATA

Le Chi Toan*
Hanoi Pedagogical University 2

ARTICLE INFO

Received: 16/3/2022
Revised: 27/4/2022
Published: 28/4/2022

KEYWORDS

Molecular phylogeny
Non-monophyly
Macrosolen
Nest
Genetic congruence

ABSTRACT

Exploring the phylogenetic relationships between taxa provides important information for science. The phylogenetic study of *Macrosolen* was conducted based on molecular data sets of 27 taxa with five DNA regions including chloroplast *rbcL*, *matK*, and *trnL-F* and nuclear ribosomal (small subunit rDNA and large subunit rDNA) regions to reconstruct the phylogenetic relationship of *Macrosolen*. The Maximum likelihood (ML) and Bayesian inference (BI) methods were used to build the phylogenetic trees. The results of molecular analyses strongly supported the non-monophyly of *Macrosolen* with two major clades within the genus. The nest of the three genera *Elytranthe*, *Lepidaria*, and *Macrosolen* in the phylogenetic tree was recognized to be congruent in their morphology, molecules and distribution, but further study is necessary to resolve generic boundaries for stable classification for the three genera. The endemic species of Vietnam *M. bidoupensis* well supported as closely related to *M. tricolor* by molecular data. *Macrosolen* from Vietnam is genetically congruent with its individuals of the same species from other countries.

NGHIÊN CỨU MỐI QUAN HỆ PHÁT SINH CỦA CÁC LOÀI *MACROSOLEN* (BLUME) RCHB. (LORANTHACEAE) Ở VIỆT NAM DỰA TRÊN DỮ LIỆU PHÂN TỬ

Lê Chí Toàn
Trường Đại học Sư phạm Hà Nội 2

THÔNG TIN BÀI BÁO

Ngày nhận bài: 16/3/2022
Ngày hoàn thiện: 27/4/2022
Ngày đăng: 28/4/2022

TỪ KHÓA

Phát sinh loài phân tử
Không đơn phát sinh
Macrosolen
Đan xen
Tương đồng di truyền

TÓM TẮT

Khám phá mối quan hệ phát sinh loài giữa các đơn vị phân loại cung cấp thông tin quan trọng cho khoa học. Nghiên cứu phát sinh loài của chi *Macrosolen* được thực hiện dựa trên bộ dữ liệu phân tử của 27 mẫu nghiên cứu với năm vùng DNA bao gồm các gen lục lạp *rbcL*, *matK*, và *trnL-F* và gen nhân (SSU rDNA và LSU rDNA). Các phương pháp Maximum likelihood (ML) và Bayesian inference (BI) được sử dụng để xây dựng cây phát sinh loài. Các kết quả phân tích dữ liệu phân tử ủng hộ mạnh mẽ rằng *Macrosolen* không phải là đơn phát sinh với hai nhánh phát sinh chính trong chi này. Sự đan xen vào nhau của ba chi *Elytranthe*, *Lepidaria* và *Macrosolen* trên cây phát sinh được ghi nhận là tương đồng với dữ liệu hình thái, phân tử và sự phân bố của chúng, tuy nhiên cần nghiên cứu thêm để giải quyết rõ ràng các ranh giới cho một sắp xếp phân loại chắc chắn cho ba chi. Loài đặc hữu *M. bidoupensis* của Việt Nam có quan hệ di truyền gần gũi với *M. tricolor* dựa trên dữ liệu phân tử. Nhìn chung, các mẫu *Macrosolen* ở Việt Nam có sự tương đồng di truyền với các mẫu của chi này từ các nước khác.

DOI: https://doi.org/10.34238/tnu-jst.5696

Email: Lechitoan@hpu2.edu.vn

1. Introduction

Loranthaceae (showy mistletoe family) comprises mostly aerial hemiparasitic plants with 76 genera and more than 1000 species [1]. Members of the family are mainly distributed in tropical and subtropical regions in the world [2]. *Macrosolen* (Blume) Rchb. is aerial hemiparasitic genus (Figure 1) including ca. 25 species from India to Southeast Asia and New Guinea. Some species of *Macrosolen* are used as local medicines such as *M. cochinchinensis, M. tricolor* [3]-[5].

