Research Article

Phylogenetic reconstruction of the genus *Triptilion* (Asteraceae, Nassauvieae) based on nuclear and chloroplast DNA sequences

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Abstract The genus *Triptilion* is endemic to central Chile, the Mendoza Province and western Patagonia in Argentina. It is currently composed of seven species: *T. achilleae, T. benaventii, T. berteroi, T. capillatum, T. cordifolium, T. gibbosum,* and *T. spinosum*. The main objectives of this paper were to determine the phylogenetic relationships of species of *Triptilion.* We also traced the evolution of annual and perennial life-forms. Historically a close relationship has been described between genera *Triptilion* and *Nassauvia.* Phylogenetic analysis of the genus *Triptilion* and more closely related genera was undertaken using two nuclear (ITS, ETS) and two chloroplast (*trnL-F, rpl32-trnL*) markers. The topology of the Bayesian inference tree shows that the genus *Triptilion* is paraphyletic, because *N. lagascae,* the only representative of *Nassauvia* section *Caloptilium* grouped with *T. achilleae,* Clade I. The other species of *Triptilion* form two clades: Clade II composed of *T. cordifolium* and *T. gibbosum* and Clade III that includes *T. benaventii, T. berteroi, T. capillatum,* and *T. spinosum*. The genus *Triptilion* originated and diverged during the Miocene. The results of the life history reconstructions indicate that the common ancestor of *Triptilion* and *Nassauvia* was perennial. The annual habit appears to be derived in *Triptilion.* The life-form of the common ancestor of *Triptilion* was ambiguous; it may have been annual or perennial.

Key words: central Chile, life-form, paraphyletic, patagonia, *Triptilion*.

1 Introduction

The Asteraceae genus *Triptilion* Ruiz & Pav. (Mutisioideae, Nassauvieae) is composed of seven species: (a) annual herbs, *T. achilleae, T. berteroi, T. capillatum, T. cordifolium* and *T. gibbosum*; (b) perennial herbs, *T. benaventii,* and *T. spinosum* (Katinas et al., 1992; Zuloaga et al., 2008; Table 1). Five of these are endemic to Chile, whereas *T. achilleae* and *T. capillatum* also extend into Argentina (Katinas et al., 1992; Fig. 1). Annual species are multi-caulinar, with elongated internodes that make the rosette of leaves less evident; the two perennial species have a rhizome from which arise rosettes of leaves and monocephalous inflorescences (*T. benaventii*) or multi-caulinar stems (*T. spinosum*). Both life forms have alternate and sessile leaves. The capitula have five flowers; blue in *T. spinosum* and *T. berteroi* and white in the remaining species. The pappus is white or yellow-green, formed by three deciduous paleaceous bristles (occasionally 2 or 4) that are lanceolate and folded longitudinally. The pappus has been the main character used to separate *Triptilion* from *Nassauvia* (Katinas et al., 2008b). However, pappus characters used to separate these genera overlap (Cabrera, 1982).

Historically a close relationship has been hypothesized between the genera *Triptilion* and *Nassauvia.* The genus *Triptilion* was described in 1797 by Ruiz & Pavón and was based on the species *T. spinosum*. In 1832, Don classified *Triptilion* as a section of *Nassauvia*; however, a year later he accepted the genus (Don, 1833). Crisci (1974) assessed discrete geographic and morphological characters and concluded that *Triptilion* and *Nassauvia* are closely related. Later Crisci (1980) used the same morphological data to produce a phylogenetic hypothesis of Mutisieae subtribe Nassauviinae. Character polarity was determined based on several criteria and trees were constructed using a Wagner tree algorithm that indicated that *Nassauvia, Triptilion* and *Calopappus* are closely related. Cabrera (1982), on the basis of the smaller number of species, the perennial habit of some *Triptilion* species and presence of characters diagnostic of certain groups of *Nassauvia,* suggested that *Triptilion* was derived from early diverging species of the section *Panargyrum* of *Nassauvia.* A more recent cladistic analysis of morphological data proposes that *Nassauvia/Calopappus* is the sister group of *Triptilion* (Freire et al., 1993).

Kim et al. (2002) used one species of *Triptilion* (*T. spinosum*) and three of *Nassauvia* (*N. digitata, N. gaudichaudii, N. lagascae*)

Table 1 Collection localities, herbarium voucher numbers and GenBank accession numbers of taxa

