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Artemisia tyuosenicola (Asteraceae), a new species from Taiwan

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ABSTRACT: A new species, *Artemisia tyuosenicola* (Asteraceae: Anthemideae), is described from Taiwan. It is found exclusively on the scree slopes of Mt. Zhongyangjian in the Central Range of Taiwan. This species is distinctive in having shallowly trilobed, trident-like leaves with densely white arachnoid-tomentose abaxial surfaces. Its leaf morphology resembles *A. tridentata*, *A. integrifolia* and *A. stolonifera* to some extent, but can be readily distinguished by its smaller leaf size, pattern of leaf lobes and relatively uniform leaf shape. Both capitulum morphology and molecular phylogenetic analysis indicate that *A. tyuosenicola* belongs to *A.* subg. *Artemisia*. A detailed description and a distribution map of *A. tyuosenicola* are also provided herein.

KEY WORDS: *Artemisia integrifolia*, *Artemisia stolonifera*, *Artemisia tridentata*, phylogeny, Tyuosenzan, Zhongyangjian.

INTRODUCTION

Artemisia L. is the largest genus in the tribe Anthemideae of the family Asteraceae, comprising over 400 species mainly distributed in the temperate regions of the Northern Hemisphere, spanning across Europe, Asia, and North America (POWO, 2023; WFO, 2023), with a few species extending to the Southern Hemisphere (Bremer and Humphries, 1993; Ling and Peng, 1998; Shultz, 2006; Kim *et al.*, 2020). This genus is well known as aromatic herbs and many species have been utilized in food, medicine and horticulture worldwide (Vallès and McArthur, 2001). Despite its common usage in human culture, the circumscription of the genus *Artemisia* and its infrageneric classification have been challenging due to tremendous species diversity (Sanz *et al.*, 2008; Hayat *et al.*, 2009; Jiao *et al.*, 2023). Historical taxonomic works have focused on capitulum morphology for infrageneric classification (De Candolle, 1838; Poljakov, 1961; Persson, 1974). By combining morphological and molecular evidences, 6 subgenera of *Artemisia* were commonly recognized (Hobbs and Baldwin, 2013), namely ***Artemisia*** Less., ***Absinthium*** (Mill.) Less., ***Dracunculus*** (Bess.) Rydb., ***Seriphidium*** (Bess.) Rouy., ***Tridentatae*** (Rydberg) McArthur, and ***Pacifica*** C.R. Hobbs & B.G. Baldwin. However, most subgenera were not supported as monophyletic in the subsequent studies (Malik *et al.*, 2017; Jiao *et al.*, 2023), with the only exception being *Pacifica*. The recent phylogenomic work utilizing nuclear genome SNPs data proposed a new 8-subgenera framework, with two new subgenera (***Pectinatae*** B.H. Jiao & T.G. Gao and ***Ponticae*** B.H. Jiao & T.G. Gao) and revised circumscriptions to reflect the update phylogenomic results (Jiao *et al.*, 2023).

In Taiwan, 17 taxa of *Artemisia* were recorded in *Flora of Taiwan* 2^{nd} *edition*, namely *A. annua*, *A. anomala*, *A. capillaris*, *A. chinensis* (=*Crossostephium chinense*), *A. chingii*, *A. fukudo*, *A. indica*, *A. japonica*, *A. kawakamii*, *A. lancea*, *A. morrisonensis*, *A. niitakayamenis*, *A. oligocarpa*, *A. simulans*, *A. somae* var. *somae, A.somae* var. *batakensis* and *A. tsugitakaensis* (Ling and Peng, 1998). Additionally, *A. filifolia* Torr. was newly added to the record as a naturalized plant in eastern Taiwan (Chung and Hsu, 2018). During a field expedition in Mt. Zhongyangjian in central Taiwan (Fig. 1), we discovered a population of *Artemisia* possessing remarkable shallow trident-shaped leaves, which were not seen in any known species from Taiwan. After careful examination of its morphology, and after comparing with other entire-leafed *Artemisia* taxa from *Flora of Taiwan*, *Flora of China*, *Flora of Japan* and relevant literature (Koyama, 1995; Ling and Peng, 1998; Shultz, 2006; Ling *et al.*, 2011), we concluded that this population bears unique leaf morphology and represents an undescribed species. During our investigation of relevant specimens deposited in Taiwanese herbaria (TAIF, TAI, HAST), we found that this population from Mt. Zhongyangjian had been previously collected twice but identified as either *A. princeps* var. *orientalis* (now as a synonym of *A. indica*; *K. S. Hsu 907*, TAIF) or an unknown species (*C. I. Huang et al. 2768*, HAST), and it was not found in any other area. The trail to Mt. Zhongyangjian is notorious for its danger and difficulty among the Taiwanese hiking community (TNPH, 2024), which may explain why this population has previously been overlooked by botanists. Here, we propose it as a new species, named *Artemisia tyuosenicola*, with a detailed morphological description.