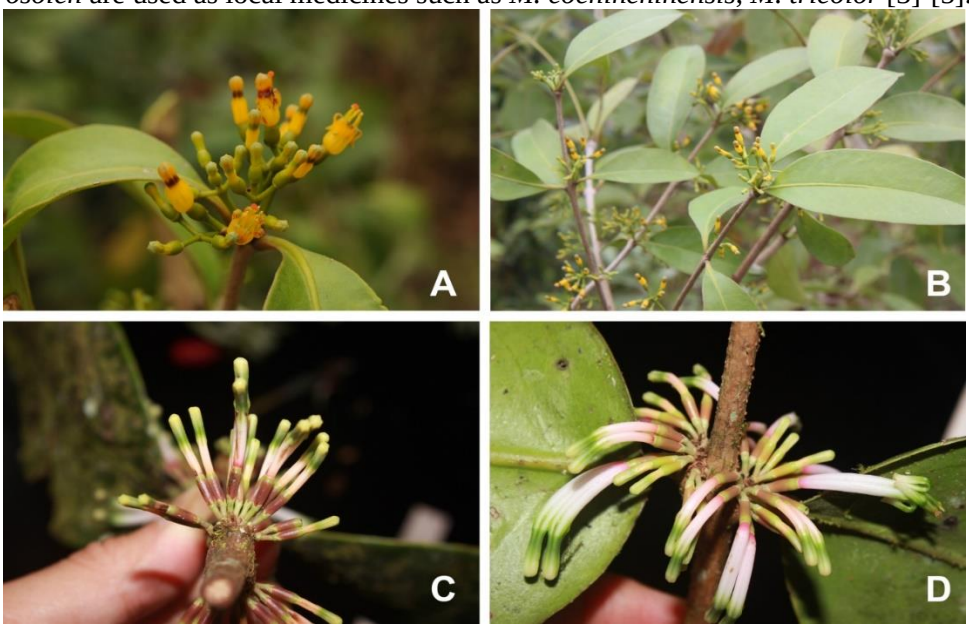

Figure 1. *Morphology of Macrosolen. A, B: M. cochinchinensis; C, D: M. bibracteolatus. Photo credits: C. T. Le*

The phylogenetic relationships within Loranthaceae have been explored in several molecular phylogenetic studies [2], [6]-[8]. Loranthaceae was supported as monophyletic, and five tribes were reconigzed within the family. Nickrent et al. [9] proposed a tribe and subtribe classification for Loranthaceae based on both molecular and morphological evidence, and the genus *Macrosolen* was placed in tribe Elytrantheae. Liu et al. [2] conducted the phylogeny and historical biogeography of Loranthaceae, this study suggested that some members of *Elytranthe* and *Lepidaria* were nested within *Macrosolen*. However, the sampling of *Elytranthe* and *Lepidaria* in this study is limited with only one species per genus, thus the relationship of the three genera needs further study.

Recently, Tagane et al. [10] described species *Macrosolen bidoupensis* from Lam Dong province, southern Vietnam. The study also provided the key for seven *Macrosolen* species in Vietnam including *M. bidoupensis*. However, the phylogenetic position of *M. bidoupensis*, and the genetic relationship of *Macrosolen* species in Vietnam has not been investigated even the *rbcL* and *matK* sequences of *M. bidoupensis* were published under this study.

