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**Article**

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# *Senecio kumaonensis* (Asteraceae, Senecioneae) is a *Synotis* based on evidence from karyology and nuclear ITS/ETS sequence data

TIAN-JING TONG[1,3], MING TANG[2], CHEN REN[1] & QIN-ER YANG[1*]
[1]*Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, Guangdong, China*
[2]*Collaborative Innovation Center of Jiangxi Typical Trees Cultivation and Utilization, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China*
[3]*University of Chinese Academy of Sciences, Beijing 100049, China*
**Author for correspondence: e-mail: qeyang@scib.ac.cn*

## Abstract

We apply a multidisciplinary approach to settle the dispute over the generic affiliation of the Himalayan species *Senecio kumaonensis* (based on *Cacalia penninervis*). Some floral micromorphological characters of this species, including shape of anther bases, configuration of anther collars, and anther endothecial tissue cell wall thickenings, are confirmed to perfectly match those of *Senecio* and this has obviously resulted in the placement of the species within that genus. Chromosome counts indicate *S. kumaonensis* has $2n = 40$, a number common to *Senecio* and *Synotis* but not occurring in *Parasenecio* (the correct generic name for the Asian species previously referred to *Cacalia*), which has $2n = 52$, 58, and 60. The presence of six subterminal-centromeric (st) chromosomes in the karyotype of *S. kumaonensis*, however, lends strong support to the close relationship of this species to *Synotis*. Our phylogenetic analyses based on ITS/ ETS sequences also place *S. kumaonensis* in *Synotis*. In this species the anther tail feature, once deemed to be diagnostic for *Synotis* (anther bases in *Synotis* with sterile, tailed auricles vs. those in *Senecio* without such auricles, obtuse to sagittate), has an exception as evidenced from phylogeny. We therefore transfer *S. kumaonensis* to *Synotis* and, as the epithet "*penninervis*" has hitherto never been used in the genus, we propose the new combination *Syn. penninervis* for this species.

**Key words:** Compositae, cytotaxonomy, Himalayan region, molecular systematics, new combination, taxonomy, Xizang

## Introduction

*Senecio kumaonensis* Duthie ex Jeffrey & Chen (1984: 357) (Asteraceae, Senecioneae; Fig. 1) is widely distributed in the Himalayan region, including Bhutan, southern Xizang (Tibet) of China, northern India, and Nepal. This species is a perennial erect herb up to 90 cm tall. Its stems are solitary or few-fasciculate, rather woody at base, leafy. Leaves are ovate or ovate-oblong, pinnately veined, densely white arachnoid abaxially. Capitula are discoid, pendulous, numerous in narrow terminal and upper axillary panicles. Achenes are cylindrical, glabrous.

The generic placement of *Senecio kumaonensis* has been uncertain. This species was initially described as *Cacalia penninervis* Koyama (1969: 180) on the basis of three gatherings, *T. Namba 1015015* (KYO) and *T. Namba 1022001* (KYO) from central Nepal, and *S. Nakao 569* (KYO) from Bhutan, with the first designated as the holotype. The author also proposed a new series, *C.* ser. *Pinnatae* Koyama (1969: 180), to accommodate this species, stating that the series was characterized by the shape and venation of leaves and by the style branches which were more or less similar to those of *Senecio* Linnaeus (1753: 866), although the other characters were mostly of *Cacalia* Linnaeus (1753: 834). Robinson & Brettell (1973) proposed the new name *Koyamacalia* Robinson & Brettell (1973: 273) to replace *Cacalia* (nom. ambig. rejic.) and thus made the new combination *K. penninervis* (Koyama 1969: 180) Robinson & Brettell (1973: 273). Koyama (1977) might have ignored or neglected this treatment and still used the name *C. penninervis* when he elaborated on the differences between this species and *S. candolleanus* Wallich ex Candolle (1838: 364). Jeffrey & Chen (1984) transferred *C. penninervis* to *Senecio*, but they pointed out that this species, although technically placed in *Senecio* by its floral characters, was very distinct in habit and achene anatomy. Hence they stressed that its relationship to the genus *Synotis* (Clarke 1876: 177) Jeffrey & Chen (1984: 285) remained to be clarified. Because of the pre-existing *S. penninervius* Candolle (1838: 395) in *Senecio*, they proposed the replacement name *S. kumaonensis*.

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Meanwhile, they raised *C*. ser. *Pinnatae* to a section in *Senecio*, i.e. *S*. sect. *Pinnati* (Koyama 1969: 180) Jeffrey & Chen (1984: 285), and also placed *S. candolleanus* to this section. The treatment on *C. penninervis* by Jeffrey & Chen (1984) has been accepted by Mathur (1995), Chen (1999), Grierson & Springate (2000), and Chen *et al.* (2011).

During our botanical trips to southern Xizang in recent years, we successfully discovered several populations of *Senecio kumaonensis*. We made careful observations on living plants and collected material for floral micromorphological, karyological and molecular systematic studies. The results, particularly those obtained from karyological observations and molecular phylogenetic analyses based on nuclear ITS/ETS sequences, clearly demonstrate that this species is a member of *Synotis*. This makes it necessary to transfer the species from *Senecio* to *Synotis*.

