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Source: Candollea, 73(2) : 261-275

Published By: The Conservatory and Botanical Garden of the City of Geneva (CJBG)

URL: <https://doi.org/10.15553/c2018v732a11>

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Enlarging the monotypic Monocarpieae (Annonaceae, Malmeoideae): recognition of a second genus from Vietnam informed by morphology and molecular phylogenetics

Tanawat Chaowasku, Anissara Damthongdee, Hathaichanok Jongsook, Dung T. Ngo, Hung T. Le, Duc M. Tran & Somran Suddee

Abstract

CHAOWASKU, T., A. DAMTHONGDEE, H. JONGSOOK, D.T. NGO, H.T. LE, D.M. TRAN & S. SUDDEE (2018). Enlarging the monotypic Monocarpieae (Annonaceae, Malmeoideae): recognition of a second genus from Vietnam informed by morphology and molecular phylogenetics. *Candollea* 73: 261–275. In English, English abstract. DOI: <http://dx.doi.org/10.15553/c2018v732a11>

Recent botanical expeditions in central Vietnam yielded an unknown species of *Annonaceae* that could not be confidently identified to subfamily, tribe, and genus. Preliminary BLAST® searches based on plastid data have suggested that this taxon is genetically closely-related to the following tribes of subfamily *Malmeoideae*: *Malmeeae*, *Fenerivieae*, *Maasieae*, *Phoenicantheae*, *Dendrokingstonieae*, *Monocarpieae*, and *Miliuseae*. Using representatives of *Piptostigmatieae*, another tribe of *Malmeoideae*, as outgroups and including representatives of all other tribes of *Malmeoideae*, molecular phylogenetic analyses of seven combined plastid markers (*rbcl*, *matK*, *ndhF*, *ycf1* exons; *trnL* intron; *trnL-trnF*, *psbA-trnH* intergenic spacers) inferred the enigmatic Vietnamese taxon as belonging to the monotypic tribe *Monocarpieae*. Detailed morphological comparisons between this taxon and its sister group, *Monocarpia* Miq., warranted the recognition of a second genus of *Monocarpieae* to accommodate our unknown taxon: *Leoheo* Chaowasku with a single species, *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le. The morphology of the new genus agrees well with the diagnostic traits of *Monocarpieae*, e.g., a percurrent tertiary venation of the leaves, a highly reduced number of carpels per flower, enlarged and lobed stigmas, multiple ovules/seeds per ovary/monocarp, considerably large monocarps with a hardened pericarp when dry, and spiniform ruminations of the endosperm. However, the new genus does not exhibit two characteristic features of *Monocarpia*: terminal inflorescences and generally distinct intramarginal leaf veins. In addition, the new genus possesses three autapomorphic characters: hairy domatia on the lower leaf surface, longitudinal ridges on the monocarp surface, and sessile monocarps with a stout stipe. The tribe *Monocarpieae* is consequently enlarged to include the genus *Leoheo*. The enlarged *Monocarpieae*, along with the recently established monotypic tribe *Phoenicantheae* and two other related tribes, *Dendrokingstonieae* and *Miliuseae*, are discussed.

Keywords

ANNONACEAE – *Monocarpieae* – *Leoheo* – Vietnam – Molecular phylogeny – New genus – New species – Taxonomy

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Submitted on November 4, 2017. Accepted on August 30, 2018.

First published online on November 23, 2018.

ISSN: 0373-2967 – Online ISSN: 2235-3658 – *Candollea* 73(2): 261–275 (2018)

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Introduction

Annonaceae, a pantropical angiosperm family characteristic of lowland rainforests (e.g. SLIK et al., 2003), comprise c. 2400 species classified in 109 genera (Guo et al., 2017; CHAOWASKU et al., 2018; XUE et al., 2018). A new classification has been recently proposed, subdividing the family into four subfamilies, viz., *Anaxagoreoideae*, *Ambavioideae*, *Annonoideae*, and *Malmeeioideae*; with the last two subfamilies constituting the majority of generic and species diversity (CHATROU et al., 2012). Similar to the realigned subfamilies, tribal delimitations within *Annonoideae* and *Malmeeioideae* have also been reconsidered. *Malmeeioideae* have been classified into seven tribes, viz. *Piptostigmatheae*, *Malmeeae*, *Fenerivieae*, *Maasieae*, *Dendrokingstonieae*, *Monocarpieae*, and *Miliuseae*. It is worthwhile to note that four (*Fenerivieae*, *Maasieae*, *Dendrokingstonieae*, and *Monocarpieae*) of the seven tribes are monotypic (CHATROU et al., 2012).

The evolutionary relationships within *Malmeeioideae* are still poorly resolved, requiring further inclusion of DNA regions suitable to resolve deeper relationships. There is, however, one strongly supported clade composed predominantly of Asian-Pacific species, i.e. a clade of *Dendrokingstonieae*–*Monocarpieae*–*Miliuseae* (CHAOWASKU et al., 2014). Later *Phoenicantheae*, an additional monotypic tribe of *Malmeeioideae*, has been proposed to accommodate the Sri Lankan endemic genus *Phoenicanthus* Alston formerly placed in *Miliuseae* because this genus has been shown to recover as the sister group of the *Dendrokingstonieae*–*Monocarpieae*–*Miliuseae* clade (Guo et al., 2017).

Recent field trips in central Vietnam (in Nam Đông and A Luoi Distr. of Thừa Thiên-Huế Prov.) resulted in the discovery of an unknown *Annonaceae*. This taxon is unique in possessing hairy domatia on the lower leaf surface, which is a rare character present only in a limited number of taxa in this family (CHAOWASKU et al., 2012a). Its percurrent tertiary venation of the leaves, reduced carpel number per flower, enlarged and lobed stigmas, and massive monocarps with a thick and hardened pericarp when dry point this taxon to be morphologically related to *Dendrokingstonieae* and *Monocarpieae*, two monotypic tribes of *Malmeeioideae* (or the canangoid clade of subfamily *Ambavioideae*; CHAOWASKU et al., 2012b). The aims of this study are to (1) elucidate the phylogenetic position of the unknown *Annonaceae* from Vietnam and (2) determine its taxonomic status by detailed morphological investigations and comparisons with its phylogenetically most closely related taxa.

