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Miliusa chantaburiana (*Annonaceae*), a new species from SE Thailand

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Abstract: *Miliusa chantaburiana* Damthongdee & Chaowasku, a new species of *Annonaceae* from SE Thailand, is described and illustrated. It belongs to a clade with campanulate flowers and inner petals that are generally tightly appressed from the base to more or less the midpoint at anthesis. The new species is remarkable in possessing a strongly recurved apex of the inner petals at anthesis and can be principally differentiated from its morphologically closest species, *M. pumila* Chaowasku and *M. filipes* Ridl., both from Peninsular Thailand, by the higher number of stamens and carpels per flower and horseshoe-shaped stigmas. *Miliusa chantaburiana* is also unique in having a 6-base-pair insertion in the plastid *matK* sequence. A revised key to species in the campanulate-flowered clade in Thailand is given.

Key words: *Annonaceae*, Chantaburi, *matK*, *Miliusa*, *Miliuseae*, new species, systematics, taxonomy, Thailand

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Introduction

Miliusa Lesch. ex A. DC., a medium-sized genus of *Annonaceae* (subfamily *Annonoideae*, tribe *Miliuseae*; Chatrou & al. 2012) currently containing about 60 species (Rajkumar & al. 2016), has received considerable taxonomic attention over the past decade as there are a huge number of new species described (Balachandran & Chakrabarty 2010; Narayanan & al. 2010, 2012; Chaowasku 2013; Chaowasku & Keßler 2013; Chaowasku & al. 2013; Chaowasku 2014; Chaowasku & Keßler 2014; Josekutty & al. 2016; Karuppusamy & Richard 2016; Murugan 2016; Page & Nerlekar 2016; Page & al. 2016; Rajkumar & al. 2016). The genus can be recognized by a suite of characters, i.e. sepals and outer petals of similar size with both much smaller than the inner petals, a densely hairy torus, stamens without a shield-like connective apex covering the thecae, and 4-parted lamelliform endosperm ruminations (Chaowasku & Keßler 2006). Members of the genus are distributed in (sub) tropical forests of the Indian subcontinent, mainland SE Asia, SE Asian islands, N Australia and New Guinea (including the D'Entrecasteaux Islands and Louisiade Archipelago) (Mols & Keßler 2003; Chaowasku & Keßler 2006, 2013; Chaowasku & al. 2013).

On the basis of molecular phylogenetic analyses, four strongly-supported major clades within *Miliusa* have been identified (clades A, B, C and D; Chaowasku & al. 2013). Members of clade B exhibit diagnostic bell-shaped flowers with the inner petals generally tightly appressed from the base to more or less the midpoint at anthesis, and there are somewhat translucent window-like structures at the inner petal base of a number of species in this clade (observable only in living plants; Chaowasku & Keßler 2013; Chaowasku & al. 2013). Species in this clade can be found in the Indian subcontinent through mainland SE

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Asia to Sumatra and Java (Mols & Keßler 2003; Chaowasku & Keßler 2013; Chaowasku & al. 2013).

Field trips in Chantaburi Province, SE Thailand, resulted in the discovery of an unknown *Miliusa* belonging to clade B. At a glance, this species resembles the recently described *M. pumila* Chaowasku from S Thailand (Chaowasku 2014). Detailed morphological examinations and comparisons with morphologically similar species in clade B support recognition of this unknown species as new to science. The aims of the present study are to describe and illustrate this new species. To strengthen the new species recognition, its plastid *matK* sequence is generated and compared with that of other species in clade B. In addition, a revised key to species of clade B in Thailand is provided.

Material and methods

Morphological data of *Miliusa pumila* and *M. filipes* Ridl. for comparisons were from Chaowasku (2014) and the collection *Kloss 6968* (holotype) at K, respectively. Morphological data of other species for reconstructing a key to species of clade B in Thailand were from Chaowasku & Keßler (2013) and collections cited therein. The indumentum terminology used follows Hewson (1988). The term "almost glabrous" means that only a few scattered hairs were observed. The word "circa" (c.) is indicative of a single observation/measurement.

DNA sequences of a plastid *matK* exon of the unknown *Miliusa* from SE Thailand and six other species in clade B (*M. balansae* Finet & Gagnep., *M. campanulata* Pierre, *M. cuneata* Craib, *M. eupoda* (Miq.) I. M. Turner, *M. pumila* Chaowasku and *M. umpangensis* Chaowasku & Kessler) were generated in the present study using the same protocols as described in Chaowasku & al. (2018). The *matK* sequences of four other species in clade B were obtained from the following publications: *M. thorelii* Finet & Gagnep. from Mols & al. (2004), *M.* cf. *indica* Lesch. ex A. DC. from Thomas & al. (2012) and *M. macrocarpa* Hook. f. & Thomson and *M. montana* Gardner ex Hook. f. & Thomson from Chaowasku & al. (2013). Sequence visualization was performed in MEGA7 (Kumar & al. 2016). The information on voucher specimens and GenBank accession numbers is provided in the Appendix (pp. 300–301); the alignments are provided in Supplementary Material online.