To understand the phylogenetic position of this new species in the genus *Artemisia*, molecular analysis utilizing sequences of nuclear ITS, ETS and two plastid DNA regions (see below) was also conducted in this study.

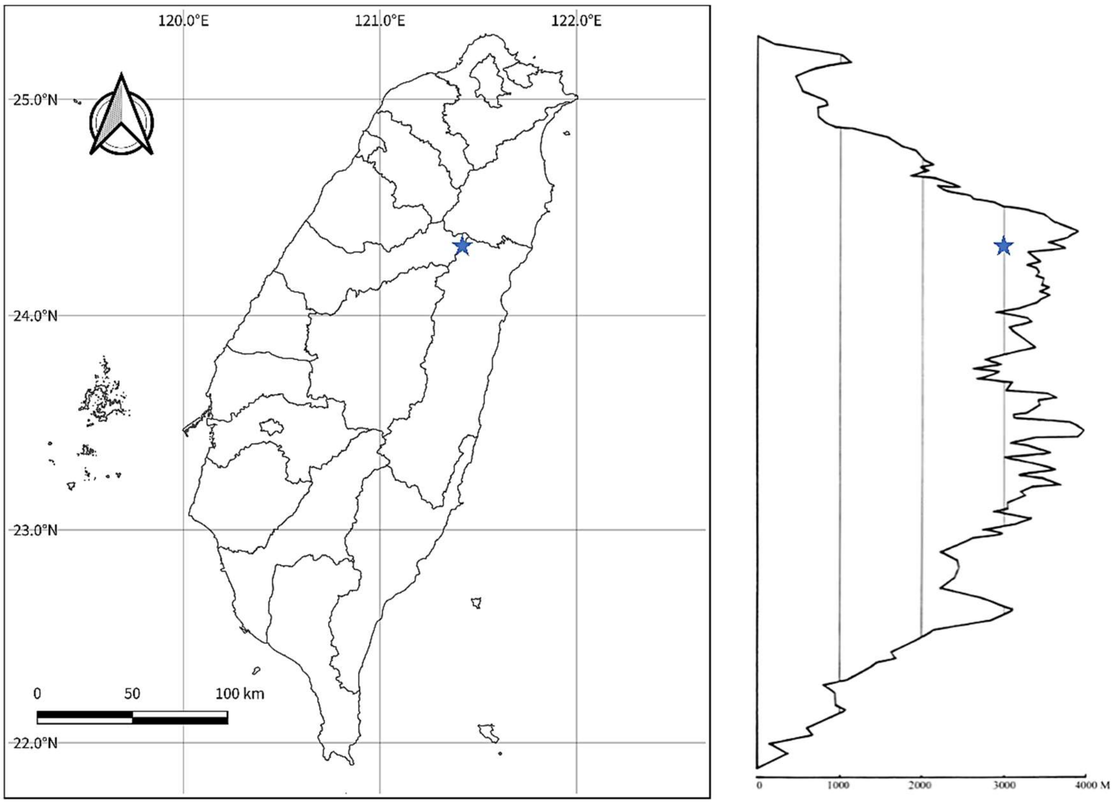

Fig. 1. Distribution map of ***Artemisia tyuosenicola.***

MATERIAL AND METHODS

Plant materials and morphological investigation

For morphological comparison, living materials of *Artemisia* taxa from Taiwan and the new species were collected, observed and further processed for specimens. Other *Artemisia* specimens available from herbaria (HAST, TAI and TAIF) were also observed for comparison. For taxa whose materials are not available in Taiwan, their morphological data were adapted from literature, chiefly the *Flora of China*, *Flora of Japan*, and *Flora of North America* (Koyama, 1995; Shultz, 2006; Ling *et al*., 2011).

For molecular phylogenetic analyses, samples of new species and other Taiwanese *Artemisia* taxa were collected from the field. Fresh leaf materials for the following molecular analyses were dried in silica gel immediately after collection. The vouchers applied for both molecular analyses and morphological investigation were deposited in TAIF herbarium. Additionally, DNA sequences of other *Artemisia* taxa were obtained from NCBI Genbank following Jiao *et al*. (2019)'s work. Studied taxa, their GenBank accession numbers, and collection information are shown in Table S1.

Genomic DNA extraction, amplification, and sequencing

Genomic DNA of the newly collected *Artemisia* leaves was extracted using CTAB method (Doyle and Doyle, 1990) with some modifications. For each sample, ca. 20 mg of dried leaf tissue were grounded in liquid nitrogen. 3X CTAB buffer (3% CTAB; 1.4 M NaCl; 20 mM EDTA; 100 mM Trizma base pH 8.0; 1% PVP; 0.4% 2-mercaptoethanol) was used to isolate DNA. Organic extraction was done by three runs of chloroform: isoamyl alcohol (24: 1) mixing and centrifugation. The supernatant was further extracted and purified with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following manufacture's protocol. Extracted DNA was stored at -20 ℃ before amplification.