Material and methods

Taxon and character sampling

Twenty-four accessions comprise the ingroup, with representatives covering all currently accepted tribes of *Malmeeioideae* except *Piptostigmatheae*, viz. *Malmeeae* (3 accessions), *Fenerivieae* (1 accession), *Maasieae* (1 accession), *Phoenicantheae* (1 acces-

sion), *Dendrokingstonieae* (3 accessions), *Monocarpieae* (3 accessions), and *Miliuseae* (10 accessions). These tribes have been recently demonstrated to retrieve as a strongly supported monophyletic group (Guo et al., 2017; XUE et al., 2018). Two accessions of the unknown taxon from Vietnam collected in the same vicinity (Nam Đông Distr.: *Chaowasku 129*, CMUB; *Chaowasku 165*, CMUB) were included. Two species (*Mwasumbia alba* Couvreur & D.M. Johnson and *Brieya fasciculata* De Wild.) belonging to the tribe *Piptostigmatheae* were assigned as outgroups. The above strategy of taxon sampling was adopted because preliminary nucleotide searches via BLAST® suggested that sequences of the unknown taxon from Vietnam were most similar to those of various species in several related tribes of *Malmeeioideae* except *Piptostigmatheae*, i.e. at least the first 80 sequences retrieved according to the E-value upon the BLAST® searches belonged to *Malmeeae*, *Fenerivieae*, *Maasieae*, *Dendrokingstonieae*, *Monocarpieae*, or *Miliuseae*; with a member of *Monocarpieae* retrieved as the first sequence in four of the seven plastid DNA markers selected. Every accession has sequences of six plastid DNA regions (*rbcl*, *matK*, *ndhF* exons; *trnL* intron; *trnL*–*trnF*, *psbA*–*trnH* intergenic spacers). Twenty-two of the 24 accessions of the ingroup have been sequenced for an additional plastid DNA region (*ycf1* exon), but at least one species per tribe are represented by the *ycf1* sequence. Previously published sequences were obtained from the following publications: MOLS et al. (2004a, 2004b), PIRIE et al. (2006, 2007), SU et al. (2008), COUVREUR et al. (2009), CHAOWASKU et al. (2012a, 2013a, 2014, 2018), and CHATROU et al. (2012). Thirty-three sequences were newly generated in the present study. All information regarding voucher specimens and GenBank accession numbers are shown in Appendix I.

In total, 6975 nucleotide plus 10 indel characters were included. The simple method of SIMMONS & OCHOTERENA (2000) for indel coding was followed. More indel structures were observed in the aligned data matrix, but only the less homoplasious and non-autapomorphic ones were chosen. An inversion of 15-nucleotide stretch in the *psbA*–*trnH* intergenic spacer is present in roughly 40% of the accessions sequenced and was changed to its reverse-complement to be homologically comparable to the remaining sequences, following PIRIE et al. (2006).

DNA extraction, amplification, and sequencing

DNA extraction was performed using the GF-1 Plant DNA extraction kit (Vivantis). A standard PCR protocol was followed throughout using the 5x Hot FirePol® Blend Master Mix with 10 mM MgCl₂ (Solis BioDyne). The final reaction volume of 10 µl (1X) contained 0.2 µl of each primer (10 µM), 2 µl of 5x Hot FirePol® Blend Master Mix, and 1 µl of template DNA (average final DNA concentration = c. 5 ng/µl). The PCR programs used consist of 35 cycles, each with 95°C:

40 seconds, 53°C–65°C: 50 seconds (optimum annealing temperature depends on each primer pair; sometimes a touchdown approach was implemented by setting the initial annealing temperature at 65°C or 60°C, then decreasing the annealing temperature by 0.5°C every cycle until reaching 57°C or 53°C, respectively, and then continuing with these temperatures until the 35th cycle), 72°C: 1 min 20 seconds, with the initial denaturation for 15 min at 95°C and the final extension for 7.5 min at 72°C. The primer sequences for amplifying and sequencing all seven plastid regions were the same as those used in CHAOWASKU et al. (2012a). Amplicons were cleaned by the GF-1 AmbiClean kit (Vivantis), and then sequenced using the BigDye® Terminator v3.1 cycle sequencing kit chemistry (ThermoFisher Scientific).

Phylogenetic analyses

Sequences were edited using the Staden package [<http://staden.sourceforge.net>] (STADEN et al., 2000) and subsequently aligned by Multiple Sequence Comparison by Log-Expectation (MUSCLE; EDGAR, 2004) in MEGA7 (KUMAR et al., 2016). The alignment was then manually checked and re-aligned (if necessary) on the basis of homology assessment using the similarity criterion (SIMMONS, 2004). Parsimony analysis was performed in TNT v1.5 (GOLOBOFF & CATALANO, 2016). All characters were equally weighted and unordered. Incongruence among regions was evaluated by analyzing each region individually, to see if there was any significant conflict in clade support (SEELANAN et al., 1997; WIENS, 1998). Multiple most parsimonious trees were generated by a heuristic search of the combined data, with 9000 replicates of random sequence addition, saving 10 trees per replicate, and using the tree bisection and reconnection (TBR) branch-swapping algorithm. Clade support was measured by symmetric resampling (SR), which is not affected by a distortion associated with some bootstrap and jackknife resampling analyses (GOLOBOFF et al., 2003). A default change probability was used. Two hundred thousand replicates were run, each with four replicates of random sequence addition, saving four trees per replicate. A clade with SR ≥ 85%, 70–84%, or 50–69% was considered strongly, moderately, or weakly supported, respectively.

Maximum likelihood analysis was performed in IQ-TREE v1.6.1 (NGUYEN et al., 2015) under partition models (CHERNOMOR et al., 2016) implemented with the “-spp” command, whereas Bayesian Markov chain Monte Carlo (MCMC; YANG & RANNALA, 1997) phylogenetic analysis was performed in MrBayes v3.2.3 (RONQUIST et al., 2012) via the CIPRES Science Gateway v3.1 (MILLER et al., 2010). The data matrix was divided into seven partitions based on DNA region identity (the *trnL* intron and the adjacent *trnL*–*trnF* spacer were combined as a single partition) plus a binary indel-coded partition. The most appropriate model of sequence evolution for each DNA partition was selected

by Akaike Information Criterion (AIC; AKAIKE, 1974) scores, using FindModel [<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>] (POSADA & CRANDALL, 1998). The General Time Reversible (GTR; TAVARÉ, 1986) nucleotide substitution model with a gamma distribution for among-site rate variation was selected for four partitions (*rbcL*, *matK*, *ndhF*, and *ycf1*), and the Hasegawa-Kishino-Yano (HKY; HASEGAWA et al., 1985) substitution model with a gamma distribution for among-site rate variation was selected for the remaining two partitions (*trnLF* [= *trnL* intron + *trnL*–*trnF* intergenic spacer] and *psbA*–*trnH*).

In the maximum likelihood analysis, the model “JC2+FQ+ASC” was chosen by corrected AIC scores for the binary indel partition. Clade support was assessed by non-parametric bootstrap resampling method (BS; FELSENSTEIN, 1985) with 2000 replicates. Similar to the discrimination of the SR values in the parsimony analysis, a clade with BS ≥ 85%, 70–84%, or 50–69% was considered strongly, moderately, or weakly supported, respectively.

