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PHYLOGENETICS

Strong incongruence between the ITS phylogeny and generic delimitation in the *Nemosenecio-Sinosenecio-Tephroseris* assemblage (Asteraceae: Senecioneae)

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ABSTRACT. The three genera *Sinosenecio*, *Nemosenecio* and *Tephroseris* form a closely knit group nested in the subtribe Tussilagininae of the tribe Senecioneae (Asteraceae). The generic limits in this assemblage remain unclear and need revision. In this study, we analysed sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA available from GenBank and sequenced 19 accessions of an additional 13 species encompassing all three genera. Phylogenetic analyses based on the ITS variation of 27 species in this assemblage and seven species from related genera of the Tussilagininae suggested that neither *Sinosenecio* nor *Tephroseris* is monophyletic. The sampled species of *Sinosenecio* were scattered in different clades or subclades of the phylogenetic tree. Four species of this genus, including the generic type species (*S. eriopodus*, *S. hederifolius*, *S. homogyniphyllus* and *S. subcoriaceus*) are clustered in a tentative clade with genera such as *Ligularia*, *Cremanthodium*, *Parasenecio, Farfugium and Tussilago*. The remaining ten *Sinosenecio* species comprise a highly supported clade together with 13 *Tephroseris* species and four *Nemosenecio* species. Within this clade, 10 *Tephroseris* species together with two *Sinosenecio* species (*S. newcombei* and *S. koreanus*) comprise a monophyletic subclade while the remaining 11 species from all of three genera are clustered into another clade with moderate statistical support. Within the latter subclade, *T. changii* was revealed to be closely related to four *Sinosenecio* species, and three *Nemosenecio* species comprising a monophyletic lineage. These two lineages form a polytomous radiation with the other two *Sinosenecio* lineages. The generic delimitations of the three genera clearly need some adjustments, which is also supported by previous studies of gross and floral morphology. Two *Sinosenecio* species (*S. newcombei* and *S. koreanus*) should be transferred to *Tephroseris*, and the genus *Sinosenecio* should be re-circumscribed to contain those species clustered in the *Ligularia—Tussilago* clade. Most of the other described species under *Sinosenecio* and *T. changii* should either be transferred to an enlarged *Nemosenecio* concept, or a new genus needs to be established to encompass them. However, the morphological distinctions between these genera require further investigation.

Keywords: Asteraceae; Internal transcribed spacer (ITS) region; *Nemosenecio*; Molecular phylogeny; *Sinosenecio*; *Tephroseris*; Tussilagininae.

INTRODUCTION

Nemosenecio (Kitam.) B. Nord., *Sinosenecio* B. Nord. and *Tephroseris* (Reichenb.) Reichenb. are closely related genera of tribe Senecioneae of the Asteraceae (Liu et al., 2006; Pelser et al., 2007). *Nemosenecio* has six species, five of which are found in China and one in Japan (Jeffrey and Chen, 1984; Nordenstam, 2007; Zhang et al., 2008). *Sinosenecio* contains about 38 species of which 37 occur in China, Korea, and Indo-China with a distinct center of diversity in the Sichuan province of China. *Sinosenecio newcombei* (Greene) J. P. Janovec & T. M. Barkley, however, is endemic to the Queen Charlotte Islands of Canada. *Tephroseris* contains around 50 species mainly found in temperate and arctic Eurasia. Six species are recognized in NW North America, one of which is endemic (Barkley and Murray, 2006). Although these three genera have traditionally been regarded as members of the subtribe Tussilagininae Dum., the *Nemosenecio-Sinosenecio-Tephroseris* assemblage has

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also been recognized at the subtribal level (Jeffrey and Chen, 1984). Tephroseridinae C. Jeffrey et Y. L. Chen was considered as distinct from the Tussilagininae by owning narrow, cylindrical anther-collars, polarized, scattered, or radial endothecial cell wall thickenings, and confluent, contiguous or separate stigmatic areas (Jeffrey and Chen, 1984). These character states are, however, certainly not unique to *Nemosenecio*, *Sinosenecio*, and *Tephroseris* and have also been observed in the Tussilagininae sensu Jeffrey and Chen (Liu, 1999). In addition, Jeffrey and Chen (1984) suggested the gametic chromosome number of 24 as diagnostic for Tephroseridinae, although a wide range of other chromosome numbers have also been observed (Jeffrey and Chen, 1984; Nordenstam, 2007; Pelser et al., 2007) and the more typical Tussilaginoid chromosome number of x = 30 has been recorded for two *Sinosenecio* species (Liu, 2004). Of the three genera, *Sinosenecio* especially shows character states of both the Tephroseridinae and Tussilagininae sensu Jeffrey and Chen (1984) (Liu, 2000). Because of the lack of diagnostic characters for Tephroseridinae and its phylogenetic position deeply nested within Tussilagininae sensu Jeffrey and Chen (1984), it is currently not recognized as a subtribe and *Nemosenecio*, *Sinosenecio*, and *Tephroseris* are placed in Tussilagininae by most authors (e.g., Bremer, 1994; Liu et al., 2006; Pelser et al., 2007).

