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On the taxonomic position of *Tanacetum funkii* (Anthemideae, Compositae)

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Abstract

Oberprieler, C. & Vogt, R. 2016. On the taxonomic position of *Tanacetum funkii* (Anthemideae, Compositae). *Anales Jard. Bot. Madrid* 73(2): e046.

In order to clarify the taxonomic position of the enigmatic SE Spanish endemic *Tanacetum funkii* Sch. Bip. ex Willk., a phylogenetic analysis based on nrDNA ITS sequence variation of representatives of Anthemideae (Compositae) was carried out together with morphological analyses of the type material. The observation of nearly identical (1 bp difference) sequences of ITS1 and ITS2 in *T. funkii* and *Anthemis cotula* L., along with the joint possession of a conical receptacle and subulate receptacular scales, argue for the conspecificity of these two taxa. As a consequence, *T. funkii* is transferred to the genus *Anthemis* L. and placed in the synonymy of *Anthemis cotula*, and a lectotype is designated.

Keywords: *Anthemis*, Asteraceae, Nomenclature, nrDNA ITS, Taxonomy.

Resumen

Oberprieler, C. & Vogt, R. 2016. Acerca de la posición taxonómica de *Tanacetum funkii* (Anthemideae, Compositae). *Anales Jard. Bot. Madrid* 73(2): e046.

Con el fin de aclarar la posición taxonómica del enigmático endemismo del sureste español *Tanacetum funkii* Sch. Bip. ex Willk., se ha llevado a cabo un análisis filogenético basado en la variación de secuencias ITS nrDNA de representantes de las Anthemideae (Compositae) junto con un análisis morfológico del material tipo. La observación de secuencias casi idénticas (diferencia de 1 pb) de ITS1 e ITS2 en *T. funkii* y *Anthemis cotula* L., junto con la posesión de un receptáculo cónico y unas escamas interflorales subuladas, aboga por la coespecificidad de estos dos táxones. Como resultado, *T. funkii* se ha transferido al género *Anthemis* L. y se ha colocado en la sinonimia de *Anthemis cotula*, a la vez que se ha designado un lectotipo.

Palabras clave: *Anthemis*, Asteraceae, nomenclatura, nrDNA ITS, taxonomía.

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INTRODUCTION

Tanacetum funkii Sch. Bip. ex Willk. was described by Willkomm (1865) based on plant material from the Sierra Nevada in SE Spain collected by Michael Funk, a physician from Bamberg in Germany, during a journey to Andalusia in 1848. The collections of Funk are owned by the Natural History Museum in Bamberg but today incorporated in the herbarium of the Botanische Staatssammlung in Munich (M) as a permanent loan. Duplicates of *T. funkii* from Funk's collection were acquired in Paris (P) with the herbarium of Carl Heinrich Schultz (-Bipontinus) and in Coimbra (COI) with the herbarium of Moriz Willkomm.

The specimens in Funk's herbarium were first studied by Schultz in 1850. He recognized the new taxon by adding handwritten labels with the name *Tanacetum funkii* that was subsequently validated by Willkomm (1865) in the second volume of the *Prodromus Florae Hispanicae*. The species was characterised as a much-branched, pubescent annual herb with very small solitary capitula at the end of branches. The 15-20 cm long stems bear pinnatipartite to bipinnatisect leaves with linear-lanceolate, mucronate segments, and shortly pedunculate capitula with pale scariously bordered involucre bracts. Shape and morphology of the achenes are unknown.

To the present day *T. funkii* has not been collected and due to the lack of additional materials showing further

relevant characters, like flowers and fruits, its taxonomic position remains unclear. Even the study of the syntype specimen kept in the herbarium of Coimbra (COI) by Blanca & al. (2004) did not yield any additional information. Referring to this issue and the need of further investigation, *T. funkii* is mentioned in all European and regional botanical inventories, e.g., in *Flora Europaea* (Heywood, 1976), the *Flora of Spain and the Balearic Islands* (Smythies, 1984), *La Flora de Sierra Nevada* (Molero-Mesa & Pérez Raya, 1987), the *Flora Amenazada y Endémica de Sierra Nevada* (Blanca & al., 2004), and the recently published *Flora Vascular de Andalucía Oriental* (Blanca & al., 2009, 2011).

In the 1997 IUCN *Red List of Threatened Plants* (Walter & Gillett, 1998), *T. funkii* was classified in category "I" (indeterminate) due to the lack of information. According to Blanca & al. (2004), the intense search for *T. funkii* in the course of the LIFE project "Recuperación de áreas con flora amenazada de Sierra Nevada" failed and additionally the habitat conditions of the classical localities were considered as heavily degraded by agricultural use. This resulted in the classification of *T. funkii* in the IUCN category "EX" (extinct) in the *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (Blanca & al., 2004) and subsequently in the *Lista Roja 2008 de la flora vascular española* (Bañares & al., 2008).

