

Eutrema nanum (Brassicaceae), a new species from Chola Shan, Southwest China

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Abstract

Eutrema nanum, a new high-elevation (4500–4600 m) species from Chola Shan, Sichuan (Southwest China), is described and illustrated. It is similar morphologically to *E. nepalense* but is readily distinguished by having oblong to elliptic or obovate to spatulate (vs. suborbicular to broadly ovate) leaves, glabrous (vs. puberulent) sepals and ovate to oblong fruit 4–7 × 2–3 mm with flattened valves (vs. ovoid to subglobose fruit 2–3 × 1.8–2 mm with rounded valves). The genetic differences amongst *E. nanum*, *E. nepalense* and other close relatives are further confirmed by phylogenetic analyses using ITS and cpDNA sequence variations. The new combination *E. sinense* is proposed.

Keywords

Cruciferae, *Eutrema nanum*, molecular phylogeny, Sichuan, *Eutrema sinense*

Introduction

The boundaries of *Eutrema* R.Br. (Brassicaceae or Cruciferae) have recently been expanded to include 38 species, several of which were previously placed in the six genera *Taphrospermum* C.A.Mey., *Thellungiella* O.E.Schulz, *Neomartinella* Pilg., *Platycraspedum* O.E.Schulz, *Chalcanthus* Boiss. and *Pegaeophyton* Hayek & Hand.-Mazz. (Al-Shehbaz and Warwick 2005; Hao et al. 2017a). The taxonomic knowledge

of this genus is still incomplete because numerous collections from the high-elevation regions in Southwest China were often overlooked and many areas remain poorly explored. We reported two new species during recent field investigations and molecular analyses (Hao et al. 2016, 2017b). Here we report the third one, *Eutrema nanum*, found in Chola Shan at a high elevation of 4500–4600 m in Sichuan Province, Southwest China. This new species is morphologically similar to *E. nepalense* (Al-Shehbaz, Kats Arai & H. Ohba) Al-Shehbaz, G.Q.Hao & J. Quan Liu but, as shown below, it is readily distinguished by several aspects of leaves and fruit. The phylogenetic studies on both species and their other relatives were also conducted herein and the results support the recognition of this novelty. In addition, one of six species which were used to determine the systematic position of *E. nanum* was found to need a taxonomic combination and a new name *Eutrema sinense* (Hemsl.) G.Q.Hao, J. Quan Liu & Al-Shehbaz is therefore proposed herein.

Material and methods

We examined morphological traits of *Eutrema nanum* and several relative species. We followed Hu et al. (2015) and Hao et al. (2017a) in examining the genetic differences between this novelty (two accessions) and the morphologically similar *E. nepalense* (one accession). In order to determine the systematic position of *E. nanum*, we further included six species (*E. scapiflorum* (Hook.f. & Thomson) Al-Shehbaz, G.Q.Hao & J. Quan Liu, *E. sinense* (Hemsl.) G.Q.Hao, J. Quan Liu & Al-Shehbaz, *E. hookeri* Al-Shehbaz & Warwick, *E. fontanum* (Maxim.) Al-Shehbaz & Warwick, *E. verticillatum* (Jeffrey & W.W.Sm.) Al-Shehbaz & Warwick and *E. deltoideum* (Hook.f. & Thomson) O.E.Schulz) in our analyses. All six species were shown to be close relatives to *E. nepalense* in our previous study (Hao et al. 2017a) and two (*E. scapiflorum* and *E. sinense*) were previously placed in the genus *Pegaeophyton*. The related *E. integrifolium* Bunge (see Hao et al. 2017a) was selected as the outgroup. The collection information of the sampled species is listed in Table 1 and Figure 3 and the voucher specimens were deposited in the Sichuan University Herbarium (SZ).