Taxa	Life form	Localities		Collection No.	Source material	GenBank No.			
		Latitude °S	Longitude °W			ETS	ITS	*trnL-F*	*rpl32-trnL*
Representatives of the genus *Triptilion*									
T. achilleae DC.	AH	38°40′	71°05′	SGO 160727	F	MG432218	MG432152	MG432174	MG432196
T. benaventii J.Rémy.	PH	37°43′	73°02′	CONC 103120	H		MG432153	MG432175	MG432197
T. berteroi Phil.	AH	34°14′	70°25′	SGO 161138	F	MG432219	MG432154	MG432176	MG432198
T. capillatum Hook. & Arn.	AH	33°00′	70°38′	SGO 161134	F	MG432220	MG432155	MG432177	MG432199
T. cordifolium Lag.	AH	33°23′	71°15′	SGO 161139	F	MG432221	MG432156	MG432178	MG432200
T. gibbosum J.Rémy.	AH	29°38′	71°17′	SGO 160726	F	MG432222	MG432157	MG432179	MG432201
T. spinosum Ruiz & Pav.	PH	33°43′	70°28′	SGO 161136	F	MG432223	MG432158	MG432180	MG432202
Representatives of genus *Nassauvia*									
N. aculeata Poepp. & Endl.	PH	38°33′	71°11′	SGO 159014	F	MG432224	MG432159	MG432181	MG432203
N. argentea Phil.	PH	38°41′	71°48′	CONC 150130	H	MG432225	MG432160	MG432182	MG432204
N. argyrophylla Speg. ex Hosseus	PH				GB		EU239264	EF530292	
N. axillaris D.Don	PH				GB		EU239273	EF530278	KM978459
N. chubutensis Speg.	PH				GB		EU239268		
N. cumingii Hook. & Arn.	PH	38°32′	71°23′	SGO 160729	F	MG432226	MG432161	MG432183	MG432205
N. darwinii O.Hoffm. & Dusén ex Dusén	PH	50°43′	72°23′	CONC 93081	H/GB		EU239283		MG581284
N. dentata Griseb.	PH	37°53′	71°23′	CONC 160266	H	MG432227	MG432162	MG432184	MG432206
N. digitata Wedd.	PH	36°54′	71°24′	CONC 150097	H	MG432228	MG432163	MG432185	MG432207
N. dusenii O.Hoffm.	PH	47°31′	72°57′	CONC 169230	H	MG432229	MG432164	MG432186	MG432208
N. fuegiana (Speg.) Cabrera	PH				GB		EU239272		
N. glomerata Wedd.	PH	35°59′	70°24′	CONC 132473	H	MG432230	MG432165	MG432187	
N. glomerulosa D.Don	PH	40°44′	67°56′	CONC 73156	H/GB		EU239280		MG432209
N. juniperina Skottsb.	PH				GB		EU239271		
N. lagascae F.Meigen	PH	38°22′	71°03′	SGO 160728	F	MG432231	MG432166	MG432188	MG432210
N. lagascae F.Meigen*	PH				GB		EU239288		FJ979736
N. looseri Cabrera	PH	39°32′	70°23′	SGO 160733	F	MG432232	MG432167	MG432189	MG432211
N. magellanica J.F.Gmel.	PH	54°08′	68°42′	CONC 142119	H	MG432233	MG432168	MG432190	MG432212
N. pinnigera D.Don	PH	38°12′	71°13′	SGO 161133	F	MG432234	MG432169	MG432191	MG432213
N. planifolia Wedd.	PH				GB		EU239281		
N. pulcherrima Cabrera	PH				GB		EU239285		
N. pygmaea Hook.f.	PH	34°42′	71°15′	SGO 161131	F	MG432235	MG432170	MG432192	MG432214
N. pyramidalis Meyen	PH	33°43′	70°29′	CONC 173824	H	MG432236	MG432171	MG432193	MG432215
N. revoluta D.Don	PH	36°01′	70°33′	CONC 167704	H	MG432237	MG432172	MG432194	MG432216
N. ulicina Macloskie	PH				GB		EU239279	EF530291	
N. uniflora Hauman	PH	33°20′	70°17′	CONC 170498	H	MG432238	MG432173	MG432195	MG432217
Representatives of the Nassauvieae tribe									
Calopappus acerosus Meyen	PH	33°21′	70°15′	SGO 160730	F/GB	MG581281	KY010360	KY223737	KY223786
Calorezia nutans (Less.) Panero	PH	36°30′	71°11′	CONC 164923	H/GB	MG581282	KY009609	KY210140	KY223781
Pleocarphus revolutus D.Don	PH				GB		KY006585	KY210141	
Representative of the family Calyceraceae									
Nastanthus scapigerus Miers	PH	38°48′	70°14′	SGO 161140	F/GB	MG581283	KY009608	KY223732	KY223779

AH, annual herbs; PH, perennials herbs; Source of material: H, herbarium; F, field; GB, GenBank. *sequence downloaded from GenBank.