In addition to difficulties concerning the subtribal delimitation, also the generic delimitation of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage has been somewhat problematic. The three genera have mainly been defined on the basis of leaf characters (Jeffrey and Chen, 1984), although Nordenstam (1978) also indicated differences in habit and floral morphology. Both *Nemosenecio* and *Tephroseris* have pinnately-veined leaves, but those of *Nemosenecio* are pinnatisect, whereas the leaves of *Tephroseris* are subentire or only shallowly lobed. In contrast to these two genera, most species of *Sinosenecio* have palmately-veined leaves with sinuate-dentate to sinuate-denticulate margins, although the leaves of *Sinosenecio hainanensis* (Chang & Tseng) C. Jeffrey & Y. L. Chen are pinnately-veined. Although *Nemosenecio* is easily distinguished by its deeply incised leaves, the differences between *Sinosenecio* and *Tephroseris* are not always clear. *Tephroseris changii* B. Nord, for example, was regarded as a member of *Tephroseris* based on its pinnately veined leaves, even though this species resembles some of the *Sinosenecio* species in habit, anther shape and phyllary number (Jeffrey and Chen, 1984).

In recent years, phylogenetic studies of DNA sequences have been successfully applied to resolve the systematic positions and generic delimitations of several Senecioneae genera (e.g., Knox and Palmer, 1995; Swenson and Bremer, 1997, 1999; Panero et al., 1999; Bain and Golden, 2000; Wagstaff and Breitwieser, 2004; Liu et al., 2006; Pelser et al., 2002, 2003, 2007; Wagstaff et al., 2006). These molecular phylogenetic studies suggested that the morphology-based generic delimitation of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage needs to be revised to resolve monophyletic genera. For example, Golden et al. (2001), prompted by the extraordinary biogeographical consequence of the transfer of *Senecio newcombei* Greene from the Queen Charlotte Islands to the otherwise largely Chinese *Sinosenecio*; Janovec and Barkley (1996) performed a phylogenetic study of ITS sequences to examine the true relationships of *S. newcombei*. These authors found that the two species of *Sinosenecio* included in their studies (*S. koreanus* (Kom.) B. Nord. from Korea and *S. newcombei*) did not form a monophyletic group and are nested within *Tephroseris*. The non-monophyly of *Sinosenecio* was also concluded in another study using ITS sequence data (Liu et al., 2006). In that study, the single species of *Tephroseris* and *Nemosenecio* and two of the three *Sinosenecio* species included formed a well supported clade in which *S. bodinieri* (Van.) B. Nord. and *S. globigerus* (Chang) B. Nord. comprised a strongly supported subclade. *Sinosenecio subcoriaceus* C. Jeffrey & Y. L. Chen, the third species included, however, appeared to be more distantly related and was placed in a large polytomy with several other Tussilagininae clades. The results of these two studies were confirmed by Pelser et al. (2007), who included the *Nemosenecio*, *Sinosenecio*, and *Tephroseris* ITS sequences generated by Golden et al. (2001) and Liu et al. (2006) and a few other accessions of *Sinosenecio* and *Tephroseris* in their Senecioneae ITS phylogeny. This study indicated with strong bootstrap support and posterior probabilities that *Sinosenecio newcombei* and *S. koreanus* are more closely related to *Tephroseris* than to the other four *Sinosenecio* species that were included. Furthermore, *Tephroseris changii* proved to be distantly related to the other *Tephroseris* species and instead formed a clade with *Sinosenecio bodinieri*, *S. globigerus*, and *S. septilobus* (Chang) B. Nord. In the same way as Liu et al. (2006), Pelser et al. (2007) found *S. subcoriaceus* to be only distantly related to the other members of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage and nested within a large, but poorly resolved clade composed of *Cremanthodium*, *Ligularia*, *Parasenecio*, and other mostly Asian Tussilagininae genera.