We extracted the total DNA and amplified and sequenced four DNA markers, the nuclear internal transcribed spacer (ITS) and three chloroplast DNA (cpDNA) regions (*psbA-trnH*, *rbcL*, *matK*), following Hu et al. (2015) and Hao et al. (2017a). The sequences firstly reported here were placed in GenBank under the accession numbers (MH702367, MH793597, MH793598, MH793599). We aligned all sequences using Clustal X (Thompson et al. 1997) and refined them manually. We concatenated three cpDNA sequences into a single matrix for Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses. We coded indels using the simple code method by Gap-Coder (Young and Healy 2003). We constructed phylogenetic relationships based on two datasets (ITS and cpDNAs) using MP analyses by PAUP* 4.10b (Swofford 2003) and ML analyses using RAxML 7.2.6 (Stamatakis 2006). MP analyses employed a heuristic search with 10,000 replicates and TBR branch swapping and bootstrap values (Felsenstein 1985) were estimated with 1000 replicates and 100 random-addition-

Table 1. The sources of materials used for molecular analyses of Himalayan *Eutrema* (all vouchers at SZ).

| Taxon | Voucher | Source | Coordinate |
|-------------------------|-----------------|-------------------------------------|------------------|
| <i>E. nanum</i> | Liu & Hao 14091 | Chola Shan, Sichuan, China | 31°55'N, 98°54'E |
| <i>E. nanum</i> | Liu 17124 | Chola Shan, Sichuan, China | 31°55'N, 98°54'E |
| <i>E. nepalense</i> | Long et al. 605 | Sikkim, India | 27°36'N, 88°12'E |
| <i>E. sinense</i> | Liu 13114 | Biluo Snow Mountain, Yunnan, China | 27°59'N, 98°47'E |
| <i>E. scapiflorum</i> | Liu & Hao 13074 | Yarla Shampo Mountain, Tibet, China | 28°51'N, 91°59'E |
| <i>E. fontanum</i> | Liu & Hao 13144 | Zhuodala Mountain, Sichuan, China | 31°24'N, 99°56'E |
| <i>E. hookeri</i> | Liu 17108a | Mila Mountain, Tibet, China | 29°49'N, 92°90'E |
| <i>E. verticillatum</i> | Liu & Hao 14094 | Maila Mountain, Sichuan, China | 30°58'N, 98°58'E |
| <i>E. deltoideum</i> | Liu 13024 | Lasa, Tibet, China | 29°42'N, 91°09'E |
| <i>E. integrifolium</i> | Liu & Hao 13049 | Tianshan Mountain, Xinjiang, China | 43°12'N, 84°49'E |

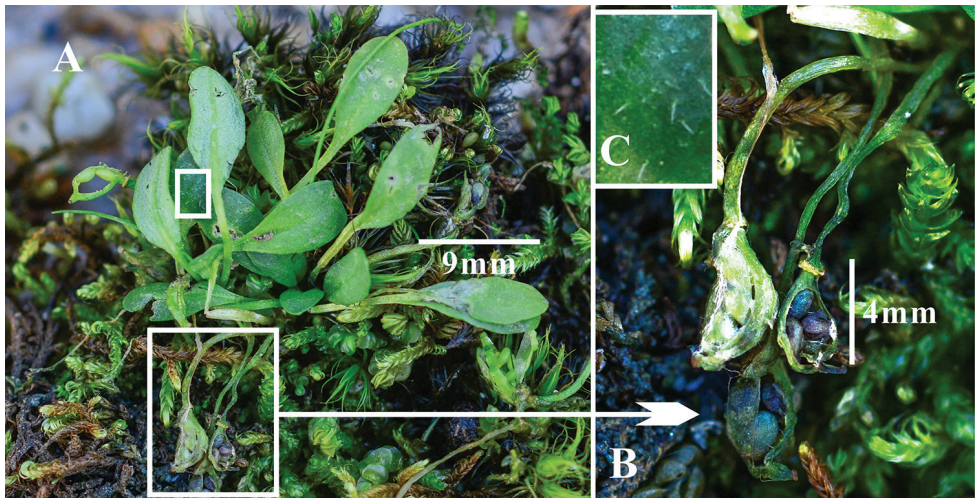


Figure 1. *Eutrema nanum*. G.Q. Hao, J.Quan. Liu & Al-Shehbaz. **A** Plant **B** Fruit **C** Leaf trichomes.

sequence replicates per bootstrap replicate. ML analyses were performed with raxml-HPC -f a -s sequence. phy -n boot2 -m GTRGAMMA -x 1234 -# 1000 -n outname. The most suitable GTRGAMMA models were used and bootstrap analyses were estimated with 1000 replicates.

Taxonomy

Eutrema nanum G.Q.Hao, J.Quan Liu & Al-Shehbaz, sp. nov.

urn:lsid:ipni.org:names:60477015-2

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Type. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., 16 August 2014, Liu & Hao 14091 (Holotype, SZ). Figures 1, 2.



Figure 2. Holotype of *Eutrema nanum*.