Following previous studies (Hobbs and Baldwin, 2013; Jiao *et al*., 2019), the nuclear ITS, ETS, and two chloroplast intergenic spacer regions (*trnL–trnF*, *psbA–trnH*) were utilized in this study to infer the phylogenetic position of *Artemisia tyuosenicola*. The primer sequences, references, and annealing temperature of the studied DNA regions are shown in Table 1. Each amplification was conducted with the mixture of 3 μL genomic DNA (10 ng/μL), 1.5 μL forward primer (10 μM), 1.5 μL reverse primer (10 μM), 9 μL sterile distilled water, and 15 μL 2X Taq Master Mix (Bioman, Taiwan). The PCR products were then commercially purified and sequenced in ABI 3730 XL DNA Analyzers by Genomics BioSci & Tech, Taiwan. Additional sequences of other *Artemisia* taxa were downloaded from the NCBI database. The GenBank accession numbers of the studied sequences and sampled taxa are provided in Table S1.

Table 1. Primers applied for PCR reactions and their references.

DNA regions	Primers	Primer Sequences (5' -->3')	Annealing temperatures	References
Nuclear regions				
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	50°C	White *et al*. 1990
	ITS4	TCCTCCGCTTATTGATATGC	50°C	White *et al*. 1990
ETS	Ast-1	CGTAAAGGTGCATGAGTGGTGT	55°C	Markos and Baldwin 2001
	18SETS	ACTTACACATGCATGGCTTAATCT	55°C	Baldwin and Markos 1998
Chloroplast regions				
trnL-trnF	c	CGAAATCGGTAGACGCTACG	55°C	Taberlet *et al*. 1991
	f	ATTTGAACTGGTGACACGAG	55°C	Taberlet *et al*. 1991
psbA-trnH	psbA	GTTATGCATGAACGTAATGCTC	55°C	Sang *et al*. 1997
	trnH-GUG	CGCGCATGGTGGATTCACAATCC	55°C	Tate and Simpson 2003

Phylogenetic analyses

The sequencing reads were visualized and assembled using MEGA X (Kumar *et al*., 2018). Alignments were carried out with MUSCLE algorithm (Edgar, 2004) under default setting and were manually adjusted. The best-fit DNA substitution models were selected with jModelTest v. 2.1.10 (Darriba *et al*, 2012). Following previous phylogenetic studies on *Artemisia* (Jiao *et al*., 2019; Hobbs and Baldwin, 2013), nuclear DNA (ITS, ETS) and chloroplast DNA (*trnL–trnF*, *psbA–trnH*) were first analyzed separately and then combined together to investigate the systemic position of *A. tyuosenicola* and congruence between datasets.