Thus, a phylogenetic analysis of *Macrosolen* with extended samples is needed to clarify the phylogenetic relationships of *Macrosolen* species in Vietnam. The study aims to (1) reconstruct the phylogeny of *Macrosolen* and its allies; (2) clarify the phylogenetic relationship of *Macrosolen* species in Vietnam.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, amplification, and sequencing

The study sampled 27 individuals representing five species of *Macrosolen*, four species of *Elytranthe* and *Lepidaria*. Two species *Decaisnina aherniana* and *D. triflora* were used as

outgroups. Three plastid markers *matK*, *rbcL*, *trnLF* and two nuclear markers small subunit ribosomal DNA (SSU rDNA), large subunit ribosomal DNA (LSU rDNA) were sequenced in this study (Table 1). The samples from Vietnam were collected during field surveys from 2019 to 2021 in Lao Cai, Ha Giang, Gia Lai, and Ba Ria - Vung Tau province. Additionally, the samples from Malaysia; China, Indonesia, Papua New Guinea were assembled from NCBI (Table 1).

Total genomic DNAs of samples were extracted from silica gel-dried leaves using a DNeasy Plant Mini Kit (Qiagen, Crawley, UK). Amplification protocol and primers for amplifying *matK*, *rbcL*, *trnLF*, SSU rDNA, LSU rDNA followed Taberlet et al. [11], Liu et al. [2], Vidal-Russell and Nickrent [7], [8].

PCR products were examined using electrophoresis and 1.0% agarose gels. The PCR products were purified with BioMed multifunctional DNA fragment purification recovery kits and then sequenced them using our amplification primers. The bidirectional sequencing was completed using an ABI 3730 DNA Sequencer (Applied Biosystems, Carlsbad, California, USA). The quality estimation and assembly for the newly generated sequences were performed in Geneious 8.0.5 [12]. The final sequences were aligned in MUSCLE 3.8.31 [13] and then adjusted them manually in Geneious.

2.2. Phylogenetic analyses

Both the two methods maximum likelihood (ML) and Bayesian inference (BI) were used to carry out the phylogenetic analyses of *Macrosolen*. The ML analysis was conducted in RAxML 8.2.12 [14], [15] using the GTR+I+G standard nucleotide substitution model generated by jModeltest 2.1.6 [16] for each DNA region and the combined dataset applying 1,000 bootstrap replicates. The BI analysis was conducted in MrBayses 3.2.6 [17] on the CIPRES using the nucleotide substitution models that estimated separately each gene region by jModeltest 2.1.6 [16]. The MCMC algorithm was run for 10 million generations with four Markov chain Monte Carlo (MCMC) and trees were sampled every 1000 generations. To check the effective sample sizes (ESSs) of all relevant parameters (>200) we used Tracer v.1.6 [18]. With the first 25% of sampled generations (2500 trees) discarded as burn-in, a 50% majority-rule consensus tree and posterior probabilities (PP) were obtained using the remaining trees.

Additionally, the proportion of variable sites in single molecular matrices was evaluated by the maximum parsimony method in PAUP* [19].

3. Results and discussion

3.1. Molecular data

This study generated 46 new sequences. The lengths of individuals data sets of *matK*, *rbcL*, *trnLF*, SSU rDNA, LSU rDNA are 1088, 827, 681, 1676, 917 pbs, respectively. The combined dataset included 5189 aligned positions for the ingroups and outgroups. In the five makers, *trnLF* and *matK* did not amplify efficiently despite optimization of PCR amplification conditions, while other DNA regions were easily amplified.

Results of the evaluation variable sites indicated that among all individual markers, *matK* possessed the highest proportion of variable sites (16.5%), followed by *trnLF* (8.37%), LSU rDNA (5.67%), *rbcL* (4.35%), SSU rDNA (3.52%) this result was also seen in Malécot and Nickrent [20]. Manzanilla et al. [21] and Linh et al. [22] suggested that the numbers of variable sites and pairwise distances are proportional to the species divergence, though a previous study suggested that the proportion of variable sites may not affect a marker's classification ability. Based on the phylogenetic results in this study (see below section), the lower numbers of variable sites in individual molecular matrices are likely related and indicate the close relationship of *Macrosolen* species and their allies.