Description. Herbs perennial, 3–6 cm tall, glabrous or puberulent; caudex slender, ca. 3–5 mm long. Leaves basal, rosulate, 20–25 per caudex; petiole 13–20 mm long, slender at base, glabrous or with few trichomes; blade oblong, elliptic,



Figure 3. Geographical distribution of the sampled populations of *Eutrema nanum* and related species.

obovate, spatulate, 6–10 × 3–4 mm, fleshy, glabrous or abaxially pubescent with trichomes, 0.3–0.6 mm long, base subattenuate, to cuneate, margin entire, apex obtuse to subrounded. Pedicels slender, 18–23 mm long at anthesis, not elongated in fruit, not persistent. Flowers 5–8 per plant; sepals ovate to oblong, 1–1.5 mm long; petals white, broadly obovate to spatulate, blade 2–3 × 1–2 mm, persistent to fruit maturity, claw-like base 0.5–1 mm long. Ovules 2–4 per ovary. Fruit latisepate, dehiscent, ovate to oblong, somewhat curved, 4–7 × 2–3 mm; valves nearly flat, extending along part of fruit length; gynophore 0.1–0.3 mm long; replum 0.3–0.4 mm wide; style 0.6–1 mm long. Seeds broadly ovate, brown, plump, 2–4 per fruit, 1.4–2 × 0.6–1 mm.

Eutrema nanum is morphologically most similar to *E. nepalense*, from which it is readily distinguished by having oblong, elliptic, obovate to spatulate leaves, glabrous sepals and ovate to oblong larger fruit 4–7 × 2–3 mm with flattened, glabrous valves. In contrast, *E. nepalense* (<https://www.gbif.org>) has suborbicular to broadly ovate leaves, puberulent sepals and ovoid to subglobose smaller fruit 2–3 × 1.8–2 mm with rounded, puberulent valves. *Eutrema nanum* was only found with around 100 individuals along a stream in a valley about 2 kilometres from the Chola Shan peak, whereas *E. nepalense* occurs across Himalayas Mountains in Bhtan, China, Nepal and India.

Phenology. Flowering: June–August. Fruiting: August–September.

Distribution and habitat. *Eutrema nanum* is currently known only from Chola Shan, part of Hengduan Mountains in West Sichuan, China (Fig. 3). It grows under rocks by streams close to glaciers, damp or gravelly scree, wet sand at a very high elevation of 4500–4600 m.

Paratype. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., Liu 17124 (SZ).

Table 2. Diagnosing sites of the aligned ITS and three cpDNA sequences between *Eutrema nanum* and *E. nepalense*.

| Species | ITS | rbcL | | | matK | | | | | | | | | | | |
|--------------------------|-----|------|-----|-----|------|-----|-----------|-----|------|-----|-----|-------|------|-----|-----|-----|
| | | 508 | 82 | 337 | 165 | 276 | 333 | 342 | 391 | 449 | 483 | 495 | 497 | 549 | | |
| <i>Eutrema nanum</i> | C | C | T | C | T | T | T | T | T | T | C | T | T | | | |
| <i>Eutrema nepalense</i> | T | A | C | T | C | G | C | A | G | C | T | C | A | | | |
| | | matK | | | | | psbA-trnH | | | | | | | | | |
| | | 601 | 603 | 633 | 638 | 657 | 28 | 40 | 48 | 92 | 114 | 115 | 138- | 212 | 228 | 235 |
| <i>Eutrema nanum</i> | C | C | T | T | G | T | C | G | - | G | C | - | 2 nt | C | C | |
| <i>Eutrema nepalense</i> | T | T | C | A | A | A | G | A | 6 nt | T | A | 74 nt | - | T | A | |

nr=nucleotide.

***Eutrema sinense* (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz, comb. nov. Based on *Braya sinensis* Hemsl., J. Linn. Soc., Bot. 30: 303. 1892.**
 urn:lsid:ipni.org:names:77190124-1

Syn.: *Eutrema robustum* (O.E.Schulz) Al-Shehbaz, G.Q.Hao & J.Quan Liu, Bot. J. Linn. Soc. 184: 219. 2017. Basionym: *Pegaeophyton sinense* var. *robustum* O.E.Schulz, Notizbl. Bot. Gart. Gerlin-Dahlem 9: 477. 1926.