in a study with plastid genes of the tribe Mutisieae and concluded that *Triptilion* should be included in *Nassauvia*. This concurs with the opinion of Don (1832). Katinas et al. (2008a) analyzed subtribe Nassauviinae using nuclear and plastid genes to study the evolution of secondary flower heads. This study which considered one species of *Triptilion* (*T. capillatum*) and four of *Nassauvia* (*N. argyrophylla, N. axillaris, N. revoluta, N. ulicina*), using Maximum Parsimony, concluded that *Triptilion* forms a polytomy with *Nassauvia*; with Maximum Likelihood it was sister to *Nassauvia*. A phylogenetic study of the genus *Perezia* by Simpson et al. (2009) using nuclear and plastid genes also included five representatives of *Nassauvia* (*N. aculeata*,
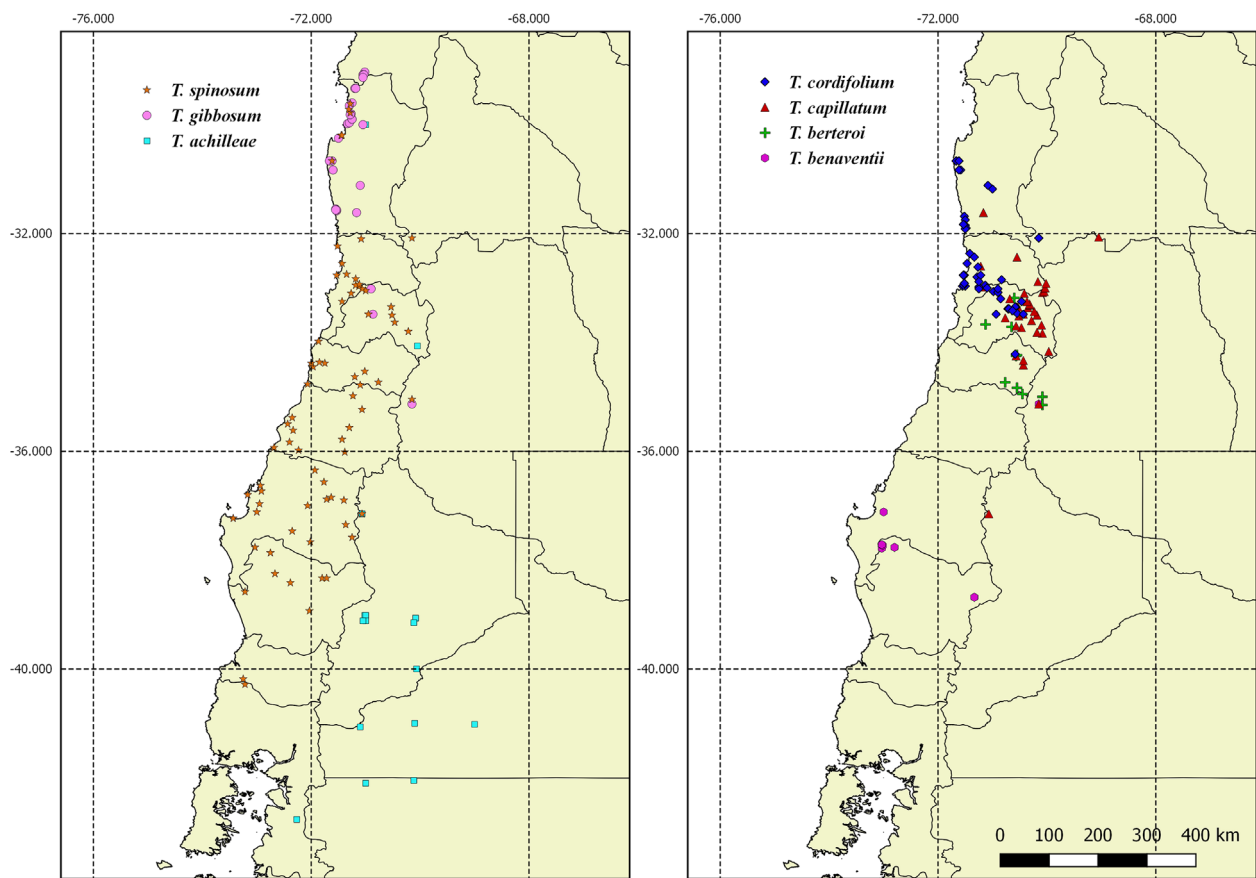
Fig. 1. The distribution of *Triptilion* in Chile-Argentina obtained from herbarium specimens (SGO, CONC) and Global Biodiversity Information Facility (GBIF). Annual herbs: *T. achilleae, T. berteroi, T. capillatum, T. cordifolium, T. gibbosum*; perennial herbs: *T. benaventii, T. spinosum.*

N. digitata, N. heterophylla, N. lagascae, N. pinnigera) and one species of *Triptilion* (*T. spinosum*); these authors proposed that *Nassauvia* is paraphyletic with respect to *Triptilion* and that the latter should be included within *Nassauvia*. In 2012, Maraner et al. published an ITS phylogeny of the genus *Nassauvia* that included 23 species of *Nassauvia* and a single species of *Triptilion* (*T. spinosum*). They concluded that *Triptilion* should be synonymized under *Nassauvia*. Although these studies showed that there is a very close relation between *Triptilion* and *Nassauvia*, in all cases only one species of *Triptilion* was considered.