Results and Discussion

Miliusa chantaburiana Damthongdee & Chaowasku, **sp. nov.** – Fig. 1–3.

Holotype: Thailand, cultivated in Bangkok [sapling originally from Khiri Than Dam, Chantaburi Province], 7 Feb 2015 [in flower], *Nakorn-Thiemchan NTC 29* (CMUB!; isotypes: B!, P!).

Diagnosis — *Miliusa chantaburiana* is morphologically close to *M. pumila* and *M. filipes*, both occurring in Peninsular Thailand (Chaowasku 2014). The new species differs mainly from *M. pumila* by having generally larger leaf blades ([9.2–]12.2–18[–19.5] × [2.8–]3.3–6 cm vs 5.4–10.5 × 2–4.1 cm), generally longer pedicels ([11–]14–22[–30] mm vs 5–11 mm), more stamens per flower (48–50 vs 38–39), and many more carpels per flower (49–71 vs 12–13). The new species primarily differs from *M. filipes* by possessing considerably more stamens (48–50 vs c. 22) and carpels (49–71 vs c. 16) per flower. In addition, *M. chantaburiana* exhibits horseshoe-shaped stigmas, whereas they are subglobose to ellipsoid(-obovoid) in *M. pumila* (Chaowasku 2014) and capitate in *M. filipes*.

Description — *Treelets* to 1.5 m tall; *young twigs* tomentose with appressed hairs. *Petiole* 2–5 mm, grooved on upper surface, glabrous, puberulous with appressed hairs on lower surface; *leaf blade* usually elliptic(-obovate), rarely obovate, (9.2–)12.2–18(–19.5) × (2.8–)3.3–6 cm, lower surface puberulous with appressed hairs, upper surface glabrous, base cuneate, apex usually caudate-acuminate, rarely (obtuse-)retuse; *midrib* raised and puberulous with appressed hairs on lower surface, (slightly) sunken and glabrous on upper surface; *secondary veins* 7–10 per side, rather prominent on lower surface, usually with inter-secondary veins forming loops, angle with midrib 51°–58° (at middle part of leaf blade). *Flowers* solitary or a pair of solitary flowers in same axil (small interval observed between two solitary flowers), axillary or in axils of fallen leaves, bisexual, buds ovoid-ellipsoid; *pedicel* (11–)14–22(–30) mm long, almost glabrous, bearing 3 or 4 bracts at base, upper one usually a bit lower than midpoint of pedicel. *Sepals* free, ovate-triangular, 1.5–2 × 1.5–1.8 mm, outside almost glabrous to puberulous with appressed hairs, inside (almost) glabrous, margin puberulous-tomentose. *Outer petals* ovate-triangular, 1.5–2 × c. 1 mm, outside puberulous with appressed hairs, inside (almost) glabrous, margin (puberulous-) tomentose; *inner petals* tightly appressed from base to ± midpoint at anthesis, elliptic, 15–18 × 7–8.5 mm, outside glabrous except basal part (c. ⅓ of inner petal length) puberulous (or more sparsely so) with appressed hairs, inside glabrous except basal part (c. ⅓ of inner petal length) puberulous intermixed with glandular dots, margin glabrous (but near margin puberulous, more densely so toward apex), base slightly saccate, with basal half ± reticulate-discoloured and rugulose, apex acute-obtuse, with apical part (between ⅓ and ½ of inner petal length) strongly recurved at anthesis. *Torus* ovoid-conical. *Stamens* 48–50 per flower, 1–1.5 mm long, connective prolongation nearly absent. *Carpels* 49–71 per flower, 1.5–2 mm long; *stigmas* horseshoe-shaped; *ovaries* puberulous or more sparsely so; *ovule* 1 per ovary, sub-basal. *Fruits* of up to 6 monocarps

Fig. 1. Holotype of *Miliusa chantaburiana* Damthongdee & Chaowasku, *Nakorn-Thiemchan NTC 29* (CMUB).