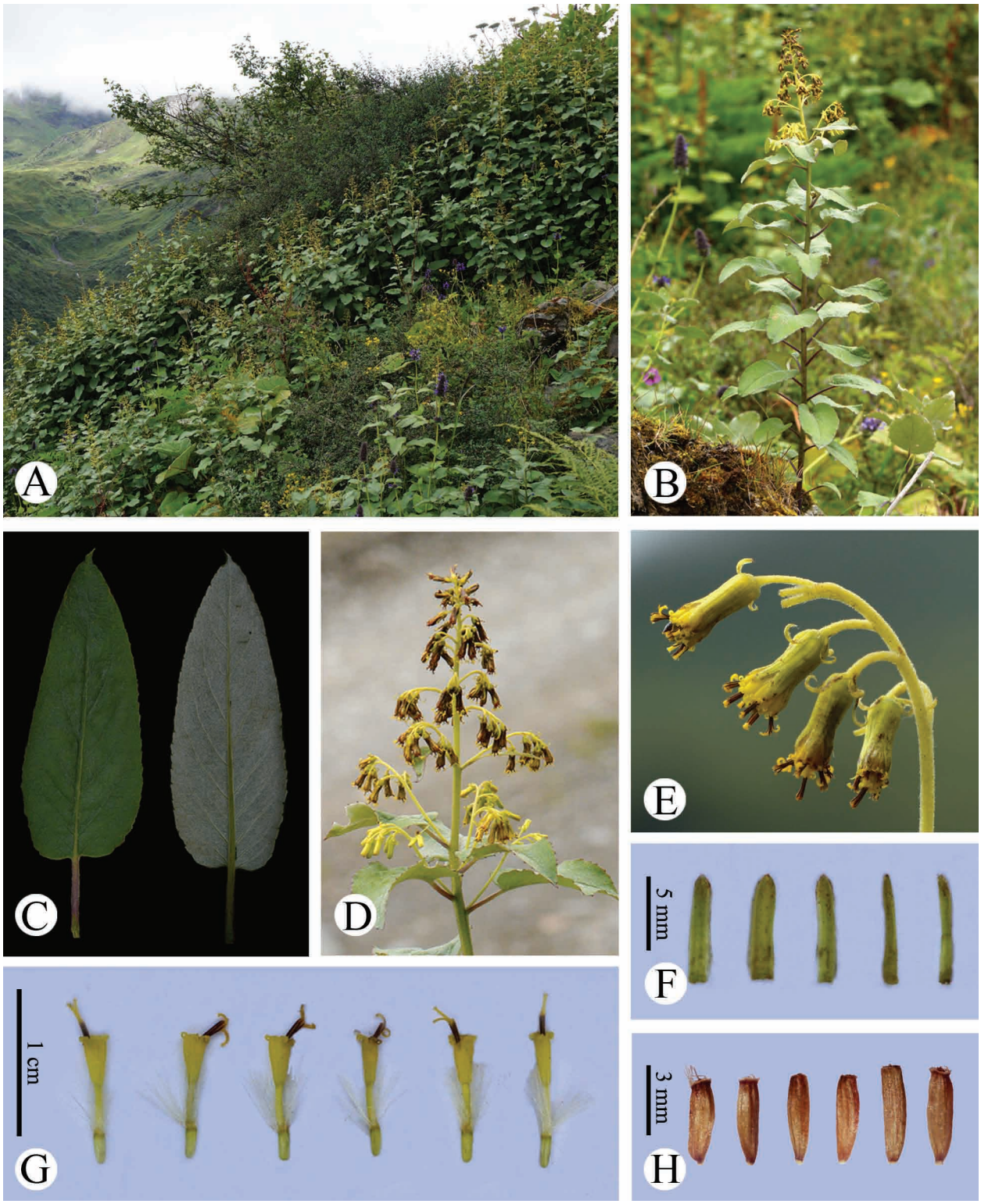


**FIGURE 1.** *Synotis penninervis* (= *Senecio kumaonensis*) in the wild. A. Habitat. B. Habit. C. Leaf blade (left: adaxial surface; right: abaxial surface). D. Synflorescence. E. Capitula. F. Phyllaries. G. Florets. H. Achenes.

## Material and methods

*Floral micromorphology*

Two populations of *Senecio kumaonensis* from southern Xizang, China (vouchers: *L. Wang & T.J. Tong 1352*, *1373*, IBSC) were examined. For observation of the anther collar and anther endothecial tissue cell wall thickenings, heads were boiled in distilled water for 3 min, and then fixed in Carnoy's solution (glacial acetic acid: absolute ethanol = 1 : 3). Mature disc florets removed from the fixed heads were dehydrated in 70% ethanol for 30 min, then placed in 99% ethanol for 1 h before they were treated with 5% NaOH overnight. The anther tissue was isolated from the florets on a slide, flooded with 50% glycerol and a cover slip was applied. Samples were then examined at 100× (anther collar) and 400× (endothecial cell wall thickenings) magnifications by light microscopy and photographed.

*Karyology*

Two populations of *Senecio kumaonensis* from southern Xizang, China (vouchers: *L. Wang & T.J. Tong 1352*, *1373*, IBSC) were examined. For chromosome observations, root tips were pretreated with 0.1% colchicine for 2.5 h before being fixed in Carnoy's solution (glacial acetic acid: absolute ethanol = 1 : 3), then macerated in a 1 : 1 mixture of 45% acetic acid and 1 M HCl at 37°C for 45 min, stained and squashed in Carbol fuchsin.

The karyotype formulae were based on measurements of mitotic-metaphase chromosomes taken from photographs. Measurements of three cells were made for karyotype analysis. We followed the acronyms proposed by Levan *et al*. (1964) to describe the karyotypes.

*Molecular phylogenetics*

*Taxon sampling*.—Based on the results of past phylogenetic analyses of the tribe Senecioneae (Pelser *et al.* 2007, 2010, Tang *et al*. 2014), we selected 79 species in 32 genera as ingroup taxa, with the majority from subtribe Tussilagininae and subtribe Senecioninae as defined by Pelser *et al.* (2007) and Nordenstam *et al*. (2009). *Abrotanella emarginata* (Gaudichaud 1825: 104) Cassini (1825: 27) (subtribe Abrotanellinae) was selected as an outgroup according to Pelser *et al*. (2007, 2010) and Tang *et al*. (2014).

*DNA extraction, amplification, and sequencing*.—Total genomic DNA of *Senecio kumaonensis* was extracted from silica gel-dried leaves by the modified CTAB method (Doyle & Doyle 1987). The primer pairs ITS4 (TCCTCCGCTTATTGATATGC)/ITS5(GCAAGTAAAAGTCGTAACAAGG) (White *et al*. 1990) and AST-1 (CGTAAAGGTGGCATGAGTGGTGT; Markos & Baldwin 2001)/18S-ETS (ACTTACACATGCATGGCTTATCT; Baldwin & Markos 1998) were used to amplify the nuclear ITS and ETS regions, respectively. Reactions were carried out in a volume of 25 µl containing 0.25U PrimeSTAR®HS polymerase, 25 µl of each primer (10 µmol $l^{-1}$), 5 µl of 5 × PrimeSTAR® buffer ($Mg^{2+}$ plus), 2 µl of 2.5 mM dNTP mixture. Amplification for all primer combinations was performed under the following conditions: initial denaturation for 3 min at 95°C, followed by 37 denaturation cycles of 40s at 94°C, annealing for 30s at 53°C, extension for 1 min at 72°C, with a final extension step of 10 min at 72°C. PCR products were checked for length and concentration on 1% agarose gels and sent to Shanghai Invitrogen Biotechnology for commercial sequencing. The DNA sequences obtained were checked and edited by using Sequencher ver. 4.1.4. Two new sequences of *S. kumaonensis* have been deposited at GenBank (accession no. ITS, KY347902; ETS, KY347901). Other sequences used in this study were downloaded from GenBank. Note that when both ITS and ETS sequences were available, they were from the same voucher specimen in all the taxa except for *Pippenalia delphiniifolia* (Rydberg 1924: 419) McVaugh (1972: 470), *S. cadiscus* Nordenstam & Pelser (2009: 29) and *Sinacalia tangutica* (Maximowicz 1881: 486) Nordenstam (1978: 15), which had ITS and ETS sequences from two different accessions. Two vouchers for these taxa are listed (see Appendix 1 for the details).

*Alignment and phylogenetic analyses*.—Two datasets, including 79 ITS and 55 ETS sequences, respectively, were separately aligned with ClustalX ver. 2.1 (Thompson *et al*. 1997, Larkin *et al*. 2007) by using the default settings of the program, and then manually adjusted by using BioEdit ver. 7.0.5.3 (Hall 1999) when necessary. Unavailable ETS sequences were treated as missing data. The complete concatenated alignment of these two regions has been deposited in TreeBASE (<www.treebase.org>; accession no. S20325; ITS region: 1–675, ETS region: 676–1202). Gaps introduced by alignment were also treated as missing; indels were not coded for phylogenetic analyses.