The earliest available epithet of this taxon at the species rank is “*sinensis*” and it should have been transferred to *Eutrema* by Hao et al. (2017a) instead of using the varietal epithet “*robustum*.”

Genetic differences between *Eutrema nanum*, *E. nepalense* and other relatives

Sequence data from *Eutrema nanum* and *E. nepalense* reveals that one nucleotide substitution in ITS, two in *rbcL*, 18 in *matK* and eight substitutions and three indels in *psbA-trnH* distinguish them very well (Table 2).

Based on sequence variations of ITS and cpDNAs (Table 3), phylogenetic analyses suggested that *Eutrema nanum* is mostly related to *E. nepalense*, *E. sinense* and *E. scapiflorum*. However, phylogenetic relationships of these four species are incongruent between ITS and plastid DNA tree (Fig. 4). In the ML analyses of ITS sequence data, *E. nanum* and *E. nepalense* formed a single cluster sister to *E. sinense* and together are sister to *E. scapiflorum* with high support values (>80%) (Fig. 4A). By contrast, in the ML analyses of cpDNAs sequences, the phylogenetic relationships were maintained between *E. nanum* and sister *E. sinense* and together as sister to *E. scapiflorum*, but *E. nepalense* fell outside that relationship and was separated from them by *E. hookeri* with medium support (>50%) (Fig. 4B). MP analyses produced almost the same tree topologies with similar bootstrap support values.

Table 3. Tree statistics for analyses of the datasets.

| Data set | ITS* | <i>psbA-trnH</i> | <i>rbcL</i> | <i>matK</i> | Combined cpDNA* |
|---|----------|------------------|-------------|-------------|-----------------|
| No. of sequences | 30 | 30 | 30 | 30 | 30 |
| Aligned length used in analyses | 698 | 455 | 506 | 779 | 1786 |
| No. of variable characters | 141 | 58 | 17 | 66 | 169 |
| No. of parsimony-informative characters | 56 | 15 | 7 | 22 | 47 |
| Tree length (steps) | 78 | 70 | 10 | 71 | 195 |
| Consistency (CI) | 0.833333 | 0.900000 | 1.000000 | 0.873239 | 0.892308 |
| Retention index (RI) | 0.803030 | 0.708333 | 1.000000 | 0.790698 | 0.764045 |
| Rescaled consistency index (RC) | 0.669192 | 0.637500 | 1.000000 | 0.690468 | 0.681763 |

*gaps were coded and included.

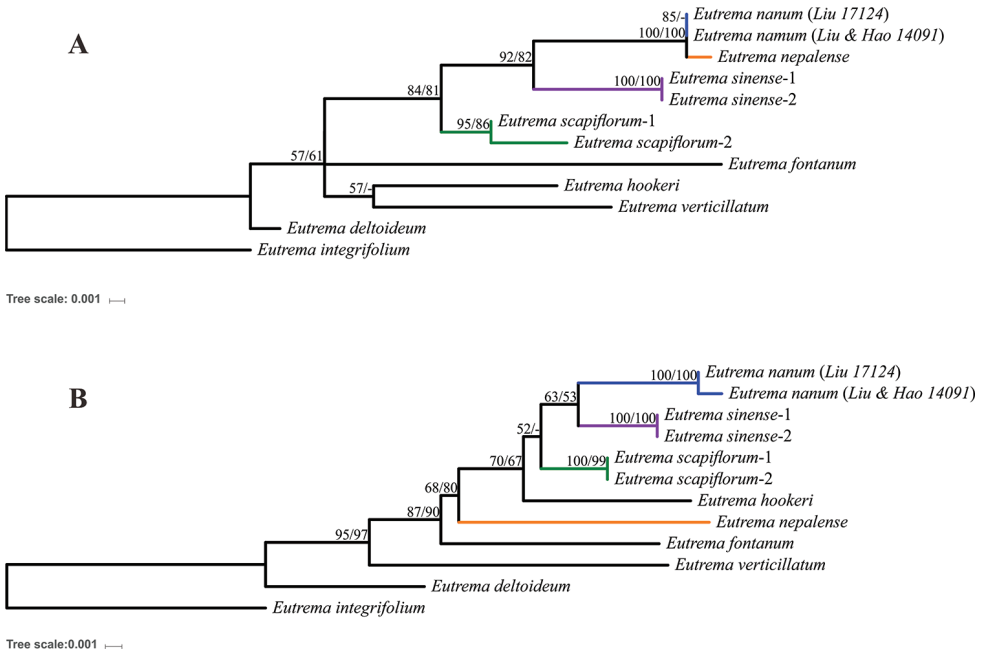


Figure 4. The Maximum Likelihood tree based on analysis of ITS (**A**) and Three cpDNA regions (**B**). Numbers above branches are maximum likelihood bootstrap support values and maximum parsimony bootstrap support values. ‘-’ represents <50%.