Table 1. *Voucher information and GenBank accession numbers for DNA sequences generated or used in this study. The sequences generated in this study begin with ON. "–" indicates missing data.*

Species	**Country of origin**	**Collector & number**	**LSU rDNA**	**SSU rDNA**	***matK***	***rbcL***	***trnL-F***
Decaisnina aherniana (Merr.) Barlow	Indonesia	*Z. D. Chen 508*	MG999386	MG999461	MG999410	–	–
Decaisnina triflora (Span.) Tiegh.	Papua New Guinea	*D. L. Nickrent 4491*	EU544364	EU544324	EU544421	EU544468	EU544484
Elytranthe albida (Blume) Blume	Vietnam	*V. D. Nguyen and S. K. Nguyen 03*	ON331622	ON331650	ON331632	ON331640	ON331658
Elytranthe albida (Blume) Blume	China	*C. Q. Liu s.n.*	MG999388	MG999464	MG999413	MG999433	MG999485
Elytranthe albida (Blume) Blume	China	*Z. D. Chen 27481*	ON331623	–	–	ON331641	ON331659
Elytranthe albida (Blume) Blume	China	*C. T. Le s.n.*	ON331624	ON331651	–	ON331642	ON331660
Lepidaria forbesii Tiegh.	Malaysia	*D. L. Nickrent 4044*	EU544378	EU544330	EU544434	–	EU544492
Lepidaria kingii (King) Danser	–	*D. L. Nickrent 4044*	OM249631	OM249631	–	–	–
Lepidaria oviceps Danser	–	*Calvin and Wilson B02-19*	–	–	–	–	DQ340602
Macrosolen bibracteolatus (Hance) Danser	Vietnam	*DMTT 107*	ON331625	ON331652	ON331633	ON331643	ON331661
Macrosolen bibracteolatus (Hance) Danser	China	*T. Yang YT00113*	ON331626	ON331653	ON331634	ON331644	ON331662
Macrosolen bibracteolatus (Hance) Danser	China	*B. Liu 2784*	MG999392	MG999467	MG999415	MG999438	MG999489
Macrosolen bidoupensis Tagane & V.S.Dang	Vietnam	*C. T. Le LCT56*	ON331627	–	ON331635	ON331645	ON331663
Macrosolen bidoupensis Tagane & V.S.Dang	Vietnam	*Tagane et al. V4083*	–	–	LC259011	LC259010	–
Macrosolen brandisianus (Kurz) Tiegh.	Thailand	*A. Chveerach P16.2*	–	–	–	JN687566	–
Macrosolen cochinchinensis (Lour.) Tiegh.	Vietnam	*C. T. Le LCT26*	ON331628	ON331654	ON331636	ON331646	ON331664
Macrosolen cochinchinensis (Lour.) Tiegh.	Vietnam	*C. T. Le LCT43*	ON331629	ON331655	ON331637	ON331647	ON331665
Macrosolen cochinchinensis (Lour.) Tiegh.	China	*T. Yang YT00043*	ON331630	ON331656	ON331638	ON331648	ON331666
Macrosolen cochinchinensis (Lour.) Tiegh.	Malaysia; China	*C. Calvin et al. s.n; J. X. Su 109*	EU544384	EU544334	EU544439	MG999439	MG999490
Macrosolen cochinchinensis (Lour.) Tiegh.	Malaysia	*C. Calvin et al. s.n.*	EU544384	EU544334	EU544439	HQ317769	EU544497
Macrosolen melintangensis (Korth.) Miq.	–	*Calvin and Wilson B02-17*	–	–	–	–	DQ340593
Macrosolen tricolor (Lecomte) Danser	China	*ByeongCheol Moon s.n.*	–	–	MW484855	–	–
Macrosolen tricolor (Lecomte) Danser	China	*ByeongCheol Moon s.n.*	–	–	MW484856	–	–
Macrosolen tricolor (Lecomte) Danser	China	*ByeongCheol Moon s.n.*	–	–	MW484857	–	–
Macrosolen tricolor (Lecomte) Danser	China	*ByeongCheol Moon s.n.*	–	–	MW484858	–	–
Macrosolen tricolor (Lecomte) Danser	Vietnam	*Z. D. Chen & C. T. Le 10*	ON331631	ON331657	ON331639	ON331649	ON331667
Macrosolen tricolor (Lecomte) Danser	Vietnam	*Z. D. Chen & C. T. Le 34*	MG999393	MG999468	MG999416	MG999440	MG999491