In the Bayesian analysis, the “coding=variable” setting was selected for the binary indel partition, which was implemented with a simple F81-like model without a gamma distribution for among-site rate variation. Three independent analyses, each using four MCMC chains, were simultaneously run; each run was set for 10 million generations. The default prior settings were used except for the prior parameter of rate multiplier (“ratepr” [=variable]). The temperature parameter was set to 0.08. Trees and all parameter values were sampled every 1000th generation. Convergence was evaluated by checking the standard deviation of split frequencies of the runs with values < 0.01 interpreted as indicating a good convergence and by checking for adequate effective sample sizes (ESS > 200) using Tracer v1.6 (RAMBAUT et al., 2013). The initial 25% of all trees sampled were discarded as burn-in, and the 50% majority-rule consensus tree was generated from the remaining trees. A clade with posterior probabilities (PP) ≥ 0.95, 0.9–0.94, or 0.5–0.89 was considered strongly supported, weakly supported, or unsupported, respectively.

Morphology

The macromorphology of the unknown *Annonaceae* from Vietnam was studied from six herbarium specimens (dried and pickled material: *HUAF collectors 2009-03-19-ND*; *Chaowasku 129, 130, 131, 165, 166*; see below for more details). The relevant morphological information of related taxa were taken from literature (HUBER, 1985; VAN HEUSDEN, 1992; KESSLER, 1993; MOLS & KESSLER, 2000a, 2000b, 2003; MOLS et al., 2004a; CHAOWASKU et al., 2012b, 2013b, 2014; TURNER, 2012) or derived from herbarium specimens for *Phoenicanthus obliquus* (Hook. f. & Thomson) Alston [*Huber 515, 540, 565* (E); *Huber 518, 577* (L)]. The indumentum terminology used follows HEWSON (1988).

Results

The parsimony analysis resulted in eleven most parsimonious trees with 1302 steps. The consistency and retention indices (CI and RI) were 0.83 and 0.77, respectively. There was no strong topological conflict ($SR \geq 85\%$) in the analysis of each DNA region. Fig. 1 shows the Bayesian 50% majority-rule consensus tree, with posterior probabilities, maximum likelihood bootstrap values, and parsimony symmetric resampling values indicated.

The ingroup, comprising *Malmeeae*, *Fenerivieae*, *Maasieae*, *Phoenicantheae*, *Dendrokingstonieae*, *Monocarpieae*, and *Miliuseae*, was recovered as a maximally supported clade. The two accessions of the unknown taxon from Vietnam were retrieved as a maximally supported clade sister to another maximally supported clade consisting of three accessions in the genus *Monocarpia*. In the latter clade, *M. euneura* Miq. is the sister group of a strongly supported clade (PP 1, BS 100%, SR 99%) composed of two accessions of *M. maingayi* (Hook. f. & Thomson) I.M. Turner. Both *Monocarpia* and the unknown taxon from Vietnam constitute a strongly supported *Monocarpieae* clade (MON.; PP 1, BS 100%, SR 99%). The *Monocarpieae* clade is weakly to strongly supported (PP 0.98, BS < 60%, SR < 59%) as the sister group of an unsupported clade (PP 0.78, BS < 50%, SR < 50%) comprising the maximally supported *Dendrokingstonieae* (DEN.) and *Miliuseae* (MIL.) clades. A clade of *Monocarpieae*–*Dendrokingstonieae*–*Miliuseae* is the sister group of the *Phoenicantheae* lineage with strong support (PP 1, BS 100%, SR 99%). The *Phoenicantheae*–*Monocarpieae*–*Dendrokingstonieae*–*Miliuseae* clade then is the sister group of an unsupported clade (PP 0.53, BS < 50%, SR < 50%) consisting of a weakly to strongly supported *Malmeeae* clade (MAL.; PP 1, BS 68%, SR 57%) and a moderately to strongly supported clade (PP 0.95, BS 76%, SR 85%) composed of *Fenerivieae* and *Maasieae* lineages.

Discussion

Phylogenetic analyses strongly support the belonging of the unknown Vietnamese *Annonaceae* to *Monocarpieae*, sister to genus *Monocarpia* (Fig. 1). However, this taxon morphologically deviates from *Monocarpia* by two main features: 1) an absence of intramarginal leaf veins (Fig. 2C) and 2) axillary inflorescences (Fig. 3A). The intramarginal leaf veins (Fig. 2A) and terminal inflorescences (Fig. 3B) have been regarded as reliable diagnostic traits for *Monocarpia* (TURNER, 2012). The absence of these features in the unknown taxon from Vietnam warrants its recognition as a distinct genus of *Monocarpieae*. In each *Annonaceae* genus, both terminal and axillary inflorescences seldom coexist (KOEK-NOORMAN et al., 1990), e.g. in *Miliusa* Lesch. ex A. DC. (CHAOWASKU et al., 2013a) and *Pseuduvaria* Miq. (SAUNDERS et al., 2004; SU et al., 2010), and

either state has been previously shown to constitute a good character for generic delimitations (CHATROU et al., 2000).

Besides the above-mentioned crucial morphological differences, the unknown taxon from Vietnam exhibits longitudinal ridges on the monocarp surface (Fig. 2D), as well as domatia on the lower leaf surface, each composed of a tuft of aggregated hairs (Fig. 4A). These two characters have never been reported to occur in *Monocarpia* (MOLS & KESSLER, 2000b; TURNER, 2012; Fig. 2B, 4B). Further, the monocarps of the unknown taxon from Vietnam are subsessile with a stout stipe (Fig. 2D), while those of *Monocarpia* are completely sessile (Fig. 2B; MOLS & KESSLER, 2000b; TURNER, 2012). Other features of the Vietnamese new genus fit well with the diagnostic traits of *Monocarpieae* (VAN HEUSDEN, 1992; MOLS & KESSLER, 2000b; CHAOWASKU et al., 2012b; CHATROU et al., 2012): enlarged stigmas (more or less peltate and lobed; Fig. 2H, 5J), a percurrent tertiary venation of the leaves (Fig. 2C, 6), a reduced carpel number to 3–4 per flower (Fig. 5A), multiple ovules per ovary (and hence seeds per monocarp) arranged in two rows, relatively large monocarps with a thick and hardened wall when dry (Fig. 2D, 5K), and spiniform endosperm ruminations (Fig. 5N). The tribe *Monocarpieae* is, therefore, enlarged to include the new genus from Vietnam.

Taxonomy

Leoheo Chaowasku, gen. nov.