Fig. 2. Leaf and flower of *Miliusa chantaburiana* – A: abaxial (lower) leaf surface; B: adaxial (upper) leaf surface; C: flower, apical view showing stamens, carpels, inner petal discolouration and translucent window-like structures; D: flower, oblique view showing strongly recurved apical part of inner petals; E: stamens attached to torus; F: carpel. – Scale bars: A = 2 cm; B = 10 cm; E = 1 mm; F = 0.5 mm. – A, B from cultivated material; C–F from *Nakorn-Thiemchan NTC 29* (CMUB – spirit material).

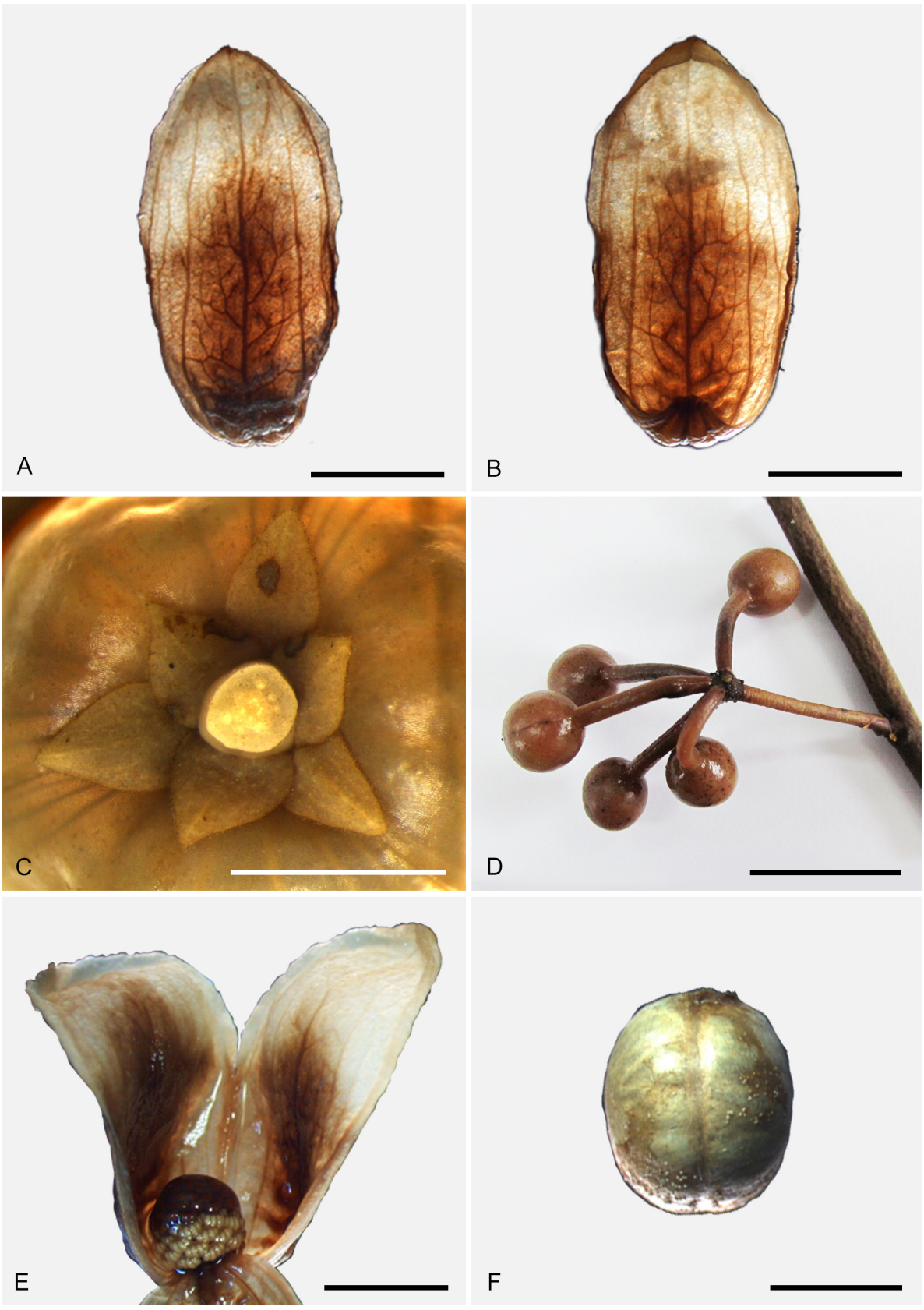

Fig. 3. Flower, fruit and seed of *Miliusa chantaburiana* – A: abaxial surface (outside) of an inner petal; B: adaxial surface (inside) of an inner petal; C: flower, basal view showing sepals and outer petals; D: fruit with five monocarps; E: flower with one inner petal pulled apart from others showing a mass of stamens and carpels; F: seed. – Scale bars: A, B, E, F = 5 mm; C = 3 mm; D = 2 cm. – A, B, E from *Chaowasku 170* (CMUB – spirit material); C from *Nakorn-Thiemchan NTC 29* (CMUB – spirit material); D from *Chaowasku 171* (CMUB – spirit material); F from *Nakorn-Thiemchan NTC 28* (CMUB – spirit material).

borne on a pedicel to 30 mm long; *monocarps* subglobose, 9.5–11 × 9–9.5 mm, smooth, apex not apiculate, base contracted into a stipe 11–18 mm long. *Seed* 1 per monocarp, subglobose, c. 8 × 7 mm, smooth.