Phylogenetic relationship was reconstructed via Bayesian inference (BI) and maximum likelihood (ML) approaches. The BI analyses were conducted on Beast v1.10.4 (Suchard *et al*., 2018) with four chains of Markov chain Monte Carlo (MCMC) 10,000,000 generations and tress were sampled every 1000 generations. The consensus BI tree and posterior probabilities (pp) were obtained from the retained trees (burn-in = 0.25). For the ML analyses, the phylogenetic trees were generated with 1000 bootstrap sampling in MEGA X (Kumar *et al*., 2018) under GTR+I+R model.

RESULTS AND DISCUSSION

Morphological comparison

Artemisia tyuosenicola exhibits distinct shallowly trilobed leaves, featuring predominantly one pair of broad teeth above the middle (Fig. 2F). This leaf morphology distinguishes *A. tyuosenicola* from other *Artemisia* species native to Taiwan. The leaf shape bears some resemblance to *A. tridentata* Nutt. from North America. However, in *A. tridentata*, the leaf cleavage is confined to the vary apical part and the lobes are rounded, whereas *A. tyuosenicola* displays one to occasionally three pairs of acute teeth along the leaf margin (Fig. 2G). Further differentiation is observed in the capitulum structure, where *A. tyuosenicola* encompasses outer female florets in addition to central bisexual florets (Fig. 2I). In contrast, *A. tridentata* exclusively presents central florets within the capitulum (Shultz, 2006). The capitulum type, coupled with the presence of a glabrous receptacle, supports the classification of *A. tyuosenicola* within the subgenus *Artemisia* (Jiao *et al*., 2023). Besides, *A. integrifolia* L. and *A. stolonifera* (Maxim.) Kom., both originating from the Far East, Russia, also possess trident-like leaves on occasion. Nevertheless, the leaves of *A. integrifolia* are usually longer and more linear, and leaves of *A. stolonifera* are substantially larger. Comparing to *A. tyuosenicola*, these two species also exhibit greater variability in leaf shape and the number of teeth throughout a plant (Ling *et al*., 2011). Moreover, the capitula usually comprise 20–40 florets in *A. integrifolia* and *A. stolonifera* (Ling *et al*., 2011) but only 8–16 in *A. tyuosenicola*. Both *A. integrifolia* and *A. stolonifera* can reach a height of more than one meter (Ling et al., 2011), whereas *A. tyuosenicola* is a low-growing shrub (Fig. 2A, 2B). Morphological comparisons are summarized in Table 2. As the morphological characteristics of *A. tyuosenicola* could not match any described *Artemisia* taxa, herein we propose it as a new species (see below).

Phylogenetic analyses

The datasets for nrDNA and cpDNA comprised 143 sequences encompassing 123 taxa, and 112 sequences covering 92 taxa, respectively, with 27 sequences being novel contributions in this study (Table S1). The alignment of ITS, ETS, *trnL-trnF*, and *psbA-trnH* regions yielded lengths of 703, 610, 904, 584 base pairs respectively. The combined dataset excluded taxa with only nrDNA data. Consequently, the composite dataset consisted of 92 taxa, totaling 2807-bp, with 292 characters being parsimony-informative (10.4%). Details regarding the alignments are presented in Table 3.

Figure 3 shows the BI tree reconstructed from the combined dataset, with bootstrap values from ML analyses indicated for corresponding nodes. Consistent with the findings by Jiao *et al*. (2019) and Hobbs and Baldwin (2013), the phylogenetic incongruence between nuclear and chloroplast data were weakly supported, and the phylogeny based on combined dataset displayed a more resolved tree topology with higher supporting value in most clades (Fig. 3, Fig. S1–S2). The conflict may be due to the low resolution of the chloroplast DNA data (Fig.