Typus: *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le.

Medium-sized to large *trees*; indumentum of simple hairs; intramarginal *leaf* veins absent, tertiary leaf venation percurrent; *inflorescences* 1- or few-flowered, axillary; bracts present; *flowers* bisexual, both petal whorls of \pm equal size; stamens 70–76, connective truncate and dilated, covering thecae; carpels 3–4 per flower, free in flower and fruit; stigmas \pm peltate and lobed; ovules many and arranged in two rows, placentation lateral; *monocarps* subsessile, cylindrical, monocarp abscission basal, pericarp thick and hardened when dry; aril absent; endosperm ruminations spiniform.

Etymology. – The generic epithet *Leoheo* is from the local Vietnamese name of “Lèo Heo” for this plant and is designated as a masculine noun of nominative singular in third declension with genitive singular “*Leoheonis*”.

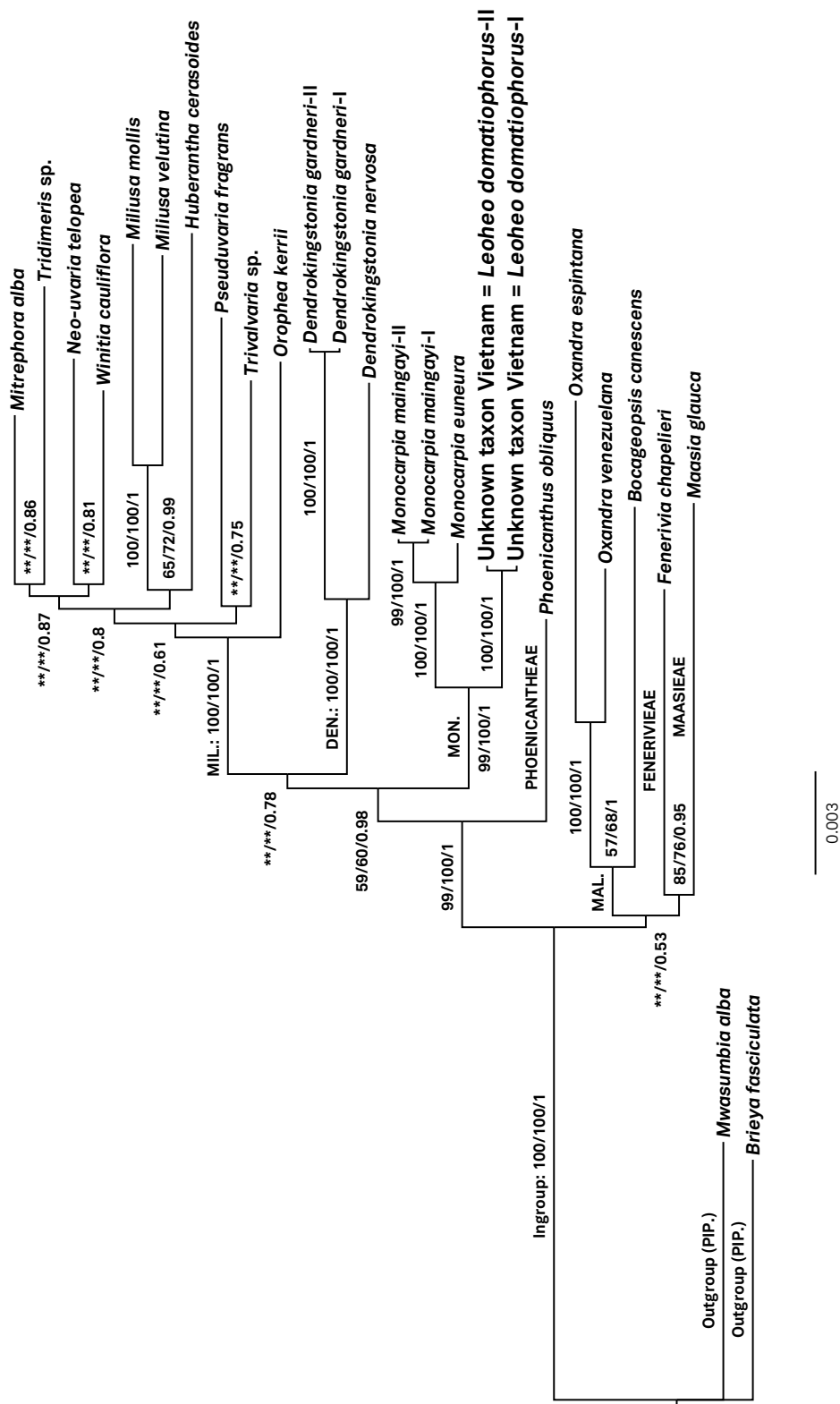


Fig. 1. – 50% majority-rule consensus phylogram derived from Bayesian inference of combined seven plastid DNA regions. Bayesian posterior probabilities (PP) indicated on the right; maximum likelihood bootstrap (BS) percentages in the middle; parsimony symmetric resampling (SR) percentages on the left [** denotes BS/SR < 50%]. DEN. = *Dendrokingstonieae*; MAL. = *Malmeeae*; MIL. = *Miliuseae*; MON. = *Monocarpieae*; PIP. = *Piptostigmatieae*. Scale bar unit = substitutions per site.

Notes. – The principal morphological differences between *Leoheo* and *Monocarpia* are highlighted in Table 1. Furthermore, in view of molecular phylogenetics, the genera *Leoheo* and *Monocarpia* each is characterizable not only by nucleotide substitutions, but also by an indel structure in the *psbA-trnH* intergenic spacer for *Leoheo* and another indel structure in the *trnL-trnF* intergenic spacer for *Monocarpia*. Although the other two species of *Monocarpia*, *M. borneensis* Mols & Kessler and *M. kalimantanensis* Kessler, have not been included in the present molecular phylogenetic analyses due to the failure in DNA amplification, their morphologies (e.g. Fig. 2A, 3B) substantially coincide with those of *M. euneura* Miq. and *M. maingayi* (Hook. f. & Thomson) I.M. Turner (Fig. 2B; TURNER, 2012), hence we are convinced that such two missing species (both or any of them) will not retrieve as the sister group of *Leoheo* or the *Leoheo-Monocarpia* clade.