Cabrera (1982) suggested that *Nassauvia* would be the ancestor of *Triptilion*. Since all species of the *Nassauvia* are perennial, the annual species of the *Triptilion* would be derived. It has been suggested that annual species of the genus *Bellis* (Asteraceae) are derived from perennial ancestors (Fiz et al., 2002). A recent family-wide analysis show that woody habitat has been derived from the herbaceous habit on several occasions in Asteraceae (Beaulieu et al., 2013) showing high life-form lability. Thus, it is interesting to ask whether the annual habit in *Triptilion* is derived.

In order to resolve relationships within the genus *Triptilion*, divergence times and trace the evolution of the annual habit in *Triptilion*, phylogenetic reconstruction was undertaken using nuclear and plastid genes. Our study includes all species of *Triptilion* and 66% of the species of *Nassauvia* representing the two subgenera and all four sections recognized by Cabrera (1982).

2 Material and Methods

2.1 Taxon sampling

DNA for *Triptilion* and species of *Nassauvia* was obtained from leaf material of individuals collected in the field and from herbarium material stored CONC (Herbarium of the Department Botany, University of Concepción) and SGO (Herbarium of the National Museum of Natural History). Samples were stored in silica gel. Vouchers for field-collected material are deposited in the herbaria CONC and SGO. We downloaded sequences for some species from GenBank (NCBI; Table 1). All *Triptilion* taxa were sampled. We also sampled 28 representatives of the tribe Nassauvieae including 25 species of *Nassauvia*, *Calopappus acerosus* Meyen, *Calorezia nutans* (Less.) Panero, *Pleocarphus revolutus* D.Don and *Nastanthus scapigerus* (J.Rémy) Miers that belongs to Calyceraceae, the sister group of the Asteraceae according to molecular studies (Tank et al., 2015; Barker et al., 2016; Huang et al., 2016; Panero & Crozier, 2016). Initially we used more than one individual per species to verify their positions in the topology.

2.2 DNA extraction, amplification, and sequencing

Genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen, Valencia CA, USA). We amplified the DNA using plastid and nuclear genes. The PCR reaction was prepared to a

total volume of 30 μL, which contained 4 μL DNA (100 ng), 8.35 μL distilled water, 3 μL MgCl$_2$ (25 mmol/L), 6 μL buffer, 2.4 μL of 2.5 mmol/L dNTP mixture, 1.8 μL of each primer (10 X), 2.4 μL BSA and 0.25 μL Taq polymerase (1 U μL^{-1}). DNA was denatured at 95 °C for 5 min, followed by 35 cycles of 45 s at 94 °C, annealing for 1 min at 50–58 °C, elongation for 1 min 30 s at 72 °C, for *rpl32-trnL* temperature change of 0.3 degree per second to the elongation temperature, and a final extension of 7 min at 72 °C. Samples were sent to Macrogen (Seoul, South Korea) for purification and sequencing. Sequences were loaded, edited and aligned using ChromasPro 2.64 (1996–1998) (Technelysium, Brisbane, Australia) and BioEdit 7.2.6.1 (Hall, 1999), and have been deposited in GenBank (Table 1).

2.3 Phylogenetic analysis, reconstruction of life history and divergence times

We performed an analysis of total sequence evidence obtained from the rDNA ITS (White et al., 1990), ETS (Linder et al., 2000) and cpDNA intron and intergenic spacer regions *trnL-F* (Taberlet et al., 1991), *rpl32-trnL* (Shaw et al., 2007). Bayesian Inference analyses were performed with MrBayes 3.2 (Ronquist et al., 2012). For the combined analysis with Bayesian Inference, four partitions were used corresponding to evolutionary models as follows: GTR+G in ITS; GTR+I+G in ETS, GTR+I in *trnL-F*; and GTR+I+G in *rpl32-trnL* selected using the Akaike information criterion in MrModeltest 2.2 (Nylander, 2004). The Tracer program v1.6 (Rambaut et al., 2014) was used to visualize output parameters in order to prove stationarity and assess convergence of duplicated runs on the same mean likelihood. Runs appeared stationary prior to 20^6 generations, and we conservatively excluded the first 2.0×10^6 generations of each run as burn-in. Nodes with ≥0.95 were considered to be supported for posterior probabilities (Ronquist et al., 2012). Life form reconstruction was performed on the combined Bayesian tree using Parsimony and Maximum likelihood methods using Mesquite 3.2 (Maddison & Maddison, 2017). Life form character was scored as annual or perennial according to the criteria of Katinas et al. (1992). The divergence times were calculated using the calibration of Panero & Crozier (2016) whose study was based on fossils of the Asteraceae. We used BEAST 1.4.8 (Drummond & Rambaut, 2007), assuming a relaxed molecular clock.

