Tongoloa arguta (Apiaceae),
a new species from southwest China

Ling-Jian Gui¹, Jun Wen¹, Yan-Ping Xiao¹,
Ting Ren¹, Hong-Yi Zheng¹, Xing-Jin He¹

¹ Key laboratory of Bio-Resources and Eco-Environment of Ministry of Education, College of Life Sciences,
Sichuan University, 610065, Chengdu, Sichuan, China

Corresponding author: Xing-Jin He (xjhe@scu.edu.cn)

Abstract
A new species Tongoloa arguta (Apiaceae) is described and illustrated in this article. The new species grows
in alpine bushes and meadows in south-western China. It resembles T. silaifolia, but differs from the latter
by the length of the stem, ultimate segments of leaf and rays of the umbel. Phylogenetic analysis, based on
nuclear ribosomal DNA internal transcribed spacer (ITS) sequences, is provided, as well as comparative
morphology between related species.

Keywords
Apiaceae, China, new species, phylogeny, Tongoloa

Introduction
Tongoloa H.Wolff (Apiaceae) is a genus comprising about 15–20 species distributed
mainly in southwest China, with a few species extending westwards to central Ne-
pal (Watson 1999; Pan and Watson 2005; Zhou et al. 2009). Tongoloa species are
characterised by having conic taproots, inflated and membranous leaf sheaths, cor-
date fruit base and filiform fruit ribs (Wolff 1925; Mukherjee and Constance 1991;
Pimenov and Kljuykov 2000; Pan and Watson 2005). Some species have been de-
scribed in Pimpinella L. due to the morphological similarity (Boissieu 1902, 1906).
The genus Tongoloa was formally established by Wolff (1925) and accepted as an inde-
ependent genus (Pimenov and Kljuykov 1995; Pimenov 2017). Molecular phylogenetic analyses, based on limited materials of nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast markers, indicated that *Tongoloa* is part of the East Asia clade of Apioideae (Apiaceae) (Zhou et al. 2009; Downie et al. 2010).

So far, 15 species of *Tongoloa* have been identified from different regions of China (Pimenov 2017), most of which being known from the Hengduan Mountains. While studying specimens in herbariums (CDBI, PE), we noticed several interesting specimens of *Tongoloa* collected from Sichuan and Yunnan, which have short stems and fewer rays of the umbel (3–8). Through field investigation and anatomical study, we confirmed that this species does not match any previously-published description of *Tongoloa* found from southwest China to central Himalaya. Further molecular analysis revealed significant differences between this species and its relatives. The results allow us to infer that these newly-collected specimens from Sichuan and Yunnan belong to a new species.

**Materials and methods**

We collected an unknown *Tongoloa* species from several populations in Yunnan and Sichuan Provinces. In addition to the samples collected in the field, the type specimens of *Tongoloa* and high-resolution type specimen photos were examined, including the specimens deposited in K, P, E, B, A, GB, LD, MW, NY, GH, W, US, PE, KUN, CDBI, WUK and HNWP. Considering the similarity between the new species and *T. silaifolia*, as well as other related species, we compared their morphological characteristics. The fresh fruits were preserved with formaldehyde-acetic acid-alcohol (FAA) for anatomical study. The mericarp transverse sections were examined using a stereomicroscope (Nikon SMZ25, Japan) after safranin O-fast green staining.

A plant genomic DNA kit (CWBIO, China) was used to extract total DNA from silica-dried leaves. Referring to the previous studies (White et al. 1990; Zhou et al. 2009), we used nuclear ribosomal DNA internal transcribed spacer (ITS) sequences for phylogenetic inference. Amplification was undertaken using a volume of 30 µl with 15 µl 2 × Taq MasterMix (CWBIO, China), 10 µl ddH$_2$O, 1.5 µl forward primer, 1.5 µl reverse primer and 2 µl total DNA. The PCR reaction was performed in Geneamp PCR System 9700 (USA) with initial denaturation at 95 °C for 2 min, 35 cycles of 94 °C for 60 s, 52.5 °C for 45 s and 72 °C for 60 s and a final extension of 72 °C for 7 min. PCR products were sent to BGI (China) for sequencing. The GenBank accession numbers and sample information of the ITS sequences used in this study are shown in Table 1.