It is noteworthy that each of the axillary inflorescences of the genus *Leoheo* often contains leaf-like bract(s) at the top of peduncle (Fig. 3A, 6), probably this feature is a transition to the terminal inflorescences characteristic for the genus *Monocarpia* (Fig. 3B). The presence of domatia on the lower leaf surface is a rare phenomenon in *Annonaceae*. It occurs only in a limited number of genera and species, for examples, *Annona* L. (VAN DEN BOS et al., 1989), *Dendrokingstonia* Rauschert (CHAOWASKU et al., 2012b), *Huberantha* Chaowasku (CHAOWASKU et al., 2012a), *Mitrephora* (Blume) Hook. f. & Thomson (WEERASOORIYA & SAUNDERS, 2010), and *Tridimeris* Baill. (ORTIZ-RODRIGUEZ et al., 2016). Of these genera, the hairy type of domatium similar to that of *Leoheo* (Fig. 4A) can be found in *Annona*, *Huberantha*, and *Mitrephora* (CHAOWASKU et al., 2012a).

The well-supported sister relationship of *Fenerivieae* and *Maasieae* is reported herein for the first time (Fig. 1). The two tribes share a number of features, e.g., axillary inflorescences, generally one ovule per ovary, and spiniform endosperm ruminations (MOLS et al., 2008; SAUNDERS et al., 2011). Their possible closest relationships were previously discussed (SCHATZ & LE THOMAS, 1990; SAUNDERS et al., 2011).

The tribes *Phoenicantheae*, *Monocarpieae*, *Dendrokingstonieae*, and *Miliuseae* constitute a strongly supported clade substantially composed of Asian-Pacific species. The fact that the Sri Lankan endemic tribe *Phoenicantheae* is the sister group of the

remainder of this clade coupled with the restricted distribution of the tribes *Monocarpieae* and *Dendrokingstonieae* might have some biogeographic implication, especially on the geographic origin of *Miliuseae*, the most diverse tribe of *Malmeeoideae* (CHATROU et al., 2012), but it is currently not possible to perform an in-depth biogeographic analysis because there are still unignorable phylogenetic uncertainties, especially the unsupported sister relationships of *Dendrokingstonieae* and *Miliuseae*, as well as of *Malmeeae* and a clade composed of *Fenerivieae* and *Maasieae* (Fig. 1). So far, no macromorphological features have yet been found to be synapomorphic for the *Phoenicantheae-Monocarpieae-Dendrokingstonieae-Miliuseae* clade. Nevertheless, some palynological correlations have been observed, i.e., any taxa of *Malmeeoideae* recovered outside *Miliuseae* possess monosulcate pollen (CHAOWASKU et al., 2012b, 2014). Currently the pollen data of *Phoenicanthus* and *Leoheo* are unavailable, but they are anticipated to exhibit monosulcate pollen based on such correlations. In addition, it is worthwhile to note that *Phoenicantheae*, *Monocarpieae*, and *Dendrokingstonieae* all possess a highly reduced carpel number to 1–4 per flower (HUBER, 1985; CHAOWASKU et al., 2012b). It is likely that the reduction in carpel number per flower is the ancestral trait of the *Phoenicantheae-Monocarpieae-Dendrokingstonieae-Miliuseae* clade. Table 2 compares the important macromorphological and pollen morphological characters of the amended *Monocarpieae* and the other three closely related tribes: *Phoenicantheae*, *Dendrokingstonieae*, and *Miliuseae*.

Monocarpia, the sister group of *Leoheo*, occurs in southern Thailand, Peninsular Malaysia, Sumatra, and Borneo (TURNER, 2012); the shortest distance of the two genera is about 1300 km away. This disjunct distribution pattern is, however, not unprecedented. In *Phaeanthus* Hook. f. & Thomson (*Malmeeoideae*, *Miliuseae*; CHATROU et al., 2012), *P. vietnamensis* Bân is the only species occurring in Indochinese Peninsula; the remaining species occur in southern Thailand Provinces bordering Malaysia (GARDNER et al., 2015), Malay Peninsula to the Philippines and New Guinea (MOLS & KESSLER, 2000a). In *Neo-uvaria* Airy Shaw (*Malmeeoideae*, *Miliuseae*; CHATROU et al., 2012), the recently described *N. laosensis* Tagane & Soulad. hitherto endemic to central Laos (TAGANE et al., 2018) is the only species disjunctly occurring north of Peninsular Thailand and

Table 1. – Principal morphological differences between *Leoheo* Chaowasku and *Monocarpia* Miq.

| Characters | <i>Leoheo</i> | <i>Monocarpia</i> |
|-------------------------------|---|-----------------------------|
| Intramarginal leaf veins | Absent | Present |
| Domatia on lower leaf surface | Present | Absent |
| Inflorescence position | Axillary | Terminal |
| Monocarp surface | With longitudinal ridges | Without longitudinal ridges |
| Monocarp base | Contracted into a short and stout stipe | Not contracted into a stipe |



Fig. 2. – **A.** Leaf of *Monocarpia kalimantanensis* Kessler, showing conspicuous intramarginal veins; **B.** Fruit of *Monocarpia maingayi* (Hook. f. & Thomson) I.M. Turner, showing monocarps without longitudinal ridges; **C–H:** *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le; **C.** Leaf without intramarginal veins; **D.** Fruit, showing monocarps with longitudinal ridges; **E.** Flowering branches; **F.** Dissected flower and young fruit; **G.** Dissected flower, showing detached stamens and stigmas; **H.** Flower, showing enlarged and irregularly lobed stigmas. [**A:** Sidiyasa et al. 3469, L; **B:** Gardner & Sidisunthorn ST0541a, L; **C–D:** Chaowasku 131, CMUB; **E–H:** HUAF collectors 2009-03-19-ND, CMUB] [Photos: **A:** Arbainsyah; **B:** S. Gardner & P. Sidisunthorn; **C–H:** D.T. Ngo]

Table 2. – Comparisons of important macromorphological and pollen morphological features of four closely related tribes in *Malmeoideae*.

| Characters | <i>Phoeniciantheae</i> | <i>Monocarpieae</i> | <i>Dendrokingstonieae</i> | <i>Miliuseae</i> |
|---------------------------------|---|---------------------------------------|---------------------------------------|--|
| Tertiary leaf venation | Reticulate | Percurrent | Percurrent | Reticulate to percurrent |
| Inflorescence position | Axillary or terminal | Axillary or terminal | Axillary | Axillary or terminal |
| Stamen number per flower | 6 or 9 | 35–76 | 9–56 | 3 to ≥ 200 |
| Stamen connective tissue | With a minute prolongation, not covering thecae | Truncate and dilated, covering thecae | Truncate and dilated, covering thecae | Without an apical prolongation; or with a minute prolongation, not covering thecae; or truncate and dilated, covering thecae; or +/- truncate, not covering thecae |
| Carpel number per flower | 1–3 | 1–4 | 1–2 | 1 to ≥ 70 |
| Pollen apertural system | Monosulcate | Monosulcate | Monosulcate | Cryptoaperturate/disulcate |

Malaysia, the nearest area where three other species of *Neouvaria* can be found (CHAOWASKU et al., 2011). Additionally, in *Disepalum* Hook. f. (*Annonoideae*, *Annoneae*; CHATROU et al., 2012) subg. *Enicosanthellum* (Bân) P.S. Li, D.C. Thomas & R.M.K. Saunders, a clade composed of two particular species: *D. petelotii* (Merr.) D.M. Johnson and *D. plagioneurum* (Diels) D.M. Johnson, both occurring in China and Vietnam (and Laos for the former; JOHNSON, 1989) is the sister group of *D. pulchrum* (King) J. Sinclair (LI et al., 2015, 2017) which can only be found in southern Thailand Provinces bordering Malaysia (CHAMCHUMROON et al., 2017) and Malay Peninsula (JOHNSON, 1989). It is interesting to understand the plausible biogeographic scenarios and other biotic/abiotic factors shaping the mentioned disjunct distribution pattern within Southeast Asia.