Discussion

Both *Eutrema nanum* and *E. nepalense* are small plants similar in flower traits and seed size. However, as discussed above, they are quite different morphologically. In addition, phylogenetic analyses of cpDNAs variations suggested these two species did not comprise a monophyletic clade. Furthermore, the Himalayan *E. nepalense* is disjunctly

separated by a distance of at least 1200 air kilometres from the Chola Shan (Sichuan, SW China), where *E. nanum* is endemic (Fig. 3). *Eutrema nanum* is also closely related to *E. sinense* in the phylogenetic analyses of the cpDNA sequence variations, but both are easily distinguished from each other. *Eutrema nanum* is a small and weak herb with entire leaves (0.5–1.5 cm long) and small flowers (petals 2–3 mm long), whereas *E. sinense* is obviously stout with entire or toothed leaves (1.5–8 cm long) and distinctly larger flowers (petals 8–15 mm long) and fruit (10–20 mm long).

Pylogenetic relationships amongst *E. nanum*, *E. nepalense* and *E. sinense* are incongruent between ITS and cpDNA trees. This incongruence may suggest possible hybridisations or incomplete lineage sorting during the rapid and recent species diversifications (Soltis and Soltis 2000, 2009). However, it is not possible at present to determine which of these two factors had caused the incongruent phylogenies observed here. More analyses and molecular data, especially based on more individuals and genomic evidence, are needed to solve these phylogenetic inconsistencies.

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References

- Al-Shehbaz IA, Warwick SI (2005) A synopsis of *Eutrema* (Brassicaceae). *Harvard Papers in Botany* 10(2): 129–135. [https://doi.org/10.3100/1043-4534\(2005\)10\[129:ASOEB\]2.0.CO;2](https://doi.org/10.3100/1043-4534(2005)10[129:ASOEB]2.0.CO;2)
- Felsenstein J (1985) Confidence limits on phylogenies, an approach using the bootstrap. *Evolution; International Journal of Organic Evolution* 39(4): 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Hao GQ, Al-Shehbaz IA, Liang QL, Liu JQ (2016) *Eutrema tianshanense* (Brassicaceae), a new species from Tian Shan Mountains of central Asia. *Phytotaxa* 286(1): 23–31. <https://doi.org/10.11646/phytotaxa.286.1.3>
- Hao GQ, Al-Shehbaz IA, Ahani H, Liang QL, Mao KS, Wang Q, Liu JQ (2017a) An integrative study of evolutionary diversification of *Eutrema* (Eutremeae, Brassicaceae). *Botanical Journal of the Linnean Society* 184(2): 204–223. <https://doi.org/10.1093/botlinnean/box024>
- Hao GQ, Zhang CB, Al-Shehbaz IA, Guo XY, Bi H, Wang JY, Liu JQ (2017b) *Eutrema giganteum* (Brassicaceae), a new species from Sichuan, southwest China. *PhytoKeys* 82: 15–26. <https://doi.org/10.3897/phytokeys.82.12329>
- Hu H, Al-Shehbaz IA, Sun YS, Hao GQ, Wang Q, Liu JQ (2015) Species delimitation in *Orychophragmus* (Brassicaceae) based on chloroplast and nuclear DNA barcodes. *Taxon* 64(4): 714–726. <https://doi.org/10.12705/644.4>

- Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences of the United States of America* 97(13): 7051–7057. <https://doi.org/10.1073/pnas.97.13.7051>
- Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60(1): 561–588. <https://doi.org/10.1146/annurev.arplant.043008.092039>
- Stamatakis A (2006) RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics (Oxford, England)* 22(21): 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Swofford DL (2003) PAUP*, phylogenetic analyses using parsimony (*and other methods), Version 4. Sunderland, MA, USA, Sinauer Associates.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface, flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25(24): 4876–4882. <https://doi.org/10.1093/nar/25.24.4876>
- Young ND, Healy J (2003) GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4(1): 6. <https://doi.org/10.1186/1471-2105-4-6>