3 Results

3.1 Phylogenetic analyses

The total evidence matrix for the 36 taxa included 4363 nucleotide characters (808 ITS, 1486 ETS, 936 *trnL-F* and 1133 *rpl32-trnL*). The Effective Sample Size (ESS) value was greater than 200 in a range between 14 452 and 35 488. The topology of the Bayesian inference tree shows that the genus *Triptilion* is paraphyletic, because *Nassauvia lagascae*, the only representative of *Nassauvia* section *Caloptilium,* is part of the genus *Triptilion.* There are three highly supported clades in *Triptilion*: Clade I comprises two species, *T. achilleae* and *N. lagascae* (two individuals were used to verify the position of *N. lagascae*; one sequence from GenBank and the other from a sample collected in the field and extracted in our laboratory); Clade II is composed of *T. cordifolium* and *T. gibbosum* and

Clade III is composed of *T. benaventii, T. berteroi, T. capillatum, T. spinosum* (Fig. 2).

3.2 Ancestral reconstruction

The results of the life history reconstructions were similar with Parsimony and Maximum likelihood. These analyses indicated that the common ancestor of *Triptilion* and *Nassauvia* was perennial with the annual habit in *Triptilion* being derived. The life-form of the common ancestor of *Triptilion* was ambiguous, that is, it may have been annual or perennial. The life-form of the common ancestor of *N. lagascae* and *T. achilleae* (Clade I) was also ambiguous. The ancestral state of Clade II, (*T. cordifolium* and *T. gibbosum*) tended toward the annual habit in the case of Maximum likelihood and annual for Parsimony Clade III composed of annuals (*T. berteroi* and *T. capillatum*) and perennials (*T. benaventii* and *T. spinosum*) is ambiguous for Maximum likelihood and annual for Parsimony, showing in this case, a reversion to the perennial life form (Fig. 3).

3.3 Divergence times

The divergence time analysis placed the separation of *Nassauvia* and *Triptilion* in the early Miocene (22.89 Ma, 31.17–16.12 Ma 95% HPD), along with the separation of Clade I from the rest of *Triptilion* (22.54 Ma, 30.81–16.06 Ma 95% HPD). The separation of Clades II and III occurred in the middle Miocene (18.66 Ma, 25.5–13.22 Ma 95% HPD). The reversion of the annual to the perennial habit took place in the late Miocene (5.83 Ma, 8.57–3.54 Ma 95% HPD; Fig. 2).

4 Discussion

Previous molecular studies show *Triptilion* to be either sister to or derived from *Nassauvia,* and the two genera collectively closely related to *Calopappus, Pamphalea,* and *Perezia* (Katinas et al., 2008a; Simpson et al., 2009; Maraner et al., 2012). Taxa in this clade are endemic to South America and like most Nassauvieae, have homogamous capitula with bilabiate white or pink to blue, rarely yellow corollas. Most species are perennial herbs or prostrate shrubs but *Pamphalea* and *Triptilion* are mostly composed of annual species. *Perezia* and *Pamphalea* are the only genera found in subtropical/tropical eastern South America whereas *Calopappus, Nassauvia* and *Triptilion* are Andean with some species also found in arid areas of eastern Patagonia. Based on the present work the sister genus of *Triptilion* is *Nassauvia,* as originally suggested by Crisci (1980) and Freire et al. (1993). The relationship of *Triptilion* as sister group of *Nassauvia* was also established by Katinas et al. (2008a). Kim et al. (2002), Simpson et al. (2009) and Maraner et al. (2012), conclude that *Triptilion* should be included in *Nassauvia*. It must be emphasized that all these studies used only one species of *Triptilion*; this is a potential problem since using only one species as representative of a genus may bias phylogenetic relationships. Also, if the genera are closely related and belong to the same tribe, they will tend to group and be separated from the genera of other tribes.