Phenology — Flowering material collected in February and May (in cultivation); fruiting material collected in November (in cultivation).

Distribution and ecology (at original locality) — Chantaburi Province, SE Thailand (Fig. 4); occurring in partially disturbed evergreen forests around a constructed dam; at an elevation of c. 205 m.

Fig. 4. Distribution of *Miliusa chantaburiana* (●).

Field notes — Flowers bell-shaped; inner petals light green, more red-purple toward base, apical part (between ⅓ and ½ of inner petal length) becoming strongly recurved at anthesis, discolouration observed inside inner petals, more or less reticulate at basal part, with translucent window-like structures at base.

Conservation status — This species is known only from a very restricted area, i.e. around Khiri Than Dam of Chantaburi Province, SE Thailand (Fig. 4). Fewer than 10 individuals were observed in the area, some of which occur adjacent to the reservoir and could be submerged in the near future, and it is believed that many more individuals have been submerged during dam construction. Further, this species has never been reported to occur in nearby areas (e.g. Khao Khitchakut National Park, Khao Soidao Wildlife Sanctuary, Namtok Phliu National Park and Namtok Khlongkaew National Park) and no specimens have been collected prior to the present study. Based on this information, *Miliusa chantaburiana* is undoubtedly a rare species; however, we believe that more exploratory data, especially from Cambodia (which is merely c. 20 km away from the dam), are required prior to the assessment of the conservation status of this species. Therefore, it is considered here as Data Deficient (DD) (IUCN 2012).

Etymology — The epithet refers to Chantaburi, the SE Thai province where this species is endemic.

Remarks — Table 1 highlights important morphological differences between *Miliusa chantaburiana* and two other morphologically similar species, *M. pumila* and *M. filipes*. On the basis of sequence comparisons of the *matK* exon, *M. chantaburiana* differs from other species in clade B by having a 6-base-pair insertion. Besides, the new species and *M. pumila* differ from each other by nine nucleotides. *Miliusa filipes* is an extremely rare species, known only from the type gathering. Our attempts to find this species alive again were not successful.

The apex of each inner petal of *Miliusa chantaburiana* is strongly recurved at anthesis (Fig. 2D). This trait is, however, not unprecedented because it is also observable in several other species in clade B, e.g. *M. codonantha* Chaowasku (Chaowasku 2013), *M. thorelii* (Chaowasku & Keßler 2013; Chaowasku & al. 2013) and *M. umpangensis* (Chaowasku & Keßler 2013). The genus *Miliusa* in Thailand is being revised for the Flora of Thailand project by the authors and it is expected that a few more new species in clades B and C will be added. Available material is currently insufficient for descriptions.

Additional specimens examined (paratypes) — Thailand: cultivated in Bangkok [originally collected as saplings from Khiri Than Dam, Chantaburi Province], *Chaowasku 170* [in flower], *Chaowasku 171* [in fruit; from the same individual as *Nakorn-Thiemchan NTC 29*] and *Nakorn-Thiemchan NTC 28* [in fruit] (all CMUB).

Table 1. Chief morphological differences between *Miliusa chantaburiana* and two other morphologically similar species: *M. pumila* and *M. filipes*.

	M. chantaburiana	*M. pumila*	*M. filipes*
Leaf blade length [cm]	(9.2–)12.2–18(–19.5)	5.4–10.5	10.9–13.8
Leaf blade width [cm]	(2.8–)3.3–6	2–4.1	2.8–4.1
Pedicel length [mm]	(11–)14–22(–30)	5–11	c. 20
Number of stamens per flower	48–50	38–39	c. 22
Number of carpels per flower	49–71	12–13	c. 16
Stigma shape	horseshoe-shaped	subglobose to ellipsoid (-obovoid)	capitate