To evaluate the congruence of the ITS and ETS datasets, we employed the incongruence length difference (ILD) test (Farris *et al.* 1995). The analysis was performed by using the PAUP* ver. 4.0b10 (Swofford 2003) with the following settings: 1000 replications, each using a heuristic search with 100 random-addition-sequence replicates and TBR (tree-bisection-reconnection) branch swapping.

Maximum parsimony (MP) and Bayesian inference (BI) methods were used to carry out phylogeny reconstruction on the combined dataset. MP analyses were implemented in PAUP* ver. 4.0b10 (Swofford 2003) by using a heuristic search with 10000 random-addition-sequence replicates and TBR branch swapping. Bootstrap (BS) values (Felsenstein 1985) were calculated with 1000 replicates with 100 random additions per replicate. Before BI analyses, Modeltest ver. 3.7 (Posada & Crandall 1998) was employed to choose optimal nucleotide substitution models for each dataset by using the Akaike information criterion (AIC). BI analyses were implemented in MrBayes ver. 3.2.1 (Ronquist *et al*. 2012) with four chains run for 3 000 000 generations until the average deviation of split frequencies reached a value below 0.01, and trees sampled every 1000 generations. Majority rule (>50%) consensus trees were constructed after discarding the first 25% of sampled trees (burn-in).

## Results and discussion

*Floral micromorphology*

The anthers of *Senecio kumaonensis* are shortly and rather obtusely sagittate at the base (Fig. 2A, C). The anther collars are balusterform, rather distinctly dilated towards the base, with somewhat enlarged basal lateral cells (Fig. 2A, C). The endothecial tissue cell wall thickenings are radial (Fig. 2B, D). These results conform to the previous observations by Jeffrey & Chen (1984), and indicate that *Cacalia penninervis* (= *Senecio kumaonensis*) should not be placed in *Parasenecio* within Tussilagininae but should be transferred to *Senecio* or *Synotis* within Senecioninae (*Parasenecio* is the correct generic name for the eastern Asian species previously referred to *Cacalia*, and *Koyamacalia* is a synonym of *Parasenecio* Smith & Small (1922: 93); see Jeffrey & Chen (1984) for details). In Tussilagininae, the anther collars are usually cylindrical, without enlarged marginal basal cells, and the endothecial tissue cell wall thickenings are polarized (Jeffrey & Chen 1984, Nordenstam *et al.* 2009, Tang *et al*. 2014). According to Jeffrey & Chen (1984), *Senecio* is distinguished from *Synotis* by the anther bases without sterile tailed auricles, obtuse to sagittate (vs. with sterile tailed auricles). It is evident that the features of the anther bases led Jeffrey & Chen (1984) to transfer *C. penninervis* to *Senecio* instead of *Synotis*. Our examination of 38 *Synotis* species indicates that their anthers are all tailed, although the tails show remarkable variation in length between species or even between populations within a species, ranging from 1/4 to 2 times the length of the anther collars (Tang 2014). As an exception, *S. kumaonensis* seems to be closer to *Senecio* than *Synotis* in respect of the features of anther bases.

*Karyology*

Chromosome counts of *Senecio kumaonensis* indicate that it has $2n = 40$ (Fig. 3A, C). Twenty chromosomes are median-centromeric (m), 14 are submedian-centromeric (sm), and six are subterminal-centromeric (st) (Fig. 3B, D). In the Yadong population, two chromosomes have a small satellite on the short arms (Fig. 3A, B). The karyotype of this species is thus formulated as $2n = 20m + 14sm (2sat) + 6st$ or $2n = 20m + 14sm + 6st$. As shown in Table 1, the chromosome number of $2n = 40$ and the presence of the six st chromosomes in the karyotype indicate that *Senecio kumaonensis* should be a *Synotis*, neither a *Parasenecio* nor a *Senecio*. The chromosome numbers in *Parasenecio* are usually $2n = 60$, rarely 52 and 58 (Ren *et al*. 2015). Although *Senecio* and *Synotis* both have $2n = 40$, they differ in karyotype constitution (Tang *et al*. 2014). The chromosomes of *Senecio* are all either m or sm, whereas there are usually six to eight, at least four, st chromosomes in the karyotypes of *Synotis*.

*Molecular phylogenetics*

The ITS region has a total length of 675 bp, of which 476 bp are variable and 308 bp are parsimony informative. The ETS region has a total length of 527 bp, of which 281 bp are variable and 182 bp are parsimony informative. The ILD test for ITS vs. ETS regions resulted in a *p*-value of 0.748, indicating they are largely congruent and can be combined in phylogenetic analyses. The optimal nucleotide substitution models for both ITS and ETS regions suggested by Modeltest are GTR + I + G.

The topologies inferred using MP (results not shown) and BI (Fig. 4) methods are similar to each other and essentially congruent with the phylogenetic framework of Senecioneae as revealed using ITS (Pelser *et al*. 2007) or combined ITS/ETS data (Pelser *et al*. 2010, Tang *et al*. 2014). The cladogram (Fig. 4) supports findings by Pelser *et al*. (2007, 2010) and Tang *et al*. (2014) that subtribe Tussilagininae s.l. is split into three clades, one of which is moderately-supported and corresponds to Tussilagininae s.s. (PP = 0.90, BS = 61%) suggested by Pelser *et al*. (2007). Subtribe Senecioninae is recovered to be monophyletic with a posterior probability (PP) and BS of 1.00 and 90%,

respectively, and is sister to subtribe Othonninae (PP = 1.00, BS = 94%). Significantly, *Senecio kumaonensis* is part of a well-supported clade (PP = 1.00; BS = 83%) with *Synotis*, which is deeply nested within subtribe Senecioninae, far from *Parasenecio* species within Tussilagininae s.s. All the other *Senecio* species are nested in a strongly supported clade of their own (PP = 1.00, BS = 100%). Our molecular phylogenetic analyses based on combined ITS/ETS data, therefore, lend strong support to the membership of *S. kumaonensis* within *Synotis*.