Pappus characteristics were used by Katinas et al. (2008b) to separate *Nassauvia* and *Triptilion*. In *Triptilion* the pappus is deciduous, suggesting loss of anemophilous dispersion. In *Nassauvia* a deciduous pappus occurs in some species, but not
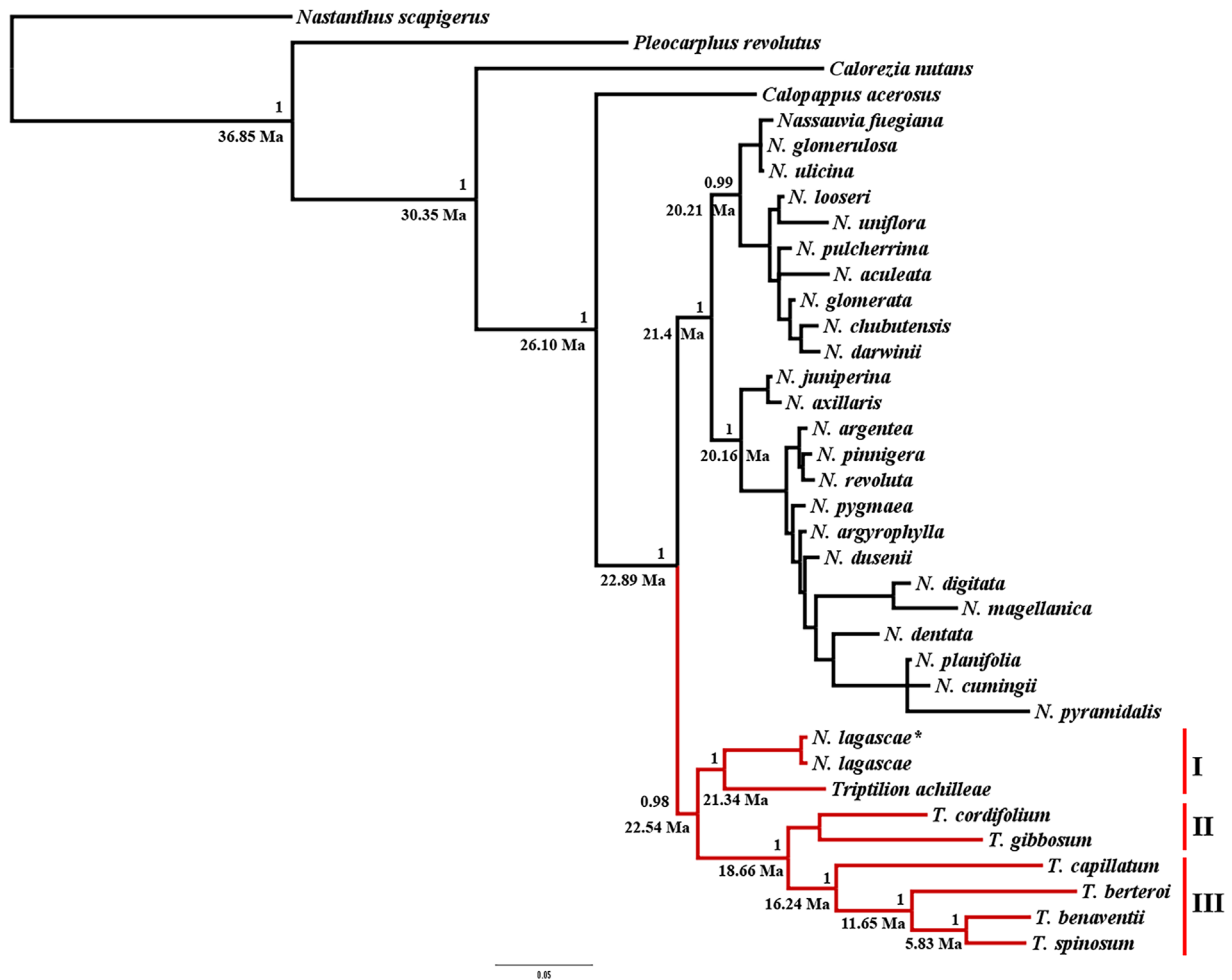
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J. Syst. Evol. 56 (2): 120–128, 2018

Fig. 2. Bayesian inference phylogenetic tree based on combined analysis (rDNA and cpDNA) for the genus *Triptilion*. Posterior probability values are found above the branches. Estimation of divergence times (millions of years, Ma) obtained with Bayesian statistics are found below the branches. The principal clades are indicated by roman numerals (I, II, III). Red lines indicate species of *Triptilion* and *Nassauvia lagascae*, and black lines indicate other taxa. *N. lagascae**, sequence downloaded from GenBank; *N. lagascae*, sequence obtained in this study.

in *Nassauvia lagascae.* In *Triptilion* the pappus has 3 to 5 lanceolate paleae, whereas in *Nassauvia* it is very variable, ranging from 3 to 15 flat paleae or feathery/ciliated bristles (Cabrera, 1982). Cabrera placed *N. lagascae* in the monotypic section *Caloptilum* because it differs from other *Nassauvia* in having a pappus with numerous, generally persistent, feathery bristles and ovate-spatulate leaves with very conspicuous parallel ribs. Our study shows that the relationship between *N. lagascae* and *T. achilleae* is strongly supported (Clado I). The relationship of *N. lagascae* to the genus *Triptilion* was also evident in the molecular study of Maraner et al. (2012) with the ITS gene, which showed that the only representative of *Triptilion* used in the study (*T. spinosum*) grouped with *N. lagascae*, and that these two species were separated from the rest of *Nassauvia*. This agrees with the results of the present study, although we used all the species of *Triptilion*. These results suggest transferring *N. lagascae* to *Triptilion*, however, we recommend first a phylogenetic study that analyzes the relation of *Triptilion* and *Nassauvia* using all species of both genera, to determine the relations with and

between these genera. In addition, a thorough morphological analysis should be performed to determine differences between them. Phylogenetic analyses of all the species with the genes used in this study would allow concluding that *Triptilion* is a paraphyletic group and that *N. lagascae* belongs in Clade I of *Triptilion*.