Revised key to species of *Miliusa* clade B in Thailand

Flowering specimens

1. Flowers unisexual or bisexual with 2–8 stamens per flower . . . . . ***M. thorelii***
– Flowers bisexual with > 15 stamens per flower . . . **2**
2. Inner petals with warty glandular structures inside apical part (c. ⅓ of inner petal length) . . . . . ***M. umpangensis***
– Inner petals without warty glandular structures inside apical half **3**
3. Base of leaf blade slightly subcordate to cordate, slightly to moderately unequal; sepals and outer petals linguiform . . . . . ***M. thailandica***
– Base of leaf blade cuneate or obtuse, usually equal; sepals and outer petals narrowly to broadly triangular(-ovate) **4**
4. Young twigs (almost) glabrous **5**
– Young twigs sparsely to densely hairy **6**
5. Discolouration on basal half of inner petals (much clearer visible inside) not (or very slightly) reticulate (much more clearly observed in living plants), window-like structures at base indistinct (in living plants; sometimes absent) . . . . . ***M. eupoda***
– Discolouration on basal half of inner petals (both sides) remarkably reticulate (much more clearly observed in living plants), window-like structures at base conspicuous (in living plants) . . . . ***M. campanulata***
6. Stamens < 30 per flower . . . . . ***M. filipes***
– Stamens ≥ 30 per flower **7**
7. Stigmas horseshoe-shaped . . . . . ***M. chantaburiana***
– Stigmas globose(-capitate), subglobose, ellipsoid (-obovoid) or terete **8**
8. Pedicels ≥ 1.5 mm long . . . . . ***M. cuneata***
– Pedicels < 1.5 mm long **9**
9. Leaf blade 5.4–10.5 × 2–4.1 cm, lower leaf surface sparsely hairy (hairs not discernible upon touching); stamens 38–39 per flower . . . . . ***M. pumila***
– Leaf blade 11–19 × 4.1–8.1 cm, lower leaf surface densely hairy (hairs discernible upon touching); stamens c. 48 per flower . . . . . ***M. hirsuta***

Fruiting specimens

Excluding species with unknown fruits: *Miliusa filipes*, *M. hirsuta* and *M. pumila*.

1. Leaf blade generally 8–14 cm wide . . . . ***M. thorelii***
– Leaf blade generally 2–7 cm wide **2**
2. Young twigs (almost) glabrous **3**
– Young twigs sparsely to densely hairy **4**
3. Stipe (18–)20–35(–43) mm long . . . . . ***M. eupoda***
– Stipe (8–)10–15(–19) mm long . . . . . ***M. campanulata***
4. Base of leaf blade slightly subcordate to cordate, slightly to moderately unequal; monocarp surface verrucose, monocarp apex usually (notably) apiculate . . . . . ***M. thailandica***
– Base of leaf blade cuneate, equal; monocarp surface smooth or (slightly) verrucose, monocarp apex usually not apiculate **5**
5. Stipe c. 5 mm long . . . . . ***M. umpangensis***
– Stipe (7–)9–20 mm long **6**
6. Monocarps 6–9 × 6–7 mm . . . . . ***M. cuneata***
– Monocarps 9.5–11 × 9–9.5 mm . . . . . ***M. chantaburiana***

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Appendix

GenBank accession numbers of *matK* sequences of *Miliusa* species in clade B, including voucher specimen information. *Taxon*: GenBank accession number; *voucher specimen*; herbarium acronym; collection locality. * = sequence newly generated for the present study.

Miliusa balansae Finet & Gagnep.: MH663442*; *Nuraliev 1052*; CMUB; Vietnam.

Miliusa campanulata Pierre: MH663443*; *Nuraliev 891*; CMUB; Vietnam.

Miliusa chantaburiana Damthongdee & Chaowasku: MH663444*; *Nakorn-Thiemchan NTC 28*; CMUB; Thailand.

Miliusa cuneata Craib: MH663445*; *Maknoi 8314*; QBG; Thailand.

Miliusa eupoda (Miq.) I. M. Turner: MH663446*; *Chaowasku 78*; CMUB; Thailand.

Miliusa cf. ***indica*** Lesch. ex A. DC.: JQ723781; *Attanayake QST 646*; HKU; Sri Lanka.

Miliusa macrocarpa Hook. f. & Thomson: JQ690499; *Grierson & Long 4095*; E; Bhutan.

Miliusa montana Gardner ex Hook. f. & Thomson: JQ690507; *Hladik 1039*; US; Sri Lanka.

Miliusa pumila Chaowasku: MH663447*; *Chaowasku 110*; L; Thailand.

Miliusa thorelii Finet & Gagnep.: AY518846; *Keßler PK 3184*; L; Thailand.

Miliusa umpangensis Chaowasku & Kessler: MH663448*; *Chaowasku 89*; L; Thailand.

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