As a first step towards arriving at a monophyletic generic delimitation of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage, an ITS phylogeny of this group is presented that includes a much larger sampling of its species (27) together with a selection of other Senecioneae genera. On the basis of this phylogeny, we discuss its incongruence with the current morphology-based generic delimitation and explore alternative classifications to obtain strictly monophyletic genera.

MATERIALS AND METHODS

Sequence data and sampled species

The ITS sequences included in this study were either newly obtained (19 accessions representing 13 species) or downloaded from GenBank. Voucher information and

GenBank accession numbers are listed in Table 1. In total, our data set comprises 42 accessions of 27 species (Table 1) representing three *Nemosenecio* species, 11 *Tephroseris* species listed by Jeffrey & Chen (1984), and 13 *Sinosenecio* species selected to represent the two sections, four subsections, and four series that Jeffrey & Chen (1984) recognized for *Sinosenecio* (Table 1). Our samples comprised all sections and subsections of *Sinosenecio*, and species which have represented the distribution range of *Tephroseris* (see details to Table 1). In addition, seven species from seven other Tussilagininae genera were included. Subtribe Senecioninae was represented with a single species (*Senecio thianshanicus* Regel et Schmalh.) and *Doronicum stenoglossum* Maxim. was selected as outgroup on the basis of the results of previous studies (Liu et al., 2006).

DNA extraction, amplification and sequencing

Total DNA was extracted from fresh or silica-gel dried leaf tissue or from leaf samples taken from herbarium specimens using the DNeasy Plant Mini kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. ITS was amplified with primers "1a" and "4" (White et al., 1990). Polymerase chain reactions (PCR) were performed in a 25-µl volume, containing 10-40 ng plant DNA, 50 mM Tris-HCI, 1.5 mM $MgCl_2$, 250 µg/mL BSA, 0.5 mM dNTPs, 2 µM of each primer, and 0.75 unit of Taq polymerase. PCR reactions were performed with the following thermocycling conditions: 5 min at 95°C, 36 cycles of 1 min at 94°C, 1 min of annealing at 52°C, and 1.25 min at 72°C, with a final 8 min extension at 72°C, and reactions were kept at 4°C until further processing. PCR products were purified using a TIANquick Midi Purification Kit following the recommended protocol (TIANGEN). Sequencing reactions were performed with the PCR primers using the ABI Prism BigdyeTM Terminator Cycle Sequencing Ready Reaction Kit. Both the forward and reverse strands of DNA were sequenced and this resulted in a minimum overlap of 70% of their length in the contigs. Sequences were aligned using CLUSTAL X (Thompson et al., 1997) with default parameter settings and were edited by hand. The boundaries of the ITS region were determined by comparison with the results of Liu et al. (2006). All new sequences have been deposited in GenBank under accession numbers EU195463-EU195532 (Table 1).

Data analysis

Phylogenetic trees were reconstructed from ITS sequences with maximum likelihood (ML) and maximum parsimony (MP) using PAUP* v 4.0b10 (Swofford, 2002) and Bayesian Inference (BI) with MrBayes 3.0 (Huelsenbeck and Ronquist, 2001; Huelsenbeck et al., 2001; Ronquist et al., 2003).

MP analyses involved a heuristic search strategy with 100 replicates of random addition of sequences, in combination with ACCTRAN character optimization, MULPARS + TBR branch swapping and STEEPEST DESCENT options on. Bootstrap values (BS; Felsenstein, 1985) were calculated from 1000 replicates using a heuristic search with simple addition with TBR and MULPARS options on.

For the ML analyses, an appropriate nucleotide substitution model was selected using the Akaike Information Criterion (AIC) implemented in MODELTEST version 3.06 (Posada and Crandall, 1998), and a heuristic search with simple addition of sequences and TBR branch swapping, MULTREES and COLLAPSE was used to produce ML trees.

The nucleotide substitution model selected with MODELTEST was also used for the BI analyses, which were carried out with four simultaneous Monte-Carlo Markov Chains (MCMC; three heated and one cold) run for two million generations. Trees were saved every 100 generations. A burn-in of 5000 trees was discarded after visual inspection of the log-likelihood values and the remaining 15 001 trees were used to construct a 50% majority rule consensus tree with posterior probabilities (PP).