3.2. Molecular data

Phylogenetic trees from individual nuclear and chloroplast partitions resulted in lower resolution of relationships within *Macrosolen* than the combined dataset. The results from ML and BI trees were highly congruent, the few differences had low support. Thus, the phylogeny of *Macrosolen* was combined in ML tree with BS and PP values, and presented in Figure 2.

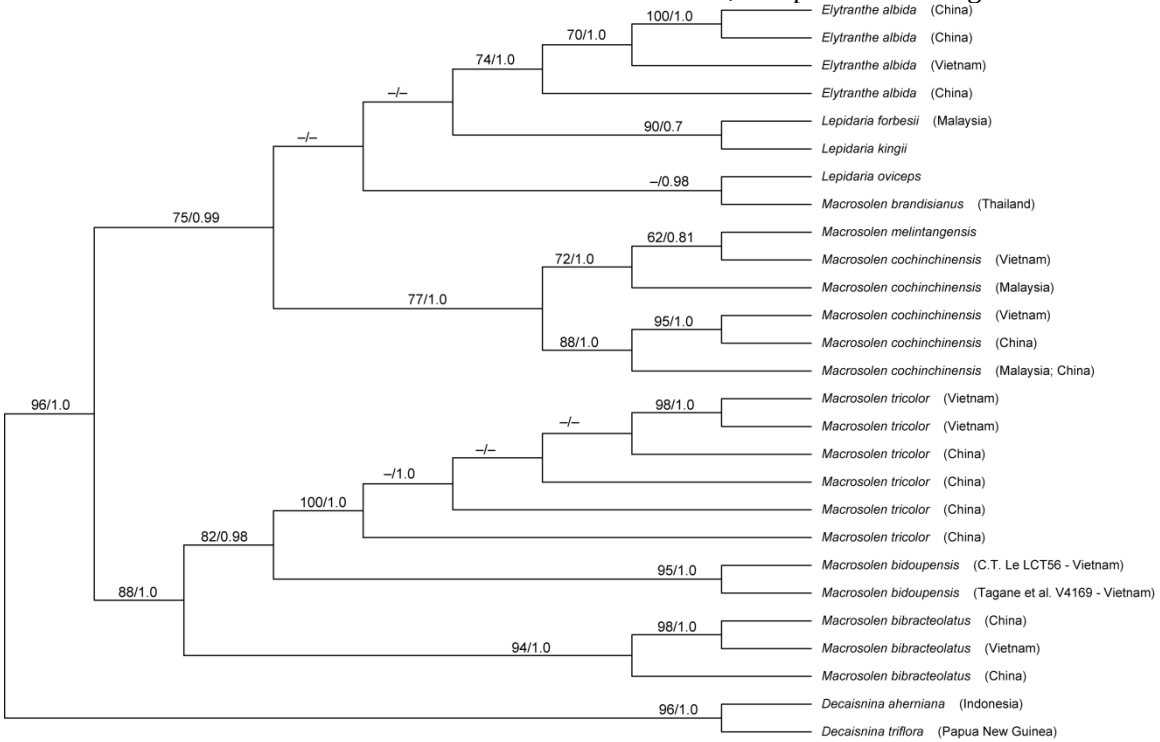

Figure 2. *Maximum likelihood (ML) tree derived from analysis of combined dataset of five genes (matK, rbcL, trnLF, LSU rDNA and SSU rDNA) representing Macrosolen and outgroups. ML bootstrap values and posterior probabilities (PP) of the BI analysis are presented above the branches. Support values less than 50% are indicated with "–".*