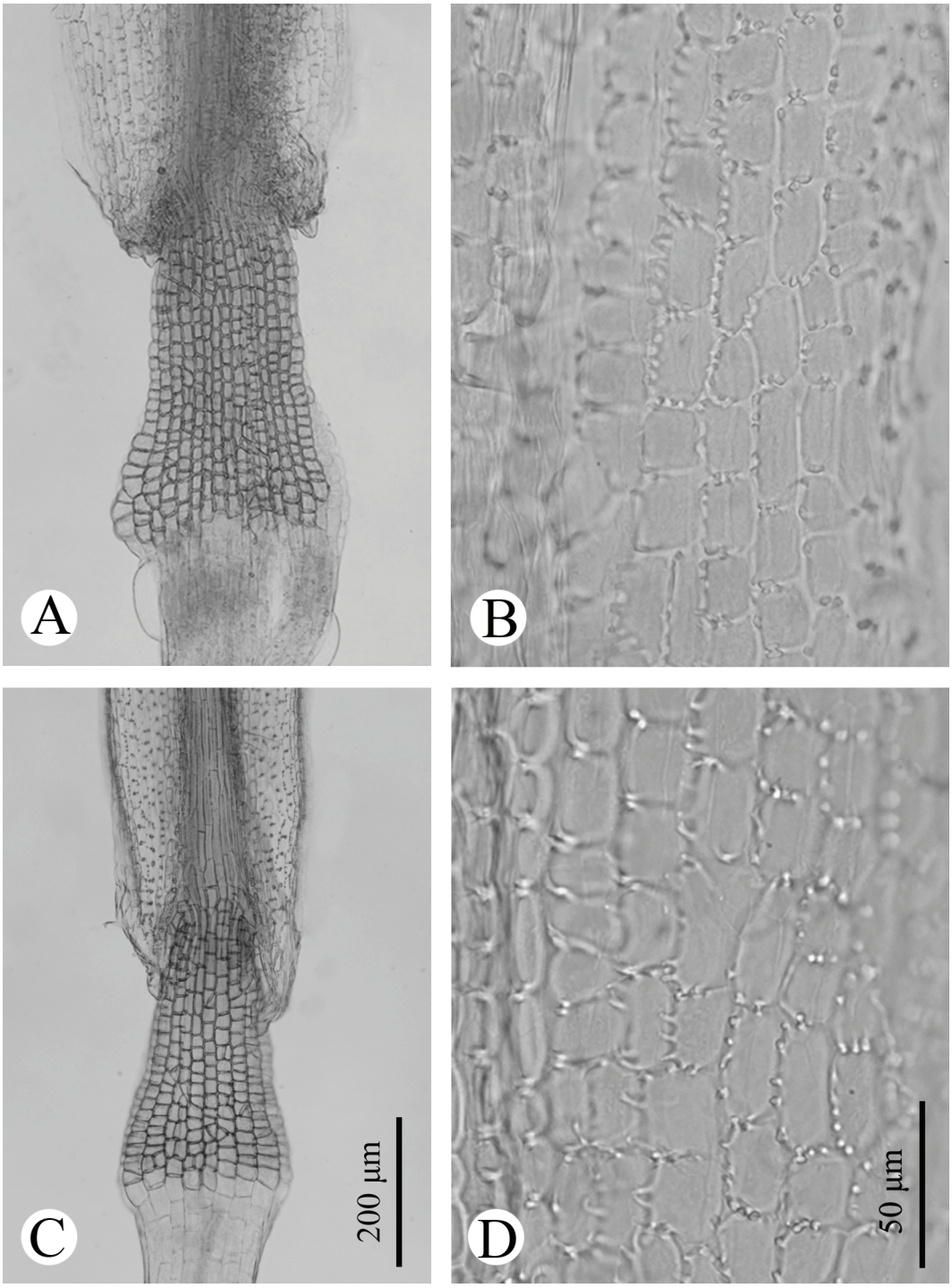


**FIGURE 2.** Floral micromorphology (A, C: anther collars, both same scale; B, D: anther tissue endothecial cell wall thickenings, both same scale) in two populations of *Synotis penninervis* (= *Senecio kumaonensis*) from Xizang, China. A, B. Yadong population (*L. Wang & T.J. Tong 1352*). C, D. Gyirong population (*L. Wang & T.J. Tong 1373*).

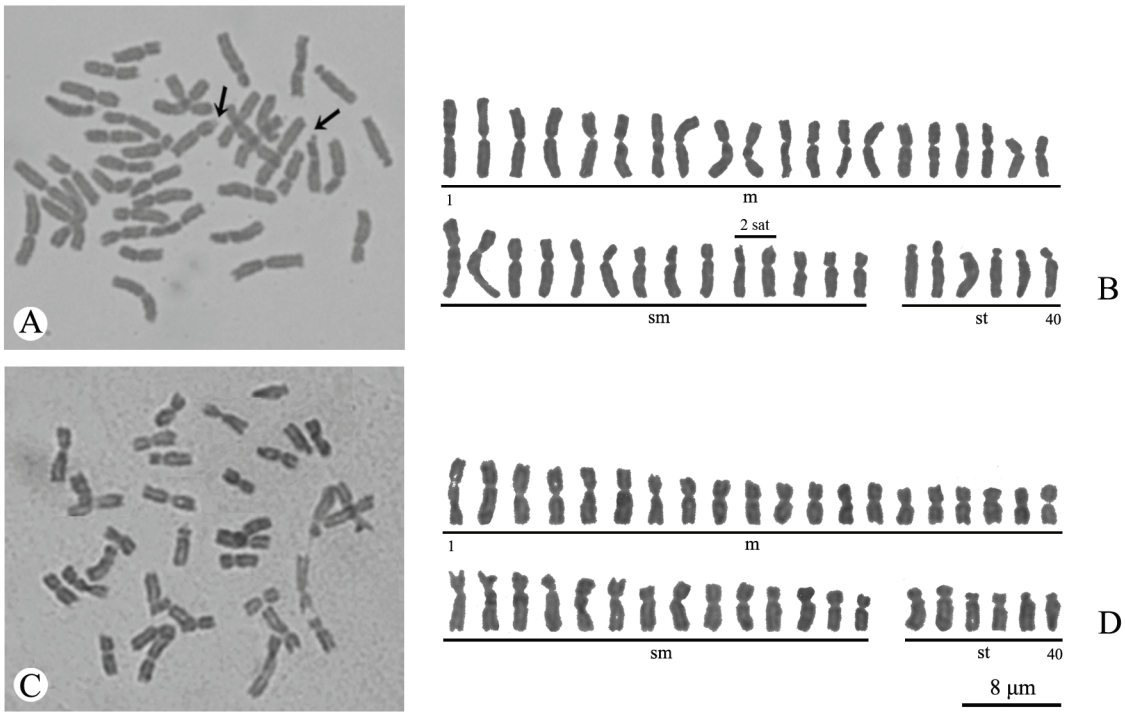


**FIGURE 3.** Mitotic metaphase chromosomes (A, C) and karyotypes (B, D) in two populations of *Synotis penninervis* (= *Senecio kumaonensis*) from Xizang, China, **a**ll same scale. A. Yadong population (*L. Wang & T.J. Tong 1352*), 2*n* = 40 (arrows indicate satellited chromosomes). B. Yadong population (*L. Wang & T.J. Tong 1352*), 2*n* = 20m + 14sm (2sat) + 6st. C. Gyirong population (*L. Wang & T.J. Tong 1373*), 2*n* = 40. D. Gyirong population (*L. Wang & T.J. Tong 1373*), 2*n* = 20m + 14sm + 6st.