Leoheo domatiophorus Chaowasku, D.T. Ngo & H.T. Le, spec. nova (Fig. 2C–H, 3A, 4A, 5–7).

Holotypus: VIETNAM. Prov. Thua Thien-Hue: Nam Đông Distr., 19.III.2009, fl., *HUAF collectors 2009-03-19-ND* (CMUB!; iso-: G!, P!).

Leoheo domatiophorus Chaowasku, D.T. Ngo & H.T. Le differs from species of the genus *Monocarpia* Miq. by the lack of intramarginal leaf veins and by having axillary inflorescences, leaf domatia, longitudinal ridges on the monocarp surface, and subsessile monocarps with a stout stipe.

Medium-sized (to large) trees, 15–25(–30) m tall; young twigs puberulous with appressed hairs. Petiole 5–8 mm long, grooved on upper surface, both sides puberulous with appressed hairs; leaf blade elliptic, 9.1–22.8 × 3.1–6.5 cm, both surfaces glabrous, base cuneate, apex caudate-acuminate; midrib raised and puberulous with appressed hairs on lower surface, sunken (becoming less so toward apex) and puberulous with appressed and erect hairs (more sparsely so toward apex) on upper surface; secondary veins 11–12 per side, angle with

midrib at middle part of leaf blade 40°–50°. Flower(s) in a 1- to 2-flowered axillary inflorescence, bisexual; peduncle 7–10 mm long (up to 17 mm long and 6–8 mm thick in fruit), indumentum puberulous-tomentose with erect hairs, bract(s) often leaf-like, placed at top of peduncle, often caducous; pedicel 2–2.5 cm long (6–7 mm thick in fruit), tomentose with erect hairs, bracts ovate, generally 2 per pedicel, one placed at \pm midpoint of pedicel, another near pedicel base, often caducous. Sepals triangular-ovate, 8–11 × 7–8 mm, both surfaces and margin tomentose with erect hairs. Outer petals obovate, 5.8–6.2 × 1.8–2.1 cm, both surfaces and margin puberulous-tomentose with erect hairs, apex obtuse-rounded; inner petals elliptic-obovate, 5.9–6.1 × 2–2.3 cm, indumentum similar to that of outer petals, apex obtuse. Torus volcano-shaped with a slightly sunken apex, glabrous. Stamens 70–76 per flower, c. 2 mm long, connective truncate, covering thecae. Carpels 3–4 per flower, 4.5–6 mm long; stigmas \pm peltate and irregularly lobed (5–6 lobes); ovaries villous with appressed hairs; ovules 15–17 per ovary, lateral, biseriate. Monocarp(s) 1–4 per fruit, subsessile (with a stout stipe), cylindrical, 5.5–7.5 × 3–3.5 cm, pericarp c. 3 mm thick, hardened when dry, surface shallowly coarsely rugose-verrucose with 7–8 longitudinal ridges (some ridges running to only midpoint of monocarps, whereas some forming loops with others), indumentum short-tomentose (often more sparsely so or almost glabrous on ridges), apex not apiculate. Seeds 9–17 per monocarp, flattened-ellipsoid to flattened-ovoid, 1.7–2.1 × 1–1.2 cm, more flattened for in-between seeds, surface pitted and slightly rugose, raphe slightly raised with a slight groove in the middle, endosperm ruminations spiniform.

Etymology. – The specific epithet *domatiophorus* is a masculine adjective in first and second declensions, referring to “domatia” on the lower leaf surface.

Habitat and phenology. – Occurring in (edges of) evergreen forests, disturbed evergreen forests, or edges of secondary



Fig. 3. – Inflorescence position of *Leoheo Chaowasku* (A) and *Monocarpia Miq.* (B). A. Axillary inflorescences/infructescences of *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le; B. Terminal inflorescence of *Monocarpia kalimantanensis* Kessler. [A: HUAUF collectors 2009-03-19-ND, CMUB; B: Sidiyasa et al. 3469, L] [Photos: A: D.T. Ngo; B: Arbainsyah]



Fig. 4. – Lower leaf surface of *Leoheo Chaowasku* (A) and *Monocarpia Miq.* (B). A. *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le, with a hairy domatium; B. *Monocarpia maingayi* (Hook. f. & Thomson) I.M. Turner, without domatia. [A: Chaowasku 131, CMUB; B: Promchua 18, CMUB]