RESULTS

The aligned ITS matrix contained 592 characters, of which 283 (47.80%) were constant and 172 (15.72%) were parsimony-informative. The best-fit model and parameters selected for this data set by the AIC in MODELTEST were: GTR + G; base = (0.2386, 0.2181, 0.2418), nst = 6, rmat = (0.7879, 2.0385, 1.2710, 0.5508, 3.5878), rates = gamma and shape = 1.2580.

MP, ML, and BI analyses resulted in trees with almost identical topologies, except for a few species that were placed in slightly different phylogenetic positions and received low BS and PP values in the MP and BI analyses. Only the ML tree is shown here (Figure 1). Two main clades were resolved for Tussilagininae, but only Clade A composed of *Nemosenecio*, *Tephroseris* and the majority of the *Sinosenecio* species, received strong BS support (99%) and PP (1.00). Four *Sinosenecio* species (*S. eriopodus*, *S. hederifolius*, *S. homogyniphyllus* and *S. subcoriaceus*), however, were not found to be part of Clade A and instead formed a poorly supported (BS < 50%; PP < 0.50) clade (B) together with *Ligularia*, *Cremanthodium* and the other Tussilagininae genera. Within clade A, one subclade (C) comprised 11 *Tephroseris* species and two *Sinosenecio* species (*S. newcombei* and *S. koreanus*) and received high BS (100%) and PP (1.00). The other subclade (D) contained 11 species from all three genera and was weakly supported (BS of 70%; PP of 0.55). Within subclade D, the three accessions of *S. guangxiensis* composed a clade sister to the remainder of D. *Tephroseris changii*, the only *Tephroseris* species of D, was found to be nested within a clade of four *Sinosenecio* species. All three species of *Nemosenecio* included in our studies comprise a monophyletic lineage within D.

Table 1. List of taxa and sources of plant material analyzed and accessions number in GenBank [Infrageneric classification following Jeffrey and Chen (1984), Chen (1999) and Liu (1989)].