Fig. 2. Morphology of ***Artemisia tyuosenicola*.** **A–B.** Habit. **C–D.** Synflorescence. **E.** Stem. **F.** Leaves. **G.** Variation of leaf morphology. **H–I.** Capitula. **J.** Phyllary. **K.** Outer florets. **L.** Central florets. **M.** Longitudinal section of central florets. **N.** Gynoecium. Scale bar: G = 1 cm; I–N = 2 mm.

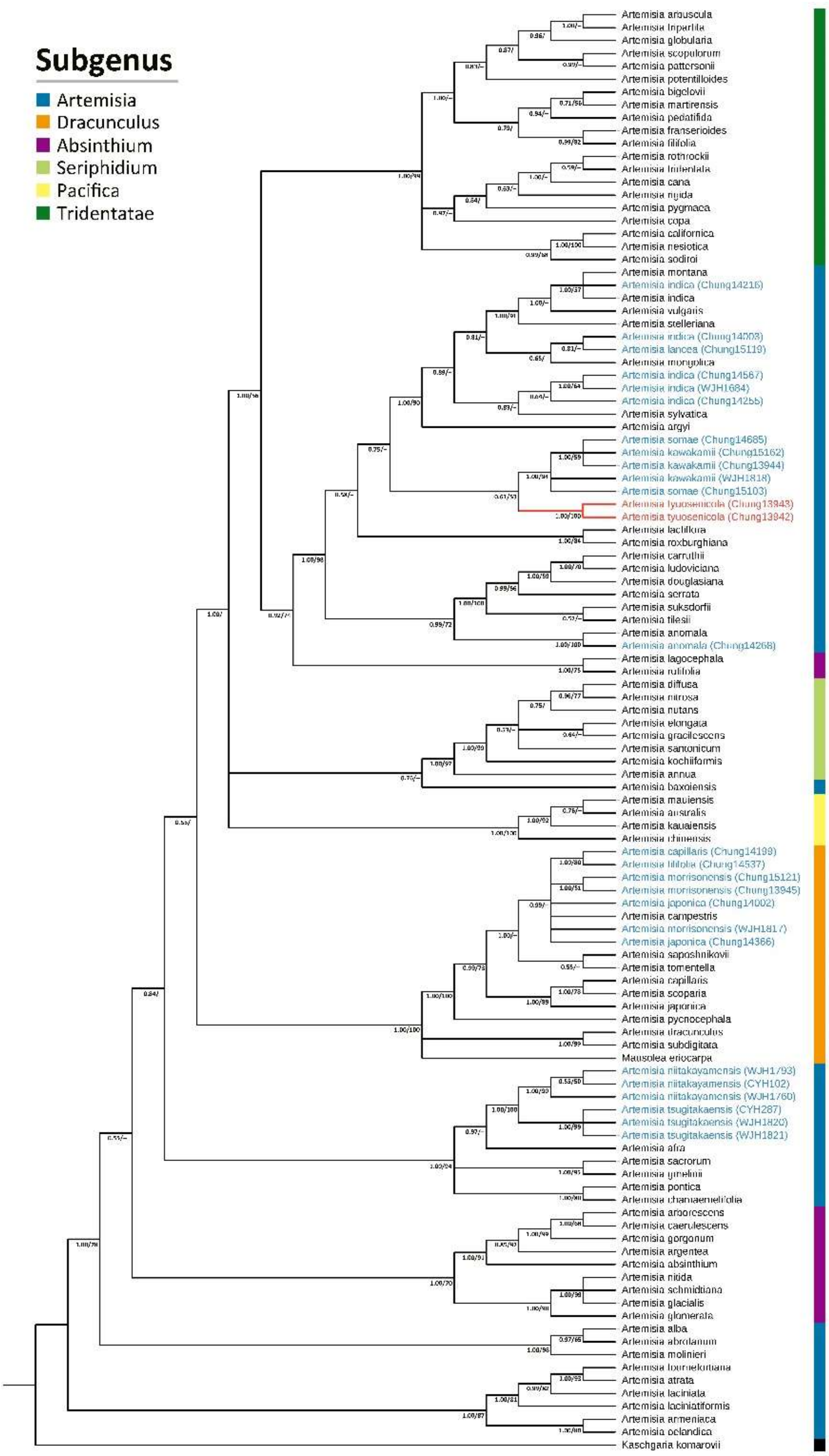

Fig. 3. The cladogram of 112 ***Artemisia*** taxa based on combined dataset (ITS, ETS, *trnL-trnF*, *psbA-trnH*) using the Bayesian Inference method. The values adjacent to nodes represent Bayesian posterior probabilities (PP) / bootstrap percentages of Maximum Likelihood analysis (BS). Nodes with PP < 0.50 were collapsed, while BS < 50% is represented by a dash (–). ***Artemisia tyuosenicola*** samples are highlighted in red, and other newly generated sequences in this study are depicted in blue. Sample collection number follow the taxa names. Subgenus annotations are based on Jiao *et al*. (2019).

Table 2. Morphological comparison of ***Artemisia tyuosenicola*** and similar species. Data of ***A. tridentata***, ***A. integrifolia*** and ***A. stolonifera*** are derived from Shultz (2006) and Ling *et al.* (2011).

Characters	*A. tyuosenicola*	*A. tridentata*	*A. integrifolia*	*A. stolonifera*
Plant height (cm)	30–80	40–200(–300)	50–120	50–120
Leaf size				
Length (cm)	1.4–3.0(–3.8)	0.5–1.2(–2.5)	4–7	6–12
Width (cm)	0.5–1.0(–1.3)	0.2–0.3(–0.6)	1.5–2.5(–3)	4–7
Leaf color				
Adaxially	Green	Gray-green	Green	Green
Abaxially	White	Gray-green	White	Green
Leaf shape	Obovate or elliptic	Cuneate or lanceolate	Elliptic, elliptic-lanceolate, linear-lanceolate or linear	Obovate-elliptic, ovate-elliptic or ovate
Leaf margin				
Pairs of main serrations or lobes	0–3, mostly 1	1 at leaf tip	0–3	0–3
Secondary serrations	Absent	Absent	Absent	Present