**TABLE 1.** Karyotypic comparison of some species in *Parasenecio*, *Senecio* and *Synotis* from China.

| Species | Karyotype formula | Reference |
|---|---|---|
| *Parasenecio deltophyllus* (Maxim.) Y. L. Chen | 2*n* = 60 = 44m + 16sm (2sat) | Liu (2000) |
| | 2*n* = 60 = 44m + 16sm | Liu (2000) |
| *Parasenecio forrestii* W.W. Smith & J. Small | 2*n* = 60 = 40m + 13sm + 7st | Tang *et al.* (2014) |
| *Parasenecio latipes* (Franch.) Y.L. Chen | 2*n* = 60 = 50m + 10sm | Liu (2000) |
| *Parasenecio roborowski* (Maxim.) Y.L. Chen | 2*n* = 60 = 50m + 10sm (2sat) | Liu (2000) |
| *Senecio argunensis* Turcz. | 2*n* = 40 = 40m | Liu (1999) |
| *Senecio faberi* Hemsl. ex Forb. & Hemsl. | 2*n* = 40 = 38m (2sat) + 2sm | Liu (1999) |
| *Senecio diversipinnus* Y. Ling | 2*n* = 40 = 36m (2sat) + 4sm | Liu (1999) |
| *Senecio thianschanicus* Regel & Schmalh. | 2*n* = 40 = 40m | Liu (1999) |
| *Senecio tricuspis* Franch. | 2*n* = 40 = 36m + 4sm | Ren & Yuan (2010) |
| *Synotis baoshanensis* M. Tang & Q.E. Yang | 2*n* = 40 = 20m + 14sm + 6st | Tang *et al.* (2013a) |
| *Synotis changiana* Y.L. Chen | 2*n* = 40 = 20m + 16sm + 4st | Tang *et al.* (2013b) |
| *Synotis chenopodiifolia* (DC.) M. Tang *et al*. | 2*n* = 40 = 24m + 10sm + 6st | Tang *et al.* (2014) |
| *Synotis penninervis* (Koyama) T.J. Tong *et. al.* (= *Senecio kumaonensis* Duthie ex C. Jeffrey & Y.L. Chen) | 2*n* = 40 = 20m + 14sm (2sat) + 6st<br>2*n* = 40 = 20m + 14sm + 6st | This study |
| *Synotis xinningensis* M. Tang, L.Y. Wang & Q.E. Yang | 2*n* = 40 + 0–1B = 22m + 14sm + 4st | Tang *et al.* (2013b) |

## Conclusion and taxonomic treatment

Our observations of the floral micromorphological characters of *Senenio kumaonensis* suggest its membership within subtribe Senecioninae, not within Tussilagininae, and that indeed this species seems to be closer to *Senecio* than *Synotis* in respect of the features of anther bases as regarded by Jeffrey & Chen (1984). Evidence from karyology and molecular systematics, however, clearly demonstrates that *S. kumaonensis* is a *Synotis*, neither a *Parasenecio* nor a *Senecio*. The following transfer is necessary.

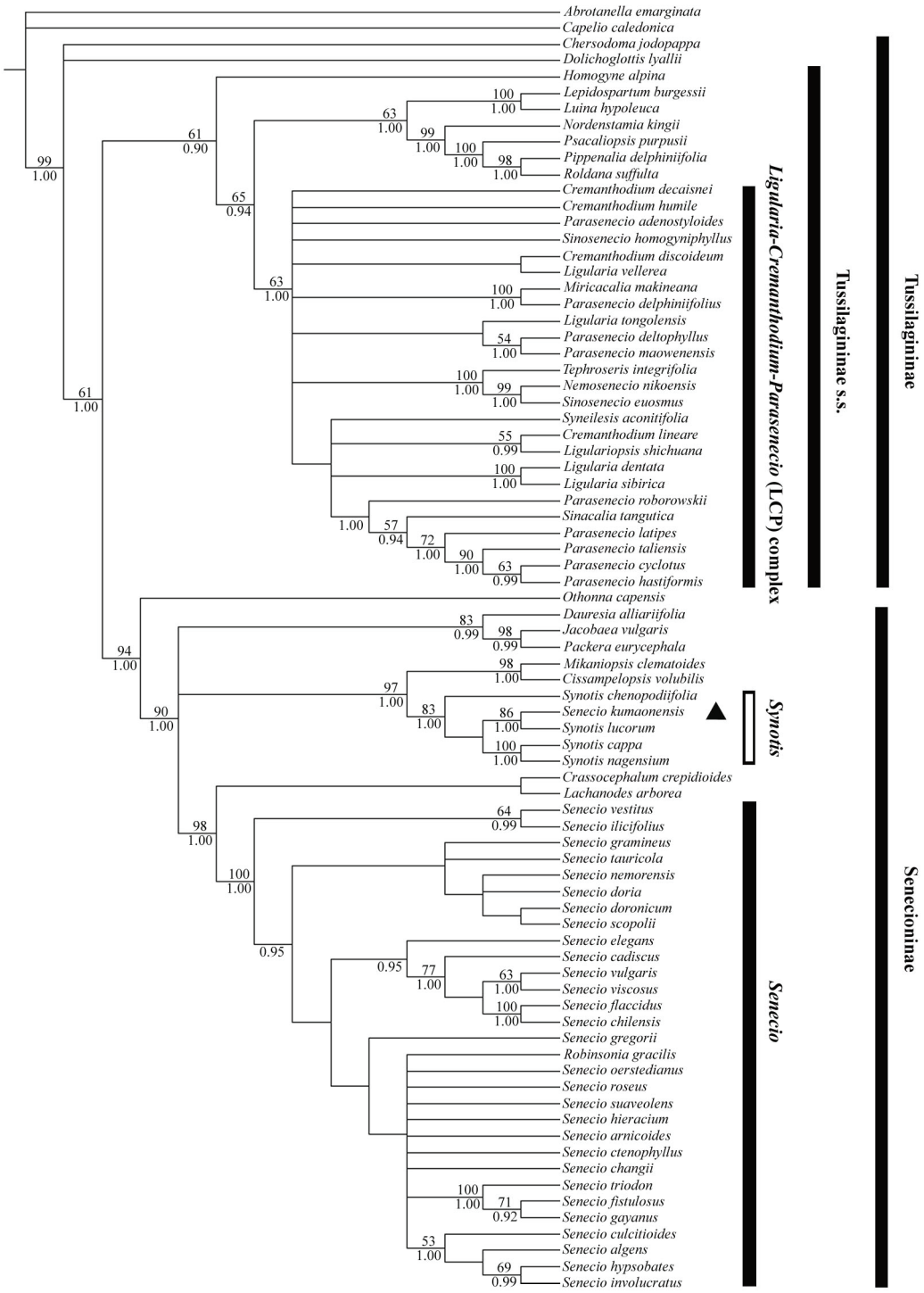


**FIGURE 4.** Cladogram inferred from combined ITS/ETS dataset using Bayesian inference (BI) method. Probabilities (> 0.70) are placed under branches, bootstrap support (> 50%) above branches. Open rectangles indicate the phylogenetic position of *Synotis*. The solid triangle indicates the phylogenetic position of *Synotis penninervis* (= *Senecio kumaonensis*).