Genus and species	Origins	Voucher number	Accessions
Sinosenecio B. Nord.			
Sect. *Sinosenecio*			
Subsect. *Sinosenecio*			
S. bodinieri (Van.) B. Nord.	Nanchuan, Chongqing	LZY001	AY176158
S. eriopodus (Cumm.) C. Jeffrey et Y. L. Chen	Sangzhi, Hunan	LKS0257	EU195467/EU195485*
S. hederifolius (Dumm.) B. Nord.	Jiange, Sichuan	Liu20050329	EU195465/EU195483*
S. subcoriaceus C. Jeffrey et Y. L. Chen	Nanchuan, Chongqing	Liu806	AY176162
Subsect. *Phalacrocarpa* C. Jeffrey et Y. L. Chen			
S. globigerus (Chang) B. Nord.	Nanchuan, Chongqing	Liu818	AY176159*
S. homogyniphyllus (Cumm.) B. Nord.	Emei, Sichuan	Liu20040620-1	EU195466/EU195484*
Sect. *Phyllocaulon* C. Jeffrey et Y. L. Chen			
Subsect. *Madarogyne* C. Jeffrey et Y. L. Chen			
S. euosmus (Hand.-Mazz.) B. Nord.	Emei, Sichuan	Liu20040620-2	EU195469/EU195487*
S. fanjingshanicus C. Jeffrey et Y. L. Chen	Songtao, Guizhou	WLK422	EU195468/EU195486*
S. koreanus (Kom.) B. Nord.	Kaongwon, Korea	Lee s.n.	AF345307/AF345315
S. septilobus (Chang) B. Nord.	Nanchuan, Chongqing	Liu800	AY176161*
Subsect. *Lasiogyne* C. Jeffrey et Y. L. Chen			
S. guangxiensis C. Jeffrey et Y. L. Chen	Longsheng, Guangxi	Gao-05001	EU195476/EU195494*
S. guangxiensis C. Jeffrey et Y. L. Chen	Longsheng, Guangxi	Gao_sino01	EU195477/EU195495*
S. guangxiensis C. Jeffrey et Y. L. Chen	Shangyou, Guangxi	Nie8305	EU195478/EU195496*
S. newcombei (Green) J.P. Janovec & T. M. Barkley	Queen Charlotte Islands, British Columbia, Canada	Bain478 (LEA)	AF161607/AF161657
S. oldhamianus (Maxim.) B. Nord.	Yongshun, Hunan	BJD0014	EU195475/EU195493*
S. oldhamianus (Maxim.) B. Nord.	Shimian, Sichuan	Liu05029	EU195470/EU195488*
S. oldhamianus (Maxim.) B. Nord.	Danba, Yunan	Liu05105	EU195472/EU195490*
Tephroseris (Reichenb.) Reichenb.			
T. atropurpurea (Ledeb.) Holub	Whitehorse, Yukon, Canada	Golden318 (LEA)	AF345306/AF345314
T. atropurpurea (Ledeb.) Holub	Mount Fairplay, Alaska, UAS	Bain493 (LEA)	L33184/L33214
T. changii B. Nord.	Genbank	Liu801	AY176164*
T. crispa Schur	Czech Republic	WML LEP 21529 L	EF538407
T. flammea (Turcz. ex DC.) Holub	Mohe, Heilongjiang	Zhu644	EU195479/EU195497*
T. fuscata Holub	Beartooth Mountains, Wyoming, USA	Golden235 (LEA)	AF345302/AF345310
T. integrifolia (L.) Holub ssp. *aurantiaca* (Hoppe ex Willd.) B. Nord. var. *leiocarpa* (Boiss.) B. Nord.	Genbank	Pelser Cult. 278 L	EF538408
T. kirilowii (Turcz. ex DC.) Holub	Genbank	Liu812	AY176165
T. palustris (L.) Fourr. subsp. *congesta* (R. Br.) Holub	Snag Junction, Yukon	Bain495 (LEA)	AF345301/AF345309
T. pierotii (Miq.) Holub	Guangfu, Jiangsu	Sun34	EU195480/EU195498*
T. rufa (Hand.-Mazz.) B. Nord..	Seda, Sichuan	Liu834	AY176166
T. yukonensis Holub	Keno City, Yukon, Canada	Golden339 (LEA)	AF345304/AF345312
Nemosenecio (Kitam.) B. Nord.			
N. incisifolius (J. F. Jeffr.) B. Nord.	Jiangchuan, Yunnan		EU195463/EU195481*
N. nikoensis (Miq.) B. Nord.	Hongshu, Japan	LJQ002	AY723279
N. nikoensis (Miq.) B. Nord.	Hongshu, Japan	H. Koyama 4079 L	EF538264*
N. yunnanensis B. Nord.	Luoping, Yunan		EU195464/EU195482*
Cremanthodium decaisnei C. B. Clarke	Xiangcheng, Sichuan	Liu2364	AY723269
Doronicum stenoglossum Maxim.	Yushu, Qinghai	Liu1791	AY176138
Farfugium japonicum (Bain and Golden) Kitam.	Nanchuan, Chongqin	Liu2143	AY176139
Ligularia dentata (A. Gray) Hara	Kunming, Yunnan	Liu2168	AY723256
Parasenecio deltophyllus (Maxim.) Y. L. Chen	Maqin, Qinghai	WAL828	AY723274
Petasites japonicus (Sieb. et Zucc.) Maxim.	Nanchuan, Chongqin	Liu854	AY176152
Syneilesis aconitifolia (Bge.) Maxim.	Beijing, China	Liu1148	AY176163
Tussilago farfara L.	Xining, Qinghai	Liu851	AY176167
Senecio thianshanicus Regel et Schmalh.	Chengduo, Qinghai	Liu857	AY176156

*Indicates new sequence reported here.

DISCUSSION

Our phylogenetic analyses confirm the results from previous ITS studies that used a much smaller sampling of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage (Golden et al., 2001; Liu et al., 2006; Pelser et al., 2007), showing that neither *Sinosenecio* nor *Tephroseris* is monophyletic. The results of this study further indicate that *Nemosenecio* is monophyletic and deeply nested within a clade composed of the majority of *Sinosenecio* species included (Clade D). These findings have been confirmed also by preliminary phylogenetic analyses of a *trn*L-*trn*F data set that contains a selection of the *Nemosenecio*, *Sinosenecio*, and *Tephroseris* species included in the present study (unpublished data) and that will be expanded in future studies. Because of the incongruence between the molecular phylogenies and the current generic classification of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage, its generic delimitation needs to be revised.