The annual species *T. achilleae* (Clade I) has characteristics that separate it morphologically from the other annuals of the genus *Triptilion*: (a) external bracts shorter than the internal ones, while the other annual species have equal bracts; (b) external bracts entire, while the other species have entire and divided external bracts; (c) floral leaves similar to the cauline leaves, while they are different for the other annuals except for *T. cordifolium*, whose leaves are similar to those of *T. achilleae*; and (d) inflorescence a pseudoracime or pseudopanicle *versus* pseudocorymb, except for *T. cordifolium* which is similar to *T. achilleae* (Cabrera, 1982; Katinas et al., 1992).

The Clade II (*T. cordifolium* and *T. gibbosum*) separates from Clade III and has different morphological characteristics, such
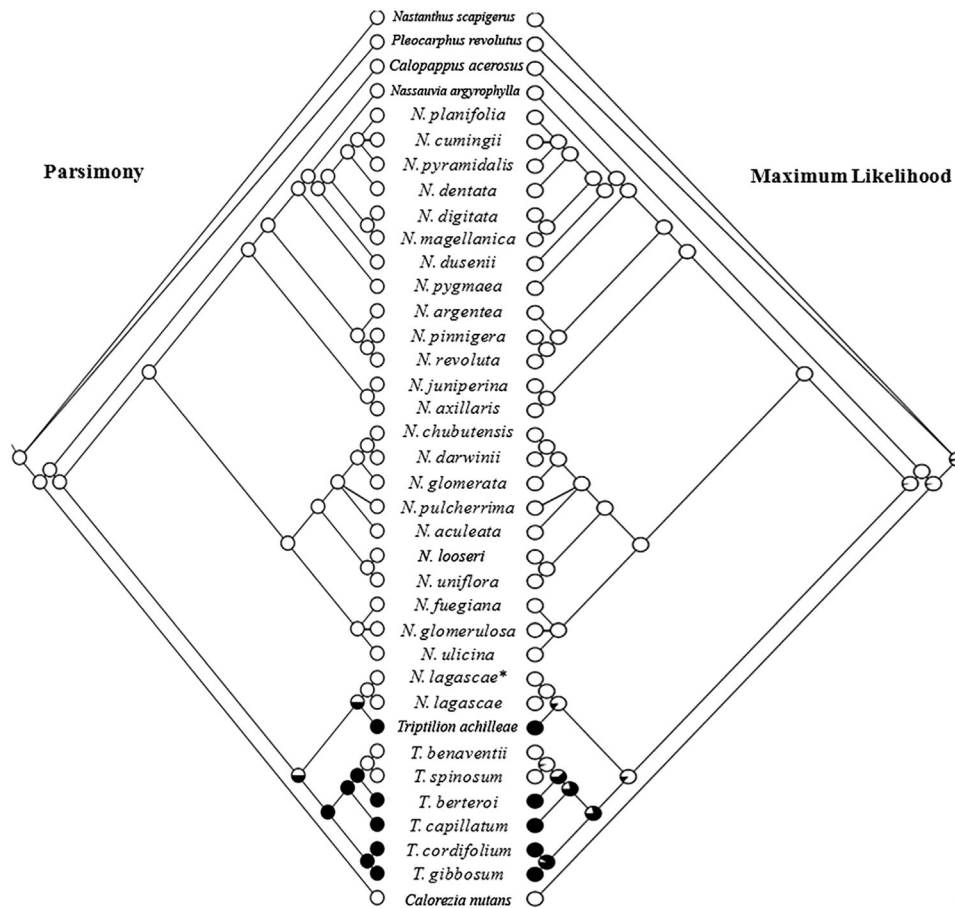
Fig. 3. Ancestral life form reconstruction for *Triptilion* on the Bayesian tree using Parsimony (left) and Maximum Likelihood (right). Inferred perennial character state shown in white circles, annual character state in black circles.

as cauline leaves glabrous to weakly pubescent and achenes weakly pubescent at maturity. The perennial species in Clade III are the only perennial species of the genus; they have basal leaves in a complete rosette, while the rest have lax rosettes; they have a pubescent receptacle, while the others have a glabrous receptacle; and they have glabrous achenes while the others are pubescent (Cabrera, 1982; Katinas et al., 1992).

Our reconstruction of the life-history character indicated that the ancestor of the *Triptilion-Nassauvia* clade was perennial and that the annual habit is derived. *Nassauvia lagascae* and *T. achilleae* are early diverging branches of the phylogeny that inhabit medium to high elevation zones in the Chilean Andes. *Nassauvia lagascae* is a perennial herb found in the high mountains from San Juan to Neuquén in Argentina and from Aconcagua to Maule in Chile. We suggest that *Triptilion* colonized the Andes then descended and diversified at lower elevations. This pattern has been observed in other taxa such as *Chaetanthera* (Hershkovitz et al., 2006), *Leucocoryne* (Jara-Arancio et al., 2014) and the *Malesherbiaceae* (Gengler-Nowak, 2002). This same perennial to annual polarity has been found in studies with nuclear genes in *Scorzoneroides* - Asteraceae (Cruz-Mazo et al., 2009), *Nemesia* - Scrophulariaceae (Datson et al., 2008) and *Leucheria* (Jara-Arancio et al., 2017).