To determine the systematic position of the new species, 37 ITS sequences with accession numbers were obtained from GenBank, including 9 species of *Tongoloa* (Table 1). Taxa of *Chamaesium* clade were selected as the outgroup (Downie et al. 2010). Maximum Likelihood (ML) analyses with GTR + G + I model and 1000 bootstrap (BS) replicates was performed using MEGA7 (Kumar et al. 2016). Bayesian Inference (BI) analysis was conducted with MrBayes version 3.2 (Ronquist et al. 2012) and the Markov Chain Monte Carlo (MCMC) search was performed for $1 \times 10^8$ generations.
Table 1. Taxa and voucher information of the used ITS sequences.

| Taxon | Locality             | Voucher information | GenBank number          |
|-------|----------------------|---------------------|-------------------------|
| Bupleurum chinense | China, Anhui, Dabieshan | CB Wang 09017 (SZ) | GU570615                |
| Bupleurum gibraltaricum | Spain, Sevilla          | S.S. Neves 35 (E) | AF479851.1              |
| Bupleurum tenuissimum | Portugal, Beira Litoral | S.S. Neves 22 (E) | AF481932.1              |
| Chaenelmis paradoxum | China, Sichuan, Daosheng-Litang | ZJ0560 (KUN) | EU236161.1              |
| Chaenelmis thalictroides | China, Sichuan, Zhangla-Caowan | ZJ0607 (KUN) | EU236162.1              |
| Chaenelmis wolfianum | China, Yunnan, Shudu Lake | ZJ0525 (KUN) | EU236163.1              |
| Changium myrioides | China, Jiangxi, Jiujiang, Pengze | PZ2 (NAS) | HJ185254.1              |
| Chaunminshen violaceum | China, Sichuan, Cangui, Xinlong nursery | J105 (KUN) | FJ385040.1              |
| Cyclorhiza peucedanifolia | China, Yunnan, YuLong, Daju, Xiahutiao | J034 (KUN) | FJ385042.1              |
| Cyclorhiza wulstonii | China, Sichuan, Derong | ZJ0536 (KUN) | EU236165.1              |
| Hansenia forbesii | China, Qinghai, Maqin | H43 (WNU) | MF78544.1               |
| Hansenia weberbaueriana | China, Yunnan, KIB nursery | ZJ0697 (KUN) | EU236180.1              |
| Haplophraea phaea | China, Yunnan, Shudu Lake | ZJ0521 (KUN) | EU236167.1              |
| Heptaptera anisoptera | Iran, Lorestan           | Pimenov et al. 438 (MW) | AY941273.1, AY941301.1 |
| Hymenadamium nanum | Kirghistan, Sarydzhbas basin | Kochevnikova s.s. (LE) | GQ379335.1              |
| Hymenolaena candollei | India, Jammu and Kashmir | Pimenov and Klyuykov 59 (MW) | FJ469958.1, FJ483497.1 |
| Hymenolaena badachshana | Tadiskistan, Badakhshan, Andarob | Sultanov 1121 (LE) | GQ379332.1              |
| Hymenolaena pimpineliformis | Kirghizia, Kyrgyz Ataoo | Pimenov 398 (MW) | FJ469959.1, FJ483498.1 |
| Komarovia anisosperma | Uzbekistan, Zeravschistan | 178 (MW) | AF077897.1              |
| Physoperennopsis delavayi | China, Yunnan, YuLong Snow Mt. | J033 (KUN) | FJ385056.1              |
| Pleuropermum anabahile | China, Yunnan, Deqin, Baimaxueshan | GLJ19100605 (SZ) | MT124614                |
| Pleuropermum francetianum | China, Sichuan | YY (WNU) | KY848849.1              |
| Pleuropermum unandense | China, Liaoning | LQX031 (NAS) | FJ778839.1              |
| Perrycyclus angelicus | China, Xizang | G19082501(SZ) | MN689078                |
| Perrycyclus rotundatus | China | G18092501-1 (SZ) | MK078059.1              |
| Sinolimprichtia alpina | China, Yunnan, Deqin, Baimaxueshan | GLJ19100702 (SZ) | MT124613                |
| S. alpina | China, Xizang | GLJ19100702 (SZ) | MT124613                |
| S. alpina Sc. | China, Sichuan, Yajiang, Jiaziwanshan | LH2018081402 (SZ) | MT124609                |
| Tongoloa arguta YN1 | China, Yunnan, Shangri-la, Daxueshan | A11 (SZ) | MT124619                |
| T. arguta YN2 | China, Yunnan, Deqin, Baimaxueshan | GLJ18082102 (SZ) | MT124599                |
| T. arguta SC1 | China, Sichuan, Yajiang, Kazilashan | GLJ18092002 (SZ) | MT124615                |
| T. arguta SC2 | China, Sichuan, Yajiang, Jiaziwanshan | GLJ19092802 (SZ) | MT124612                |
| Tongoloa dunnii | China, Hubei, Shennongjia | GLJ18091102 (SZ) | MT124601                |
| Tongoloa elata | China, Sichuan, Songpan, Huangshengguan | GLJ19080404 (SZ) | MT124607                |
| Tongoloa elata | China, Sichuan, Songpan, Huangshengguan | GLJ19080404 (SZ) | MT124607                |
| Tongoloa loloensis | China, Yunnan, Eryuan, Baicaoluou | GLJ18103002_1 (SZ) | MN630615                |
| Tongoloa loloensis | China, Fujian, Taining, Huangranfeng | GLJ18090802_2 (SZ) | MN630614                |
| Tongoloa silatifolia | China, Chongqing, Chengkou | JQP19081607_2 (SZ) | MT124617                |
| Tongoloa sp. | China, Qinghai, Yushu, Jiangxigou | GLJ19092201 (SZ) | MT124610                |
| Tongoloa taeniophylla | China, Sichuan, Kangding, Paomashan | GLJ18082902 (SZ) | MT124598                |
| Tongoloa tenuifolia | China, Yunnan, YuLong Snow Mt. | J075 (KUN) | FJ385066.1              |
| Trachydium roylei | Pakistan, Hazara | B. Dickore, 13244 | FJ469972.1, FJ483510.1  |
| Trachydium simplicifolium | China, Yunnan, LiJiang, Yulongxueshan | GLJ19111401 (SZ) | MT124618                |
| Trachydium souliei YN1 | China, Yunnan, Deqin, Baimaxueshan | GLJ18082103 (SZ) | MT124603                |
| T. souliei YN2 | China, Yunnan, NW part, Degen Co. | Pimenov et al. 472 (MW) | FJ469973.1, FJ483511.1  |