***Synotis penninervis*** (Koyama 1969: 180) T.J. Tong, M. Tang, C. Ren & Q.E. Yang, **comb. nov.** *Cacalia penninervis* Koyama (1969: 180). *Koyamacalia penninervis* (Koyama 1969: 180) Robinson & Brettell (1973: 273). *Senecio kumaonensis* Duthie ex Jeffrey & Chen (1984: 357). (Figs. 1, 5)

Type:—NEPAL. Central Nepal, 4500 m, *T. Namba 1015015* (holotype KYO, not seen).

For a full description of this species see Koyama (1977), Jeffrey & Chen (1984), Chen (1999), and Chen *et al*. (2011).

**Phenology:**—Flowering in July–September, fruiting in October.

**Distribution and habitat:**—*Synotis penninervis* is distributed in Bhutan, China (southern Xizang), northern India, and Nepal (Fig. 6). It grows on forest margins, streamsides, mountain slopes or in shady places in ravines at elevations of 3500–4500 m above sea level.

**Additional specimens examined:**—BHUTAN. Upper Mo Chu District, *I.W.J. Sinclair & D.G. Long 5416* (E). CHINA. Xizang: Cona, *Y.S. Chen et al. 13-0952* (PE), *G.X. Fu 612* (PE), *L. Wang & T.J. Tong 1290* (IBSC); Gyirong, *L. Wang & T.J. Tong 1373* (IBSC), *Xizang Med. Pl. Exped. 556* (PE); Lhünzê, *L. Wang & T.J. Tong 1268* (IBSC); Yadong, *M. Tang and C. Ren 626* (IBSC), *L. Wang & T.J. Tong 1352* (IBSC). INDIA. Sikkim: Chaethangtaka, *Ribu & R. Lepcha 5738* (E); Tarkarpo, *R. Lepcha 132* (E); Sherabthang, *C. H. Cave s.n.* (E). NEPAL. Dojam Khola, *O. Polunin et al. 3586* (E); Khading, *L. Dhevoj 240* (E); Annapurna Himal, *Stainton et al. 6594* (E); above Sauwala Khola, *Stainton et al. 3610* (E).

**Notes:**—When Jeffrey & Chen (1984) transferred *Cacalia penninervis* to the genus *Senecio*, they had to propose the replacement name *S. kumaonensis* because of the pre-existing *S. penninervius*. However, the epithet *penninervis* can be used in combination with *Synotis*, i.e. *Syn. penninervis*.

Although *Senecio* sect. *Pinnati* can be conveniently treated as a section of *Synotis*, here we refrain from doing so because the phylogenetic relationships within the genus are still unclear. According to Jeffrey & Chen (1984), the genus *Synotis* is divisible into two well-marked sections, *S.* sect. *Synotis* and *S.* sect. *Atractylidifoliae* Jeffrey & Chen (1984: 338); all but one of the species, i.e. *Syn. atractylidifolia* (Ling 1937: 24) Jeffrey & Chen (1984: 338), fall within *S.* sect. *Synotis*, which itself is divisible into five not very clearly differentiated series. This classification is only morphology-based, not as yet tested by molecular work.

*Senecio candolleanus* is most closely similar to *Synotis kumaonensis*, differing only in having pubescent (vs. glabrous) achenes (Koyama 1977), and Jeffrey & Chen (1984) referred both of them to *S.* sect. *Pinnati*. As we have not as yet checked the chromosomes of *S. candolleanus* and included it in any molecular analysis, we do not formally transfer it to *Synotis* herein.

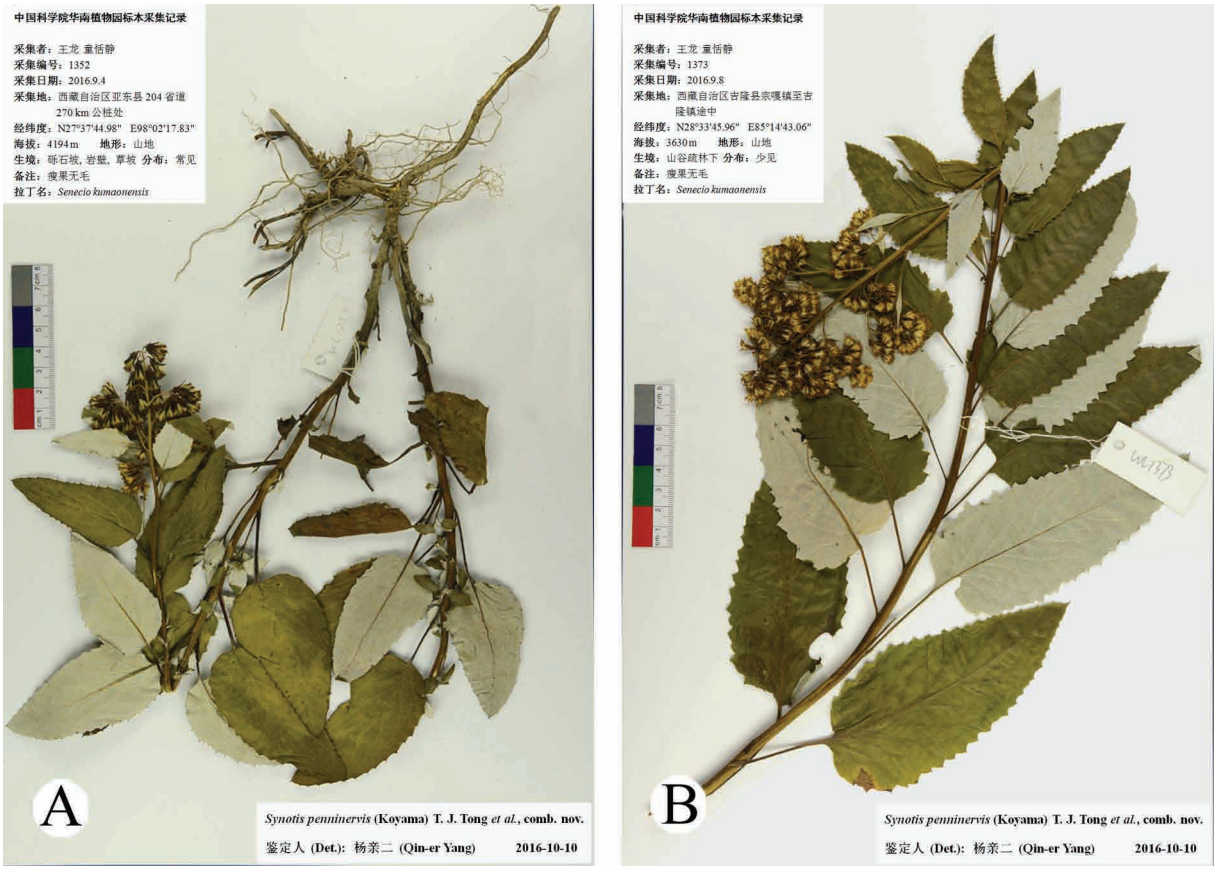


**FIGURE 5.** Specimens of *Synotis penninervis* (= *Senecio kumaonensis*). A. China, Xizang, Yadong, *L. Wang & T. J. Tong 1352* (IBSC). B. China, Xizang, Gyirong, *L. Wang & T. J. Tong 1373* (IBSC).