The molecular results indicated that *Macrosolen* was strongly supported as non-monophyletic (BS = 96% and PP = 1.0; Figure 2), the two genera *Elytranthe* and *Lepidaria* were nested within *Macrosolen*. Two major clades were recognized within *Macrosolen* (Figure 2). First clade includes three species *M. bibracteolatus*, *M. bidoupensis* and *M. tricolor*. The second clade includes the three genera, *Macrosolen cochinchinensis* represents the earliest diverging lineage in this clade, while *M. brandisianus*, *Elytranthe albida* and *Lepidaria* were nested in the crown of this clade. The position of *Macrosolen brandisianus* in the phylogenetic tree is likely uncertain (BS = 62%, PP = 0.82) (Figure 2), this result may be from the missing data of this species in the present molecular matrix (Table 1). The phylogenetic tree indicated that the individual of *M. bidoupensis* V4083 collected in Lam Dong Province by Tagane et al. is placed together with the individual (LCT56) of this species collected from Gia Lai Province by Chi Toan Le. *M. bidoupensis* was recognized as closely related to *M. tricolor* by molecular data with a high support (Figure 2). Besides, the author also found several differences in sequences of the two species (Figure 3). Based on the observation in the field and suggestion from Tagane et al. [10] the two species share the morphology of leaf and inflorescence but differ in the morphology of the veins, lengths, and color of corolla. Based on the results here, *M. bidoupensis* and *M. tricolor* are congruent in both morphology and molecule. However, the differentiation between the two species is stable.

Danser [23] and Liu et al. [2] documented a close relation in the genetics of the three genera *Elytranthe*, *Lepidaria*, and *Macrosolen*. Meanwhile, Barlow [24] treated *Elytranthe* and *Macrosolen* as congeneric based on morphological data and distribution. The species *Macrosolen cochinchinensis* is widely distributed in Asia [4], thus it's likely that the phylogenetic position of *M. cochinchinensis* has resulted from the congruence in genetics, morphology, and distribution of the three genera. However, the relationships between the three genera are at present unclear, thus further molecular work with comprehensive taxon sampling and data is necessary to resolve generic boundaries for stable classification.