Note: Province's names were indicated near the names of the species if two or several different samples were used for the molecular analysis. YN = Yunnan, SC = Sichuan, XZ = Xizang (Tibet).
**Results and discussion**

**Phylogenetic analysis**

The total length of ITS sequence alignment with gaps was 467 bp (without 5.8S rDNA genes). *Tongoloa arguta* yielded high sequence divergence values with related species, such as *T. silaifolia* (3.2%–4.0%), *T. elata* (3.7%–4.6%) and *T. taeniophylla* (5.0%–5.5%). Analysis of the data using ML and BI methods obtained similar trees with high ML BS and BI posterior probability (PP). Phylogeny reconstruction showed that *T. arguta* positioned in the *Tongoloa* clade and different populations of this species formed a strongly-supported monophyletic group (ML BS ≥ 90% and BI PP ≥ 0.90) (Fig. 1).

These results supported *T. arguta* as an undescribed and distinct species of *Tongoloa*.

**Taxonomy treatment**

*Tongoloa arguta* L.J.Gui & X.J.He, sp. nov.

urn:lsid:ipni.org:names:77212299-1

Figs 2, 3, Table 2

**Type.** China. Sichuan: Kangding, Zheduoshan Pass, 4300 m alt., 30°4’N, 101°48’E, 26 Sep 2019, Lingjian Gui GLJ19092601 (holotype: SZ).

**Diagnosis.** *Tongoloa arguta* sp. nov. is morphologically similar to *T. silaifolia*. However, the new species can be distinguished from the latter by its short stems (10–50 cm), while *T. silaifolia* has longer ones (28–60 cm); The ultimate segments of the lower leaf of *T. arguta* are acute and short (1–4 mm), while those of *T. silaifolia* are linear and longer (5–18 mm). The umbels of *T. arguta* have 3–8 rays, which are significantly less than those of *T. silaifolia* (8–22).