The re-circumscription of *Tephroseris*

All sampled *Tephroseris* species (10/11) except for *T. changii* constitute a well supported monophyletic lineage together with two *Sinosenecio* species (*S. newcombei* and *S. koreanus*) (Figure 1). Because the *Tephroseris* species not included in this study have a habit and morphology that is similar with the species sampled here, it is highly likely that most species described under *Tephroseris* will be part of this lineage. Therefore, a newly circumscribed *Tephroseris* should contain most species previously placed in this genus (Jeffrey and Chen, 1984; Jeffrey, 1992) and the two *Sinosenecio* species.

Sinosenecio koreanus has a small distribution area in north Korea and the adjacent part of Jilin in NE China, far outside the center of diversity of *Sinosenecio*. Although its habit is *Sinosenecio*-like, the leaf-blades are not distinctly cordate as is common in *Sinosenecio*, but rather subtruncate to cuneate and not distinctly palmately veined; thus in these characters it more closely resembles *Tephroseris*. The petioles are not clearly winged like in many *Tephroseris* species, but they are at least basally expanded. The ray-florets of *S. koreanus* exceed the phyllaries in number (c. 18 and c. 13, resp.), another character unusual in *Sinosenecio*, where phyllaries usually equal or exceed rays in number.

The phylogenetic affinities of *S. newcombei* have long been unclear and this species has previously been included in *Senecio* (Greene, 1897) and *Packera* (Weber and Löve,

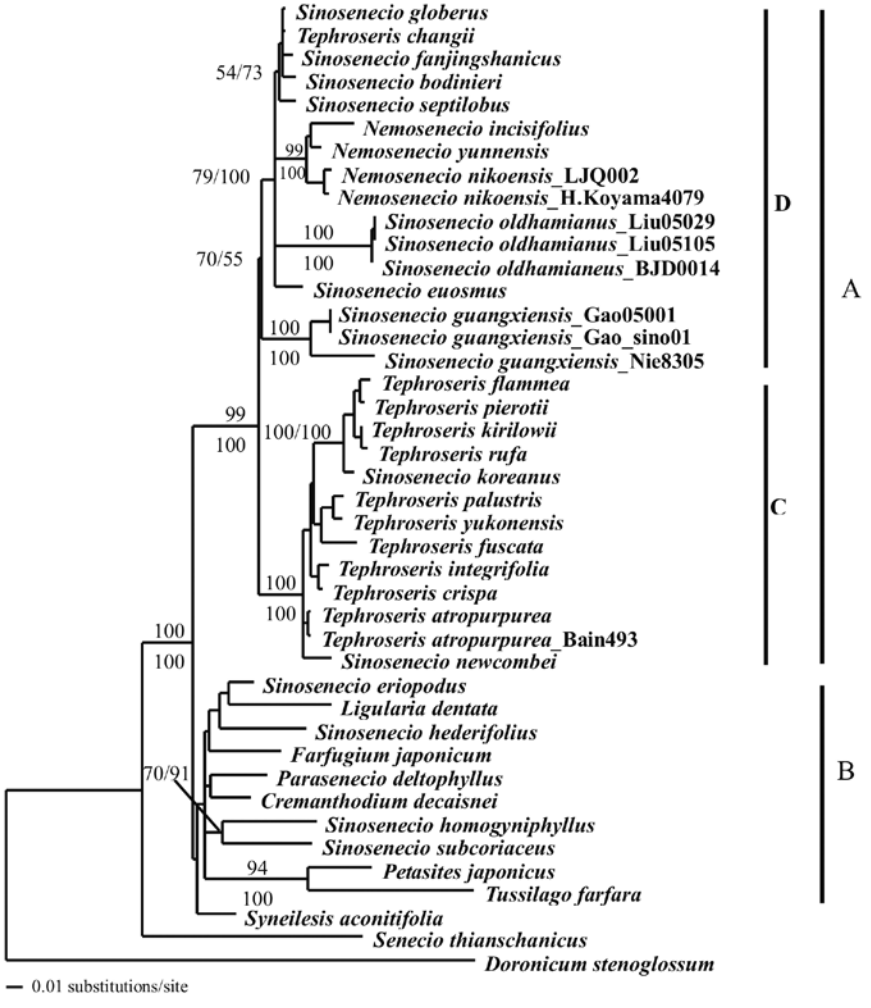

Figure 1. The single ML tree based on ITS sequences for the *Sinosenecio-Nemosenecio-Tephroseris* assemblage and other genera of the subtribe Tussilagininae of the tribe Senecioneae. Letters A and B next to the bars represent for two major clades of the ML tree, while C and D stand for the two sub-clade of *Nemosenecio-Sinosenecio-Tephroseris* assemblage (for details, please refer the Result and Discussion part). Bootstrap values from the most parsimony analyses with 1000 replicates (above branch) and Bayesian posterior probabilities appear at branch nodes (under branch).