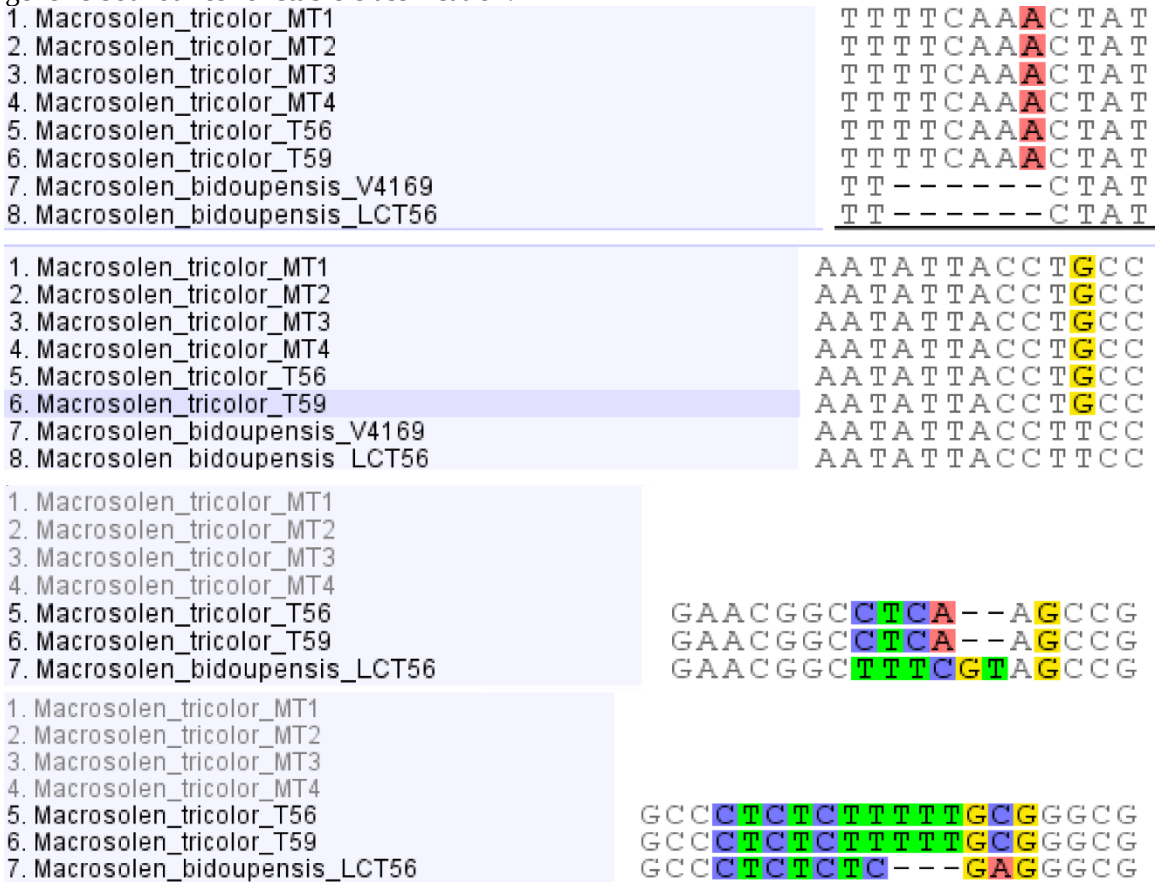

Figure 3. *Differences in sequences genes of Macrosolen bidoupensis and Macrosolen tricolor*

4. Conclusions

The study supported the non-monophyly of *Macrosolen* based on molecular data. Results of this study indicated that the nest of the three genera *Elytranthe*, *Lepidaria*, and *Macrosolen* in the phylogenetic tree based on molecular data is linked to their morphology and distribution, but further study is necessary to resolve generic boundaries for stable classification for the three genera. The endemic species of Vietnam *M. bidoupensis* well supported as closely related to *M. tricolor* by molecular data. *Macrosolen* from Vietnam is genetically congruent with its individuals of the same species from other countries.

Acknowledgements

The author thanks Dr. Nguyen Sinh Khang, Dr. Nguyen Van Du from Institute of Ecology and Biological Resources (IEBR) – Vietnam Academy of Science and Technology and Dr. Tua Yang, Dr. Zhiduan Chen from State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences for the samples collection.

REFERENCES

[1] D. L. Nickrent, "The Parasitic Plant Connection," 1997 onwards. [Online]. Available: http://parasiticplants.siu.edu. [Accessed March 2022].

[2] B. Liu, C. T. Le, R. L. Barrett, D. L. Nickrent, Z. D. Chen, L. M. Lu, and R. Vidal-Russell, "Diversification agrees with emergence of tropical forests and radiation of songbirds," *Molecular Phylogenetics and Evolution*, vol. 124, pp. 199-212, 2018.

[3] H. H. Pham, *An Illustrated Flora Vietnam*, vol. 2, Ho Chi Minh City: Young Publishing House, 2003.

[4] H. S. Qiu and M. G. Gilbert, "Loranthaceae," in *Flora of China*, vol. 5, Z. Y. Wu, P. H. Raven and D. Y. Hong, Eds., Beijing: Science Press, St. Louis: Missouri Botanical Garden Press, 2003, pp. 220-239.

[5] J. Kuijt, "Santalales," in *The families and genera of vascular plants Flowering plants: Eudicots; Santalales, Balanophorales*, vol. 12, J. Kuijt and B. Hansen, Eds., Cham, Switzerland: Springer, 2015, pp. 137-141.

[6] C. A. Wilson and C. L. Calvin, "An origin of aerial branch parasitism in the mistletoe family, Loranthaceae," *American Journal of Botany*, vol. 93, pp. 787-79, 2006.

[7] R. Vidal-Russell and D. L. Nickrent, "Evolutionary relationship in the showy Mistletoe family (Loranthaceae)," *American Journal of Botany*, vol. 95, pp. 1015-1029, 2008a.