**Description.** Plants 10–50 cm. Root usually long-conic. Stem thinly ribbed, glabrous, purplish to green, branched. Leaf sheaths inflated, membranous; blade triangular in outline, 3–5 × 2–3.5 cm, 2–3-ternate/pinnate; ultimate segments lanceolate, 1–4 × 1–2 mm, apex acute. Umbels terminal or lateral; bracts often absent or sometimes 1, leaf-like, ca. 2–4 × 1 cm, bracteoles absent; rays 3–8; umbellules 13–25-flowered. Calyx teeth minute; petals obovate, white to purple, apex obtuse; stylodium depressed, dark purple; styles short, reflexed. Fruit broadly ovoid, ca. 2 × 1.7 mm, base cordate; ribs 5, filiform; vittae 3 in each furrow, ca. 4 on commissure. Ventral surface of endosperm slightly concave to plane.

**Etymology.** The species epithet “arguta” was given to describe the acute tips of the ultimate segments of leaves.

**Phenology.** The species was observed flowering from August to September and fruiting from September to October.
Figure 1. Phylogenetic tree of *Tongoloa* and related groups inferred from ITS based on ML and BI methods. ML BS / BI PP values were shown above the branches. Asterisks (*) denoted strong support (ML BS ≥ 90% and BI PP ≥ 0.90).

**Distribution and habitat.** *Tongoloa arguta* is distributed from Sichuan (Kangding, Yajing) to Yunnan (Deqin, Shangri-la) in south-western China. It grows in alpine bushes and meadows from 4000 m up to 4500 m alt.

**Additional specimens examined.** China. Sichuan: Kangding, Xinduqiao, Zheduoshan, 4000 m alt., 3 Sep 1982, *Taichang Wei* 29664 (CDBI0095011);
Kangding, north slope of Zheduoshan, 4000 m alt., 22 Sep 1984, Yongjiang Li 454 (CDBI0172327); Yajiang County, Jianziwanshan, 4400 m alt., 28 Sep 2019, Lingjian Gui & Chang Peng GLJ19092802 (SZ); Yajiang County, Kazilashan, 4400 m alt., 20 Sep 2018, Lingjian Gui GLJ18092002 (SZ). Yunnan: Deqin County, Baimaxueshan pass, 4350 m alt., 21 Aug 2018, Lingjian Gui GLJ18092002 (SZ); Shangri-la, Daxueshan pass, 4340 m alt., Yanping Xiao A11 (SZ); Zhongdian, Deqen, Beima Shan, on the south side of road, 4675 m alt., 25 Sep 1994, ACE 1287 (PE00755697).

Figure 2. *Tongoloa arguta* sp. nov. **A** species habitat (Mt. Jianziwanshan, Sichuan, China) **B** plant in the bush surrounded by snow **C** plant **D–F** roots **G, H** basal leaf, ventral and dorsal view **I** membranous sheath of basal leaves **J** middle leaf **K** upper leaf with membranous petiole **L** umbel and bract **M–O** flowers; **P–R** fruits **S** mericarp transverse section.
Conservation status. *Tongoloa arguta* is common in some alpine bushes and meadows at an altitude of about 4300 m in Yunnan and Sichuan, where human activities and especially yak grazing pose a potential threat to its survival. We categorise *T. arguta* as Near Threatened (NT), according to IUCN (2019).
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Table 2. Morphological comparison between *Tongoloa arguta* and similar species.

| Characters          | *T. arguta* | *T. silatifoila* | *T. elata* | *T. gracilis* |
|---------------------|-------------|------------------|------------|---------------|
| Height              | 10–50 cm    | 28–60 cm         | 20–75 cm   | 25–75 cm      |
| Root                | long-conic  | conic            | conic      | slender       |
| Stem                | purplish    | purplish         | purplish   | purplish      |
| Lower leaves        | 2–3-ternate/pinnate, ultimate segments lanceolate, 1–4 mm, apex acute | 2–3-ternate/pinnate, ultimate segments linear, 5–18 mm, apex acute | 3–4-ternate/pinnate, ultimate segments linear, 5–15 mm | 3-ternate/pinnate, ultimate segments linear-lanceolate, 3–15 mm |
| Bracts              | often absent, sometimes 1, leaf-like | absent | absent | absent |
| Bracteoles          | absent | usually absent or 1–5, linear | absent | absent |
| Rays                | 3–8 | 8–22 | 6–16 | 5–11 |
| Petal               | apex obtuse | apex obtuse | apex obtuse-rounded | apex with incurved tips |
| Fruit               | broadly ovoid | broadly ovoid | broadly ovoid | oblong-ellipsoid |
| Ribs                | filiform | filiform | slender | filiform |
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