1981) from where it was placed in *Sinosenecio* (Janovec and Barkley, 1996) to now eventually find a home in *Tephroseris*. *Sinosenecio newcombei* has a chromosome number of n = 24 (Taylor and Mulligan, 1968), which is characteristic of *Tephroseris*, although also found in *Sinosenecio*. On overall morphological features *S. newcombei* is better positioned in *Tephroseris* than in *Sinosenecio*.

The newly defined *Tephroseris* does not have morphological characters that allow easy distinction from *Sinosenecio*. Its species have leafy stems, but these are also found in *Sinosenecio* and the basic chromosome number of x = 24 or 12 (Liu, 2004) found in *Tephroseris* is also observed in *Sinosenecio*. Although most *Tephroseris* species have pinnately-veined leaves, these are palmately-veined in *S. newcombei* and *S. koreanus*. Anther-collars are cylindrical and endothecial cell wall thickenings are mainly polar although a few cells close to the connective tissue bear radial thickenings as well (Jeffrey and Chen, 1984; Golden et al., 2001; Liu, 2001) as is also the case in the other genera of the Tusslagininae (Liu, 1999). The petioles of all these species are indistinct from the lamina (Jeffrey and Chen, 1984; Golden et al., 2001), a feature characteristic of *Tephroseris*.

The polyphyly of *Sinosenecio*

The ITS phylogenies (Figure 1) all indicate that *Sinosenecio* is polyphyletic and remains an unnatural group even if *S. koreanus* and *S. newcombei* are transferred to *Tephroseris*. Four of the *Sinosenecio* species included in our studies (*S. eriopodus*, *S. hederifolius*, *S. homogyniphyllus*, and *S. subcoriaceus*) form a clade with *Cremanthodium*, *Farfugium*, *Ligularia*, *Parasenecio*, *Petasites*, *Syneilesis*, and *Tussilago* (Clade B). This clade is sister to Clade A, which includes *Nemosenecio*, *Tephroseris*, and the other *Sinosenecio* species (Figure 1). As observed in other studies (Liu et al., 2006; Pelser et al., 2007) resolution in Clade B is poor and relationships do not conform well to the current generic delimitation. This is also found for the four *Sinosenecio* species in Clade B of which only *S. homogyniphyllus* and *S. subcoriaceus* form a clade. These two species are morphologically similar and no doubt closely related, and it should be noted that the former is the generic type. Although Clade B lacks diagnostic morphological characters, many of its species have a basic chromosome number of n = 30 (Liu, 2004) which is also found in *S. hederifolius* and *S. subcoriaceus* (Liu, 1999 and unpublished data). Reports of chromosome counts for *S. eriopodus* are not known to us, but Liu (1999) reported 2n = 24 from roots of *S. homogyniphyllus*. This finding, however, may need confirmation, because it is quite different from other counts for members of Clade B. Just like *Farfugium*, another genus in Clade B, all four *Sinosenecio* members of this clade have young leaves with involute leaf margins. Other *Sinosenecio* species with involute leaf margins, such as *S. cyclaminifolius* (Franch.) B. Nord. and *S. dryas* (Dunn) C. Jeffrey et Y. L. Chen, may be closely related to these four *Sinosenecio* species, although this needs to be confirmed in future molecular and morphological studies. Because *S. homogyniphyllus* is the type species of the genus (Nordenstam, 1978), *Sinosenecio* has to be more narrowly defined to include only those species that are part of Clade B, or even a selection of them if more detailed studies indicate that these species do not form a monophyletic group.