Floret number				
Outer female ones	2–4	0	10–15	10–12
Central bisexual ones	6–12	3–8	20–30	12–15

Table 3. Sequence characteristics of the regions used in this study.

Alignment	No. of sequences	No. of taxa	Aligned length (bp)	Variable characters (bp)	Parsimony-informative characters (bp)	Evolutionary model
nrITS	143	123	703	187 (26.6%)	128 (18.2%)	GTR+I+G
nrETS	143	123	610	193 (31.6%)	121 (19.8%)	GTR+I+G
trnL-trnF	112	92	904	55 (6.1%)	33 (3.7%)	GTR+I+G
psbA-trnH	112	92	584	53 (9.1%)	28 (4.8%)	GTR+G
Combined	112	92	2807	451 (16.1%)	292 (10.4%)	GTR+I+G

S2). In the tree based on cpDNA data only, the delimitation of subgenera and the phylogenetic position of *A. tyuosenicola* were not well resolved (Fig. S2). In the combined phylogeny, both BI and ML trees showed that samples of *A. tyuosenicola* were grouped together (Fig. 3; PP = 1.00, BS = 99%) and were weakly resolved as sister to the group consisting of *A. somae* var. *somae*, *A.somae* var. *batakensis*, and *A. kawakamii* (Fig. 3: PP = 0.61, BS = 53%), all of which have pinnatisect to bi-pinnatisect leaves and are endemic to the Taiwanese montane area (Ling and Peng, 1998). As morphological data suggested, *A. tyuosenicola* appeared to be included in *Artemisia* subgenus *Artemisia*. Furthermore, *A. tyuosenicola* was not clustered with any other morphologically similar species: *A. tridentata*, which also has trident-like leaves but lack outer female florets in capitula, fell into subgenus *Tridentatae* with other North America taxa (Fig. 3; PP = 1.00, BS = 99%); Both *A. integrifolia* and *A. stolonifera* have nuclear data only (Table S1). In the nrDNA tree, although they belong to subgenus *Artemisia*, they were grouped together with *A. indica, A. montana* and *A. vulgaris* (Fig. S1, uppermost clade; PP = 1.00) and were not closely related to *A. tyuosenicola*.