Desert and Mediterranean zones favor annual species that avoid drought by combining a relatively short life cycle and a

high growth rate during the rainy season (Rivero et al., 2007). It has been suggested that the annual life cycle evolves frequently in desert environments due to its resistance to the inter-annual variation in precipitation and by having seeds as its only source of annual renewal (Schaffer & Gadgil, 1975). Arroyo et al. (1995) showed that the frequency of annual species in the Mediterranean climate of central Chile increases with the degree of summer aridity, reaching nearly 25% of the species in the extreme north. It has been hypothesized that the annual cycle could arise in species as a response to desert environments and those with high coefficient of variation in annual precipitation (Evans et al., 2005), as in the case of *Oenothera* that is associated with the coefficient of variation of winter precipitation (Evans et al., 2005). Perhaps the evolution of the annual habit at or near the origin of *Triptilion* was a response to arid conditions, and the later reversion to perennial was a response to an increasingly humid climate. Biologists thought for a long time that annual species were derived from perennial species and that this change was unidirectional (Stebbins, 1957). However, phylogenetic reconstructions using molecular data have shown that sometimes the change is from annual to perennial. For example, Bena et al. (1998) showed that the perennial species of *Medicago* were derived from an annual lineage, and Tank & Olmstead (2008) showed that the perennial clade of *Castilleja*

(Orobanchaceae) with 160 species was derived from an annual ancestor, indicating that the perennial-annual transition may occur in both directions as appears to have happened in *Triptilion*.

The divergence times allow tracing the evolution of the genera *Triptilion-Nassauvia* in relation to the climate changes that occurred in southern South America during the Tertiary. An overall understanding needs consideration of the origin of the family Asteraceae. Funk et al. (2005) proposed that the separation of the Asteraceae-Calycereae and Goodeniaceae lineages occurred when South America separated from Australia-Antarctic, 60–66 Ma. According to these authors, the later separation of the most ancient subfamily of the Asteraceae, the Barnadesioideae, occurred in South America, followed by an explosive radiation along the incipient Andes Range. This suggests that the ancestral group of Asteraceae may have been part of the subtropical paleoflora of South America in the early Eocene (Hinojosa & Villagrán, 1997). Kim et al. (2005) estimated that the Asteraceae originated in the middle Eocene (42–47 Ma). Barreda et al. (2008) found fossils in the lower Miocene which indicates that the basal members of the Asteraceae, the subfamilies Barnadesiodeae (*Chuquiraga, Doniophyton, Fuseniella* and *Dasyphyllum*) and Mutisioideae had already differentiated by then. In a later study, Barreda et al. (2010) described a fossil of the Asteraceae that suggests the existence of the family at 50 Ma. They discounted the fossil belonging to the subfamily Barnadesioideae, and suggested that it belongs to the subfamily Mutisioideae or the Carduoideae. The date of this fossil led the authors to postulate that the Asteraceae may have been part of the Gondwana subtropical flora during the Eocene, thus existing before the separation of the continents. Barreda et al. (2015) estimated the origin of the Asteraceae at 76–66 Ma, while Panero & Crozier (2016) estimated the divergence of the families Calyceraceae and Asteraceae at 69.5 Ma, and suggested that the 13 subfamilies of the Asteraceae originated between the Paleocene and the Eocene (64.75–43.2 Ma).

The results obtained in this study suggest that the separation of *Nassauvia* and *Triptilion* occurred in the lower Miocene (22.89 Ma), and the separation of Clade I from the rest of *Triptilion* at 22.54 Ma. A warming tendency which began at 27 Ma reduced the extension of ice in the Antarctic, reaching its maximum at the end of the middle Miocene (17–15 Ma) (Zachos et al., 2001). Later Clades II and III separated in the middle Miocene (18.66 Ma), with the reversion of the annual life-form to the perennial life-form in *Triptilion* occurring in the upper Miocene (5.83 Ma). These dates correspond to the cooling after the climatic optimum of the middle Miocene, and with the uplifting of the Andes, which according to Blisniuk et al. (2005) by 16.5 Ma would have formed a rain shadow sufficient to generate an arid climate in Patagonia (see also Iglesias et al., 2011). The proto-Mediterranean climate of Central Chile was generated by the rain shadow effect of the rising Andes mountains in the early Miocene and the decrease in temperature toward the end of the Miocene (Rundel et al., 2016). The development of aridity in Patagonia on the eastern side of the Andes and the formation of the Mediterranean climate in central Chile on the western side led to more open vegetation, producing an explosive diversification of the Asteraceae and diversification in life-forms.

Acknowledgements

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