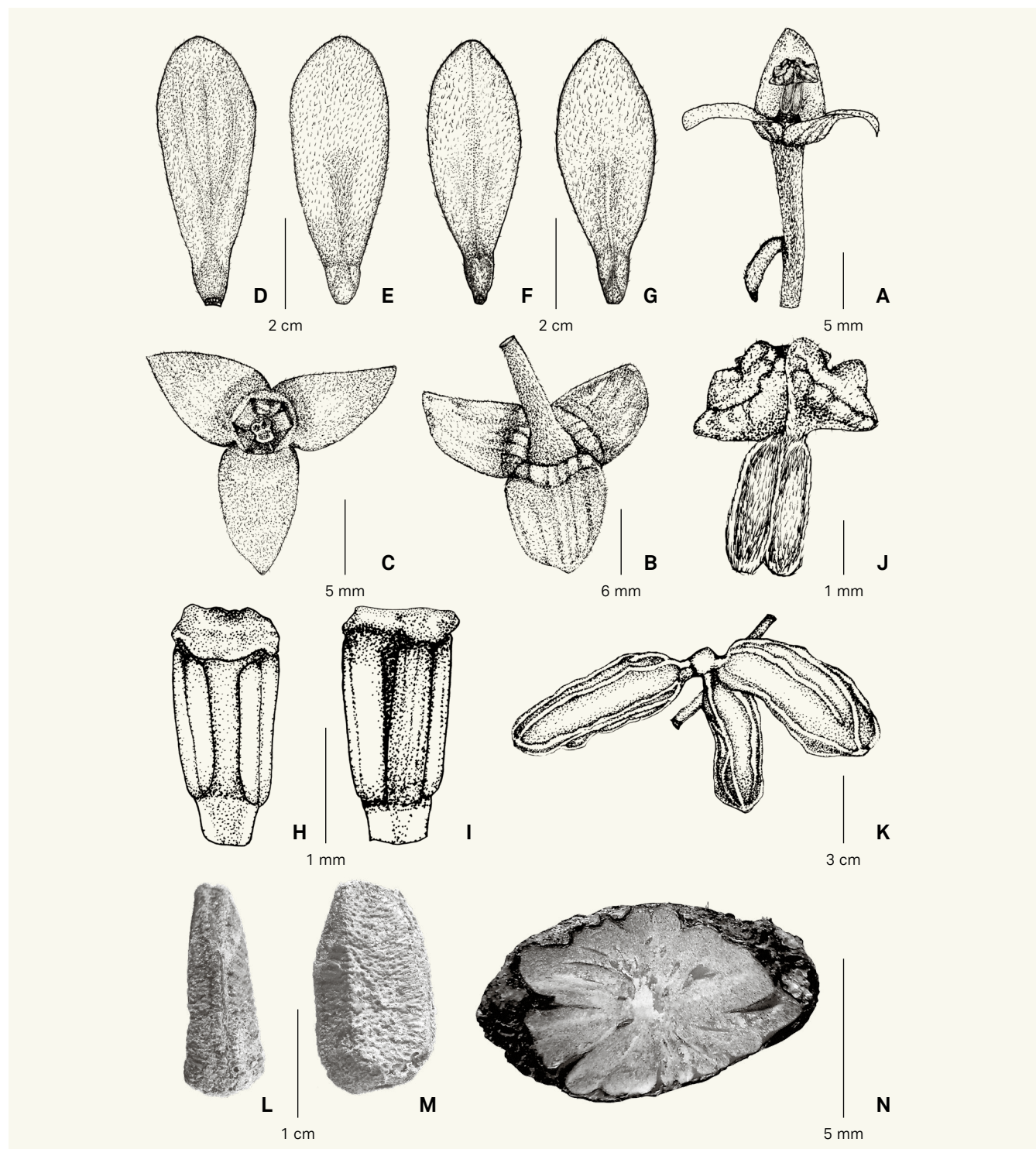


Fig. 5. – Reproductive organs of *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le: **A.** Flower with petals and stamens removed; **B.** Flower with petals, stamens, and carpels removed, back view, showing outer side of sepals; **C.** Same as (**B**), but on another side, showing a volcano-shaped torus and inner side of sepals; **D.** Inner side of an outer petal; **E.** Outer side of an outer petal; **F.** Inner side of an inner petal; **G.** Outer side of an inner petal; **H.** Stamen, abaxial side; **I.** Stamen, adaxial side; **J.** Carpels, showing enlarged and irregularly lobed stigmas; **K.** Fruit, showing longitudinal ridges on monocarp surface; **L.** Seed, lateral view, showing a raphe; **M.** Seed, lateral view, showing a pitted and slightly rugose surface; **N.** Cross section of a seed, showing spiniform endosperm ruminations.

[**A–J:** HUAF collectors 2009-03-19-ND, CMUB; **K:** Chaowasku 131, CMUB; **L–N:** Chaowasku 165, CMUB] [Drawing: A. Damthongdee]



Fig. 6. – *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le, showing habit with inflorescences and flowers. [HUAf collectors 2009-03-19-ND, CMUB] [Drawing: A. Damthongdee]

forests adjacent to the primary ones; on a steep hillside or near a stream at 270–350 m. Flowering material collected in March. Fruiting material collected in July.

Conservation status. – *Leoheo domatiophorus* grows in lowland rainforests of Nam Đông (where part of Bach Ma National Park is located) and A Luoi Districts of Thua Thien-Hue Province (Fig. 7), and has also been observed in the adjacent Quang Tri and Quang Nam Provinces as well. This habitat has been continuously destroyed for *Acacia* Mill. cultivation and other kinds of deforesting land use. Furthermore, only a small part of the habitat of *L. domatiophorus* is

protected in the Bach Ma National Park. Due to its restricted range in threatened lowland rainforests of central Vietnam, the new species qualifies as “Vulnerable” [VU B2ab(iii)] using the IUCN Red List Categories and Criteria (IUCN, 2012).

Paratypes. – VIETNAM. Prov. Thua Thien-Hue: Nam Đông Distr., 16°08'N 107°37'E, VII.2014, ster., *Chaowasku* 129 (CMUB); A Luoi Distr., 16°01'N 107°31'E, VII.2014, ster., *Chaowasku* 130 (CMUB); *ibid. loco*, VII.2014, fr., *Chaowasku* 131 (CMUB); Nam Đông Distr., 16°08'N 107°37'E, VII.2016, fr., *Chaowasku* 165 (CMUB); *ibid. loco*, VII.2016, ster., *Chaowasku* 166 (CMUB).

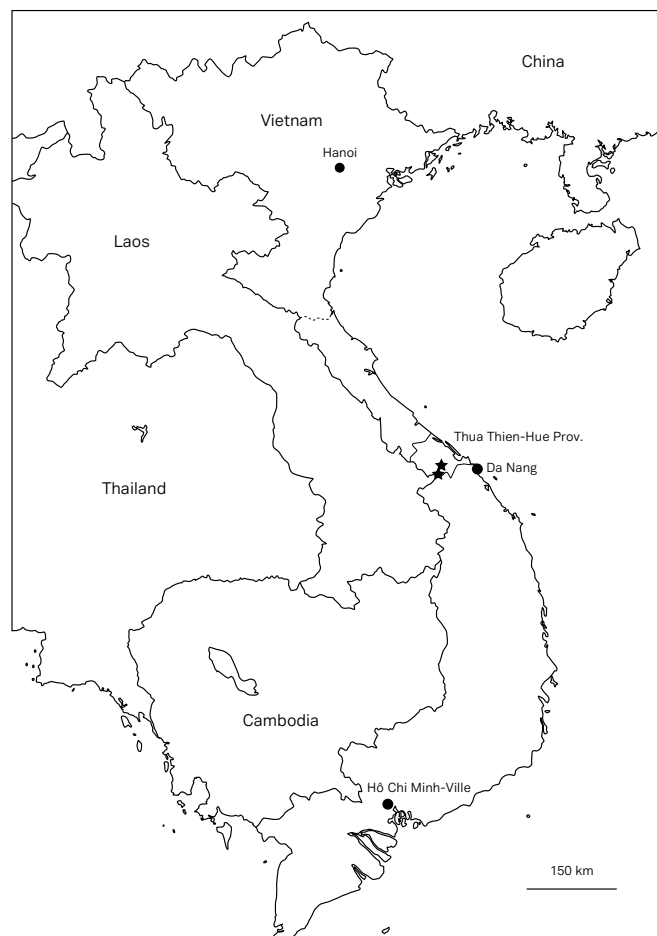


Fig. 7. – Distribution of *Leoheo domatiophorus* Chaowasku, D.T. Ngo & H.T. Le (stars).