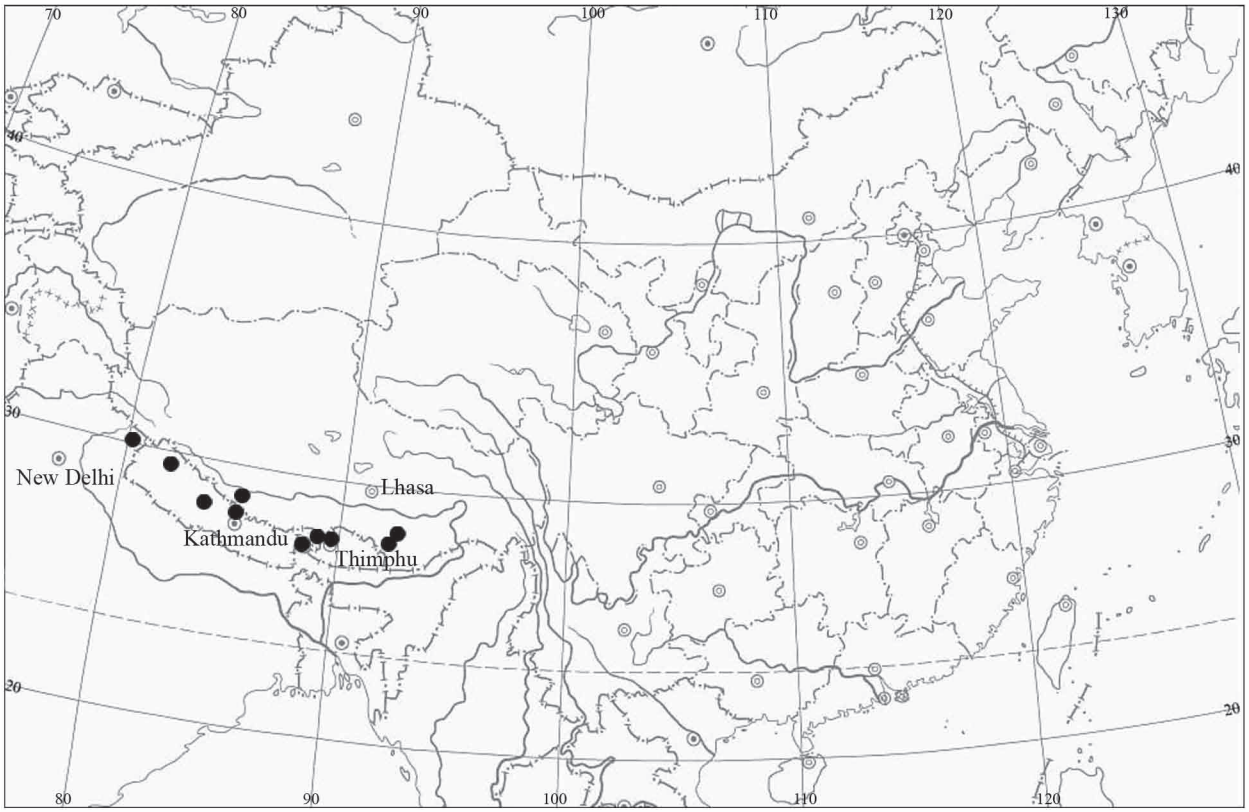


**FIGURE 6.** Distribution of *Synotis penninervis* (= *Senecio kumaonensis*) (●).

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**Appendix 1.** List of taxa and sources of plant material used for the molecular study [taxon, GenBank accession no. (ITS/ETS), source and voucher data]. Note that ITS and ETS sequences came from two different collections in *Pippenalia delphiniifolia*, *Senecio cadiscus* and *Sinacalia tangutica*; they are listed separately after the corresponding accession number.

***Abrotanella emarginata* (Gaudich.) Cass.**, EF538143/GU818108, *R.N.P. Goodall & J. Wood 3352*; ***Capelio caledonica* B. Nord.**, GU818508/GU818130, *B. Nordenstam 9644*; ***Chersodoma jodopappa* (Sch. Bip.) Cabrera**, EF538167/GU818136, *I. Hensen 2617*; ***Cissampelopsis volubilis* (Blume) Miq.**, EF538172/GU818140, *Carvalho 3175*; ***Crassocephalum crepidioides* (Benth.) S. Moore**, AF459968/GU818144, *Pelser cult. 354*; ***Cremanthodium decaisnei* C.B. Clarke**, AY723269/—, *Liu 2364*; ***Cremanthodium discoideum* Maxim.**, AY723271/—, *Liu 833*; ***Cremanthodium humile* Maxim.**, EF538175/GU818145, *H. Kurschner & M. Sonnentag 01-436*; ***Cremanthodium lineare* Maxim.**, AY176134/—, without voucher information; ***Dauresia alliariifolia* (O. Hoffm.) B. Nord. & Pelser**, AF457413/GU818148, *Muller & Tilson 907*; ***Dolichoglottis lyallii* (Hook. f.) B. Nord.**, GU818546/GU818153, *A. Strid 22172*; ***Homogyne alpina* (L.) Cass.**, GU817576/GU818183, *C.H. Uhink 98-56*; ***Jacobaea vulgaris* Gaertn.**, AF459941/GU818190, *Pelser cult. 6*; ***Lachanodes arborea* (Roxb.) B. Nord.**, GU818574/GU818193, *R. Cairns-Wicks s.n.*; ***Lepidospartum burgessii* B. L. Turner**, EF538250/GU818197, *R.D. Worthington 12382*; ***Ligularia dentata* (A. Gray) Hara**, AY176147/—, without voucher information; ***Ligularia sibirica* (L.) Cass.**, AY176146/—, without voucher information; ***Ligularia tongolensis* (Franch.) Hand.-Mazz.**, JF976832/—, *PG090946*; ***Ligularia vellerea* (Franch.) Hand.-Mazz.**, DQ272336/—, *02249*; ***Ligulariopsis shichuana* Y.L. Chen**, AY176148/—, without voucher information; ***Luina hypoleuca* Benth.**, GU818593/GU818201, *W. Greuter 17706*; ***Mikaniopsis clematoides* (Sch. Bip. ex A. Rich.) Milne-Redh.**, GU818595/GU818205, *W.J.J.O. de Wilde & B.E.E. de Wilde-Duyfjes 9006*; ***Miricacalia makineana* (Yatabe) Kitam.**, GU818596/GU818206, *Kobayashi 33484*; ***Nemosenecio nikoensis* (Miq.) B. Nord.**, EF538264/ GU818212, *H. Koyama 4079*; ***Nordenstamia kingii* (H. Rob. & Cuatrec.) B. Nord.**, EF538267/GU818215, *B. Stahl 5572A*; ***Othonna capensis* L.H. Bailey**, AF459960/GU818218, *Pelser cult. 106*; ***Packera eurycephala* (Torr. & A. Gray ex Torr. & A. Gray) W. A. Weber & Á. Löve**, GU818608/GU818219, *M.A. Vincent 8581*;