[8] R. Vidal-Russell and D. L. Nickrent, "The first mistletoes, origins of aerial parasitism in Santalales," *Molecular Phylogenetics and Evolution*, vol. 47, pp. 523-537, 2008b.

[9] D. L. Nickrent, V. Malécot, R. Vidal-Russell, and J. P. Der, "A revised classification of Santalales," *Taxon*, vol. 592, pp. 538-558, 2010.

[10] S. Tagane, V. S. Dang, N. V. Ngoc, H. T. Binh, N. Komada, J.S. Wai, A. Naiki, H. Nagamasu, H. Toyama, and T. Yahara, "*Macrosolen bidoupensis* (Loranthaceae), a new species from Bidoup Nui Ba National Park, southern Vietnam," *PhytoKeys*, vol. 80, pp. 113-120, 2017.

[11] P. Taberlet, L. Gielly, G. Pautou, and J. Bouvet, "Universal primers for amplification of three non-coding regions of chloroplast DNA," *Plant Molecular Biology*, vol. 17, pp.1105-1109, 1991.

[12] A. Drummond, B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer, and A. Wilson, "Geneious," version 5.4, 2011. [Online]. Available: http://www.geneious.com. [Accessed December 2021].

[13] R. C. Edgar, "MUSCLE: multiple sequence alignment with high accuracy and high throughput," *Nucleic Acids Research*, vol. 32, pp. 1792-1797, 2004.

[14] A. Stamatakis, "RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models," *Bioinformatics*, vol. 2221, pp. 2688-2690, 2006.

[15] A. Stamatakis, P. Hoover, and J. Rougemont, "A Rapid Bootstrap Algorithm for the RAxML Web Servers," *Systematics Biology*, vol. 575, pp. 758-771, 2008.

[16] D. Darriba, G. L. Taboada, , R. Doallo, and D. Posada, "jModelTest 2: more models, new heuristics and parallel computing," *Nature Methods*, vol. 9, pp. 772, 2012.

[17] F. Ronquist, M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck, "MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space," *Systematic Biology*, vol. 61, pp. 539-542, 2012.

[18] A. Rambaut, M. A. Suchard, D. Xie, and A. J. Drummond, "Tracer v.1.6," 2014. [Online]. Available: http://tree.bio.ed.ac.uk/software/tracer. [Accessed January 2022].

[19] D. L. Swofford, *PAUP*: Phylogenetic analysis using parsimony (* and other methods), ver. 4.0.b10*, Sunderland: Sinauer Associates, 2002.

[20] V. Malécot and D. L. Nickrent, "Molecular phylogenetic relationships of Olacaceae and related Santalales," *Systematic Botany*, vol. 33, pp. 97-106, 2008.

[21] V. Manzanilla, A. Kool, N. L. Nguyen, V. H. Nong, T. T. H. Le, and H. De Boer, "Phylogenomics and barcoding of *Panax*: Toward the identifcation of ginseng species," *BMC Evolutionary Biology*, vol. 18, pp. 1-14, 2018.

[22] N. L. Nguyen, L. B. H. Pham, T. T. H. Huynh, H. H. Nguyen, H. H. Ha, D. T. Nguyen, and T. T. H. Le, "Species discrimination of novel chloroplast DNA barcodes and their application for identifcation of *Panax* (Aralioideae, Araliaceae)," *PhytoKeys*, vol. 188, pp. 1-18, 2022.

[23] B. H. Danser, "Miscellaneous notes on Loranthaceae," *Blumea*, vol. 2, pp. 34-59, 1936.

[24] B. A. Barlow, "Loranthaceae," in *Flora Malesiana Series 1*, vol. 13, C. Kalkman, P. F. Stevens, D. W. Kirkup, W. J. J. O. de Wilde, and H. P. Nooteboom, Eds. Leiden: National Herbarium of the Netherlands, 1997, pp. 209-401.