Additionally, the *A. filifolia* collected from Taiwan (*Chung 14537*) was clustered in subgenus *Dracunculus* and was not grouped with the other *A. filifolia* sample from Genbank (subgenus *Tridentatae*) in our phylogenetic tree (Fig. 3), suggesting the need for further evaluation of the identity of this Taiwanese population.

In conclusion, both the morphological characters and molecular data support the new species as distinct.

TAXONOMIC TREATMENT

Artemisia tyuosenicola S.W. Chung, W.J. Huang & T.C. Hsu, ***sp. nov.*** 中央尖山艾

Type: Taiwan. Taichung City: Zhongyangjian Stream shelter to Mt. Zhongyangjian, 21 August 2019, *S. W. Chung 13942* (holotype: TAIF; isotype: TAI).

Description: Perennial herbs or subshrubs, 30–80 cm tall. **Stems** ascending to erect, sparsely puberulous or glabrescent. **Leaves** alternate, short petiolate or subsessile; leaf blades obovate to elliptic, 1.4–3.0(–3.8) × 0.5–1.0(–1.3) cm, adaxially green, sparsely tomentose, abaxially densely white arachnoid tomentose, apex acute, base cuneate, usually with 1 pair of acute teeth or small lobes above the middle; lower and middle stem leaves sometimes with 2(–3) pairs of teeth; upper stem leaves sometimes entire; midrib and veins impressed on adaxial surface; lateral veins 1–3 pairs, sparsely white arachnoid tomentose. **Synflorescence** narrowly paniculate. **Capitula** ovoid or ellipsoid, 3.0–4.0 × 2.0–3.0 mm; peduncle 3–13 mm long; phyllaries in 3–4 series, subcoriaceous with scarious margin, 2.0–2.5 mm long, outermost phyllaries glabrescent, inner phyllaries densely arachnoid tomentose; receptacle flat, glabrous. **Outer**

florets pistillate, 2–4, 2.5–3.5 mm long; corolla tubular, 2-toothed, basally glandular; style divergent, exerted. **Central florets** bisexual, 6–12, 2.5–3.5 mm long; corolla tubular, 5-toothed, basally glandular; apical anther appendages acute, triangular, basal anther appendages 2, obtuse; style as long as corolla, ca. 2 mm long, divergent. **Achenes** obovoid or oblong; pappus absent.

Distribution and ecology: *Artemisia tyuosenicola* is endemic to Taiwan and confined to Mt. Zhongyangjian located at the border of Taichung City and Hualien County. The plants scatter on exposed scree slopes along streams at 2850–3400 m elev. Flowering and fruiting were observed from August to October.

Etymology: The specific epithet "*tyuosenicola*" refers to the mountain where the new species was collected. Mt. Zhongyangjian (中央尖山), also known as Mt. Chungyangchien or Central Range Point, was spelled as "Tyuosenzan" at first in the old specimens.

Additional specimens examined: Taiwan. Taichung City: Nanhutashan to Chungyangchienshan, 3400 m, 25 August 1970, *K. S. Hsu 907* (TAIF); en route from Chungyangchienshan shelter to Chungyangchienshan, ca. 3000 m, 2 August 2006, *C. I. Huang et al. 2768* (HAST); Chungyangchien Stream shelter to Mt. Chungyangchien, 2850 m, 21 August 2019, *T. C. Hsu 11956, 11957* (TAIF); same loc., 21 August 2019, *S. W. Chung 13943* (TAIF).

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