The remaining seven *Sinosenecio* species included are members of subclade D, in which *Tephroseris changii* and *Nemosenecio* take nested positions (Figure 1). It is not surprising that *T. changii* appears to be closely related to *S. septilobus*, *S. bodinieri*, *S. fangjingshanicus*, and *S. globigerus*, because all of these species are scapigerous and have similar habit, anther shape and phyllary number (Jeffrey and Chen, 1984). Subclade D is characterized by scattered or radial endothecial cell wall thickenings (Jeffrey and Chen, 1984; Liu, 2001), although these character states are also found elsewhere in Tussilagininae, and chromosome numbers of 2n = 24, 48 or 72 (Liu, 1999, 2004). Because of their distant relationship with the type of *Sinosenecio* (*S. homogyniphyllus*), *Tephroseris changii* and the species of *Sinosenecio* in sublade D need to be accommodated in another genus, separate from *Sinosenecio*.s.s.. This could be achieved by transferring the seven *Sinosenecio* species and *T. changii* to *Nemosenecio*. Alternatively, in order to preserve *Nemosenecio* in its current circumscription, the clade composed of *S. septilobus*, *S. bodinieri*, *S. fangjingshanicus*, *S. globigerus*, and *T. changii* could be described as a new genus as well as the remainder of the subclades found in subclade D. In our opinion, however, subclade D is currently too poorly resolved to follow the latter taxonomic option and requires more detailed molecular and morphological studies before taxonomic changes should be made.

In conclusion, our studies indicate that the morphology-based generic delimitation of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage is strongly incongruent with the ITS phylogeny. This most likely means that the morphological characters used to define genera (e.g., leaf venation) show widespread homoplasy. Future morphological and cytological studies could reveal new diagnostic characters for clades and may therefore aid in revising the generic delimitation in the *Nemosenecio-Sinosenecio-Tephroseris* assemblage. Although the resolution and support in the ITS phylogeny allow for the transfer of *Sinosenecio koreanus* and *S. newcombei* to *Tephroseris*, the phylogenetic relationships of other *Sinosenecio* species are currently poorly supported. More detailed studies, involving sequence data from additional DNA regions, are therefore needed before taxonomic changes are made in *Sinosenecio*. In addition, a larger taxon sampling for *Sinosenecio* and closely related genera which would include several new species of *Sinosenecio* that were recently reported (for example, Zhang et al., 2008) should be used to arrive at a stable classification of the *Nemosenecio-Sinosenecio-Tephroseris* assemblage.

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狗舌草亞族複合群（菊科：千里光族）的屬間界限與 ITS 分子證據的衝突

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傳統菊科千里光族狗舌草亞族主要包括狗舌草屬、華千里光屬和羽葉千里光屬三屬，這裏我們暫稱爲“狗舌草亞族複合群”。目前這三個屬的親緣關係和系統位置存在較大分歧，需要進一步修訂。在本研究中，我們新報導了 13 個種的 19 條核糖體內轉錄間隔區 ITS 序列；並結合 Genbank 已報導序列，對該複合群 27 個種（覆蓋了這三個屬所有的組和亞組）和款東亞族內近緣屬的 7 個代表種的核糖體內轉錄間隔區 ITS 構建了分子系統發育樹。研究發現華千里光屬和狗舌草屬均非單系起源。華千里光屬四個種，包括該屬的模式種（*S. eriopodus*, *S. hederifolius*, *S. homogyniphyllus* 和 *S. subcoriaceus*）與橐吾屬、垂頭菊屬和蟹甲草屬等近緣屬的代表種聚爲一支，即分支 B，但支援率不高；而該屬其他 10 個代表種則與狗舌草屬的 13 個種和羽葉千里光屬的 3 個種共同組成分支 A，並得到較高的自展支持。主要分支 A 含有兩個穩定的亞分支 C 和 D：其中亞分支 C 包括狗舌草屬的 10 個種與華千里光屬的兩個種 *S. newcombei* 和 *S. koreanus*，而亞分支 D 包括了所有三個屬的其他 11 個種。另外，亞分支 D 內，*T. changii* 與華千里光屬的 4 個代表種聚爲一支，而羽葉千里光屬的 3 個代表種則單獨組成一個單系分支。結合先前的有關外部宏觀和微觀性狀特徵的研究與本研究中的 ITS 分子證據，這三個屬的屬間界限需要進行適當調整：*S. newcombei* 和 *S. koreanus* 兩個種應放在狗舌草屬內；而華千里光屬也應包括本研究中分支 B 內一些近緣屬的代表種；本研究所涉及到華千里光屬和 *T. changii* 應歸併至羽葉千里光屬，或成立一個新屬。但無論如何，這三個屬間界限仍需要更多形態學和分子證據，需要進一步的調查和研究。

關鍵詞：菊科；款東亞族；華千里光屬；狗舌草屬；羽葉千里光屬；分子系統發育；核糖 ITS 區域。