***Parasenecio adenostyloides*** **(Franch. & Sav. ex Maxim.) H. Koyama**, AF459970/GU818224, *Koyama 408*; ***Parasenecio cyclotus*** **(Bur. & Franch.) Y.L. Chen**, AY723277/—, *Liu 2330*; ***Parasenecio delphiniifolius*** **(Sieb. & Zucc.) H. Koyama**, EF538281.1/—, *H. Koyama 4135*; ***Parasenecio deltophyllus*** **(Maxim.) Y.L. Chen**, AY723274/—, *WAL 823*; ***Parasenecio hastiformis*** **Y.L. Chen**, AY723278/—, *Liu 2084*; ***Parasenecio latipes*** **(Franch.) Y.L. Chen**, AY176150/—, without voucher information; ***Parasenecio maowenensis*** **Y.L. Chen**, AY723275/—, *Liu 2006*; ***Parasenecio roborowskii*** **(Maxim.) Y.L. Chen**, AY176151/—, without voucher information; ***Parasenecio taliensis*** **(Franch.) Y.L. Chen**, AY723276/—, *Liu 2198*; ***Pippenalia delphiniifolia*** **(Rydb.) McVaugh**, GU818627, *Spellenberg & Bacon 11048*/GU818230, *D.E. Breedlove & F. Almeda 59032*; ***Psacaliopsis purpusii*** **(Greenm. ex Brandegee) H. Rob. & Brettell**, GU818629/GU818235, *Panero et al. 2607*; ***Robinsonia gracilis*** **Decne.**, EF538290/GU818242, *T. Stussey 6560*; ***Roldana suffulta*** **(Greenm.) H. Rob. & Brettell**, GU818631/GU818246, *Rzedowski 36569*; ***Senecio algens*** **Wedd.**, EF538296/GU818249, *S.G. Beck 2879*; ***Senecio arnicoides*** **Hook. & Arn.**, EF538298/GU818250, *O. Zoellner 3474*; ***Senecio cadiscus*** **B. Nord. & Pelser**, GU818506, *Rourke 1118/*GU818128, *P. Goldblatt & J. Manning 10690*; ***Senecio changii*** **C. Ren & Q.E. Yang**, KU499905/KU499902, *C. Ren et al. WL146*; ***Senecio chilensis*** **Cabrera**, EF538313/GU818254, *O. Zollner 2958*; ***Senecio ctenophyllus*** **Phill**, EF538322/GU818255, *O. Zoellner 3959*; ***Senecio culcitioides*** **Sch. Bip.**, EF538312/GU818253, *B. Ollgaard & H. Balslev 8822*; ***Senecio doria*** **K. Koch**, AF459946/—, *Pelser cult. 129*; ***Senecio doronicum*** **(L.) L.**, JX895355/—, *J. Calvo 4000*; ***Senecio elegans*** **L.**, GU818642/GU818257, *Cron & Goodman 687*; ***Senecio fistulosus*** **Poepp. ex Less.**, EF538335/GU818258, *S.G. Beck & M. Liberman 9672*; ***Senecio flaccidus*** **Less.**, EF538336/GU818259, *J. Thuret s.n.*; ***Senecio gayanus*** **DC.**, GU818649/GU818261, *M. Rosas 2157*; ***Senecio gramineus*** **Harv.**, GU818650/GU818262, *F.K. Hoener 2104*; ***Senecio gregorii*** **F. Muell.**, GU818651/GU818263, *D.E. Albrecht 7091*; ***Senecio hieracium*** **J. Rémy**, GU818652/GU818265, *M. Baeza & L. Finot 3695*; ***Senecio hypsobates*** **Wedd.**, EF538348/GU818268, *B. Ollgaard & H. Balslev 9863*; ***Senecio ilicifolius*** **L.**, GU818662/GU818269, *Cron & Goodman 686*; ***Senecio involucratus*** **(Kunth) DC.**, EF538150/—, *B. Nordenstam 9438*; ***Senecio nemorensis*** **L.**, AF459937/GU818278, *Pelser cult.102*; ***Senecio oerstedianus*** **Benth.**, EF538362/GU818281, *B. Nordenstam 9160*; ***Senecio roseus*** **Sch. Bip.**, EF538373/GU818292, *J. Garcia P. 250*; ***Senecio scopolii*** **Hoppe & Hornsch.**, JX895384/—, *J. Calvo 4715*; ***Senecio suaveolens*** **(L.) Elliott**, EF538222/GU818298, *D.C. Dister s.n.*; ***Senecio tauricola*** **V.A. Matthews**, GU817570/GU818301, *Budak et al. 1735*; ***Senecio triodon*** **Phil.**, GU818707/GU818303, *F. Luebert & S. Teillier 2266*; ***Senecio vestitus*** **(Thunb.) P.J. Bergius**, GU818708/GU818305, *W. Greuter 21766*; ***Senecio viscosus*** **L.**, AF459925/GU818306, *P.B. Pelser 300*; ***Senecio vulgaris*** **L.**, AF459924/JN789732, *Pelser cult. 188*; ***Sinacalia tangutica*** **(Maxim.) B. Nord.**, AY176157, without voucher information/GU818309, *Y.S. Lian et al. 93-54*; ***Sinosenecio euosmus*** **(Hand.-Mazz.) B. Nord.**, GU818710/GU818310, *D.E. Boufford et al. 27625*; ***Sinosenecio homogyniphyllus*** **(Cumm.) B. Nord.** KT149913/—, *2009075-1;* ***Syneilesis aconitifolia*** **(Bge.) Maxim.**, AY176163/—, without voucher information; ***Synotis cappa*** **(Buch.-Ham. ex D. Don) C. Jeffrey & Y.L. Chen**, EF538402/—, *Tessier-Yandell 86*; ***Synotis chenopodiifolia*** **(DC.) M. Tang *et al.***, KJ851593/KJ851592, *M. Tang & C. Ren 532*; ***Synotis lucorum*** **(Franch.) C. Jeffrey & Y.L. Chen**, AY723255/—, *Liu 2177*; ***Synotis nagensium*** **(C. B. Clarke) C. Jeffrey & Y.L. Chen**, AF459922/GU818315, *Bartholomew et al. 1991*; ***Synotis penninervis*** **(Koyama) T.J. Tong *et al.* (= *Senecio kumaonensis* Duthie ex C. Jeffrey & Y.L. Chen)** KY347902/KY347901, *M. Tang & C. Ren 626*; ***Tephroseris integrifolia*** **(L.) Holub**, GU818724/GU818319, *Budak & Hamazaoglu 1688*.