Acknowledgements

The first author would like to thank the Thailand Research Fund (TRG5880118) and IAPT Research Grants Program in Plant Systematics 2013 for financial support. Directors and curators of Edinburgh (E) and Leiden (L) herbaria kindly permitted the first author to study their material. Prapanth Iamwiriyaikul is gratefully acknowledged for his invaluable advices on nomenclature of the new taxa. Daniel C. Thomas and Sven Buerki provided many useful comments and suggestions for the improvement of an earlier draft of this article. Arbainsyah, Simon Gardner, and Pindar Sidisunthorn kindly allowed us to use their beautiful photographs.

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| Taxon | Voucher | Herbarium | Location | rbcl | trnLF | matK | ndhF | psbA-trnH | ycf1 |
|---------------------------------------|--------------------------------|-------------|--------------------------|-----------|-------------|-----------|-----------|-----------|-----------|
| <i>Bocageopsis canescens</i> | Maas et al. 9243 | U | Brazil | JQ690407 | JQ690408 | JQ690409 | JQ690410 | JQ690411 | JQ690412 |
| <i>Brieya fasciculata</i> | Jongkind et al. 1862 | WAG | Ghana | AY841647 | AY841725 | MH585796* | MH585822* | AY841497 | - |
| <i>Dendrokingstonia gardneri</i> [I] | Gardner & Sidisunthorn ST 2214 | L | Thailand | KJ418381 | KJ418406 | KJ418391 | KJ418385 | KJ418399 | KJ418378 |
| <i>Dendrokingstonia gardneri</i> [II] | Gardner & Sidisunthorn MY 067 | L | Malaysia [Peninsular] | MH585807* | MH585812* | MH585797* | MH585823* | MH585803* | MH585816* |
| <i>Dendrokingstonia nervosa</i> | Rogstad 961 | L | Malaysia [Peninsular] | KJ418382 | KJ418407 | MH585798* | KJ418386 | KJ418400 | MH585817* |
| <i>Fenerivia chapelieri</i> | Rabevahitra et al. 4439 | MO | Madagascar | MH585808* | KJ418403-04 | KJ418393 | KJ418387 | KJ418397 | MG264598 |
| <i>Huberantha cerasoides</i> | Chalermglin 440214-4 | L | Thailand | AY319017 | AY319131 | AY518854 | JQ889985 | JQ889980 | JQ889975 |
| <i>Leotheo domatiophorus</i> [I] | Chaowasku 165 | CMUB | Vietnam | MH585809* | MH585813* | MH585799* | MH585824* | MH585804* | MH585818* |
| <i>Leotheo domatiophorus</i> [II] | Chaowasku 129 | CMUB | Vietnam | MH585810* | MH585814* | MH585800* | MH585825* | MH585805* | - |
| <i>Maasia glauca</i> | Chaowasku 169 | CMUB | Thailand | MG264581 | MG264576 | MG264584 | MG264589 | MG264571 | MG264594 |
| <i>Miliusa mollis</i> | Keßler PK 3207 | L | Thailand | AY318990 | AY319102 | AY518851 | JQ690503 | JQ690504 | JQ690505 |
| <i>Miliusa velutina</i> | Pholsena & Koonkhunthod 2842 | L | Thailand | AY318993 | AY319105 | AY518847 | JQ690536 | JQ690537 | JQ690538 |
| <i>Mitrephora alba</i> | Chalermglin 440304-1 | BKK [TISTR] | Thailand | AY318994 | AY319106 | AY518855 | JQ889983 | JQ889978 | JQ889973 |
| <i>Monocaropia euneura</i> | Slik 2931 | L | Indonesia [Borneo] | AY318998 | AY319111 | AY518865 | AY841412 | AY841477 | - |
| <i>Monocaropia maingayi</i> [I] | Kaewruang 1 | L | Thailand | JQ690395 | JQ690396 | JQ690397 | JQ690398 | JQ690399 | JQ690400 |
| <i>Monocaropia maingayi</i> [II] | Gardner & Sidisunthorn ST 0541 | L | Thailand | MH585811* | MH585815* | MH585801* | MH585826* | MH585806* | MH585819* |
| <i>Mwasumbia alba</i> | Couvreur 85 | WAG | Tanzania | EU747680 | EU747674 | MH585795* | MH585821* | MH585802* | - |
| <i>Neo-uvaria telopea</i> | Chaowasku 77 | L | Thailand | JX544755 | JX544783 | JX544751 | JX544778 | JX544791 | JX544766 |
| <i>Orophea kerrii</i> | Chalermglin 440416-1 | L | Thailand | AY319008 | AY319121 | AY518818 | JQ690419 | JQ690420 | JQ690421 |
| <i>Orophea espiintana</i> | Chatrou et al. 133 | U | Peru | AY319066 | AY319180 | DQ018260 | MH585827* | AY841487 | MH585820* |
| <i>Oxandra venezuelana</i> | Chatrou et al. 120 | U | Costa Rica | AY841645 | AY841723 | JQ690413 | JQ690414 | AY841495 | JQ690415 |
| <i>Phoenicanthus obliquus</i> | Huber 518 | L | Sri Lanka | MG264582 | MG264579 | MG264587 | MG264592 | MG264574 | MG264597 |
| <i>Pseuduvaria fragrans</i> | Chaowasku 27 | L | Thailand | EU522341 | EU522231 | EU522286 | JX544829 | EU522176 | JX544815 |
| <i>Tridimeris</i> sp. | Maas 8646 | U | Cult. MO | JX544753 | JX544782 | JX544750 | JX544773 | JX544786 | JX544761 |
| <i>Trivalvaria</i> sp. | Chaowasku 35 | L | Thailand | JX544822 | JX544794 | JX544824 | JX544828 | JX544805 | JX544814 |
| <i>Winitia cauliflora</i> | Anon s.n. | L | Cult. BO (Acc. XV-A-196) | AY319054 | AY319168 | AY518800 | JX544776 | JX544789 | JX544764 |

Appendix 1. – List of sequences used in this study. Taxa, voucher information, location and GenBank accession numbers for the plastid DNA regions; * = sequences produced for this study.