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In the course of molecular phylogenetic studies of the tribe Anthemideae Cass. in general (Oberprieler & al., 2006, 2007, 2009) and of the genus *Tanacetum* L. (Sonboli & Oberprieler, 2010, 2012; Sonboli & al., 2012) in particular, we also came across *T. funkii*, which was listed by Bremer & Humphries (1993) in their generic monograph of the tribe and continued to be assigned to the genus until present (Euro+Med, 2015; Aedo & al., 2015). Morphological studies carried out on the type material of *T. funkii* gave rise to considerable doubts concerning the generic position of this species and motivated the present study for determining the taxonomic position of *T. funkii* within the tribe Anthemideae.

MATERIAL AND METHODS

DNA was extracted from the type specimen of *T. funkii* (M 0032588) by using the CTAB DNA extraction protocol described by Doyle & Doyle (1987) and Doyle & Dickson (1987). PCR amplification of nrDNA ITS regions was carried out with primers 18Sf (Rydin & al., 2004) and P2Br (White, 1990) for ITS1 and P3f (White, 1990) and 28Sr (Rydin & al., 2004) for ITS2 utilising the *Taq* RED Polymerase (Ampliqon A/S, Odense, Denmark) according to the manufacturer's suggestions. Sanger sequencing was done with the CEQ DTCS Quick Start Kit (Beckman-Coulter, Krefeld, Germany). Electropherograms were checked for base call errors using Chromas Lite v2.0 (Technelysium Pty Ltd, South Brisbane, Australia) and the newly established sequences for *T. funkii* were combined with nrDNA ITS sequences from previous phylogenetic analysis in the Anthemideae (i.e., Oberprieler & Vogt, 2000; Oberprieler, 2001, 2004a, 2004b; Oberprieler & al., 2007; Lo Presti & Oberprieler, 2009; Sonboli & al., 2012) into an alignment using BioEdit v7.2.5 (Hall, 1999). Gaps were coded as binary characters using the simple gap coding method of Simmons & Ochoterena (2000) implemented in the software programme GapCoder (Young & Healy, 2003). This resulted in a combined data set that was divided into two partitions (i.e., nrDNA ITS sequence data, coded gaps of nrDNA ITS).

The program Modeltest v3.06 (Posada & Crandall, 1998) was used to find the model (among the 56 models tested) that best fits the underlying sequence information according to the Akaike Information Criterion (AIC). This resulted in the acceptance of a General Time-Reversible (GTR) model with a parameter describing the proportion of invariable sites and a gamma distribution of substitution rates over the sites (GTR+I+ Γ), the base frequencies being freqA = 0.2340, freqC = 0.2283, freqG = 0.2461, and freqT = 0.2916, the proportion of invariable sites I = 0.0975, a gamma distribution shape parameter of α = 0.9344 and a substitution rate matrix of R[A-C] = 1.1504, R[A-G] = 2.3286, R[A-T] = 1.5896, R[C-G] = 0.3966, R[C-T] = 5.1490, and R[G-T] = 1.0. Substitution models and rates of substitution were allowed to vary among the parameters ("unlink" command and "ratepr = variable") and a binary model ("Lset coding = variable") was applied to the coded gaps. These parameters were used in a Bayesian inference (BI) approach to phylogeny reconstruction with the software programme MrBayes v3.2.2 (Ronquist & al., 2012). Two runs with eight Metropolis-coupled Markov chain Monte Carlo (MCMC) chains with incremental heating temperature of 0.2 were

run for 5,000,000 generations and sampled every 1,000th generation. The burn-in period was set to 25% of the 5,000 sampled trees and the first 1,250 trees were discarded. Estimation of tree topology and posterior probabilities of clades were based on the remaining 3,750 trees.

RESULTS AND DISCUSSION

In the phylogenetic analysis based on nrDNA ITS sequence variation (fig. 1), *T. funkii* is found in a well-supported monophyletic group (posterior probability PP = 1.0) together with *Anthemis cotula* L. and *Anthemis tigreensis* J. Gay ex A. Rich. While in nrDNA ITS1 we observed two substitutions distinguishing between *T. funkii* and *Anthemis tigreensis*, there is no sequence difference detectable between the former and *Anthemis cotula*. In the nrDNA ITS2 region, a single nucleotide difference between *T. funkii* and *Anthemis cotula* was found (at position 512 of the total alignment), while three indels and four single nucleotide substitutions discriminate between *T. funkii* and *Anthemis tigreensis*. These results overwhelmingly argue for the inclusion of *T. funkii* in the genus *Anthemis* and its close relationship with *Anthemis cotula*; the single nucleotide difference in nrDNA ITS2 (position 512) between the two accessions being easily attributable to a plesiomorphic condition (G) in *T. funkii* and an apomorphic one (A) in the widespread and more likely genetically polymorphic *Anthemis cotula*, for which the accession for the present analysis came from an Italian population [Toscana: Grosseto, Padule di Punta Ala, Romi & Marchetti s.n., 15 June 1992 (Hort. Bot. Siena); cultivated in Hort. Bot. Berol. 103-03-93-10 (B 10 0603656)]. However, the observation made in many molecular phylogenetic studies at or below the species level for the nrDNA ITS marker showing intraspecific variation on the one hand (e.g., Lorenz-Lemke & al., 2005) or interspecific identity on the other hand (e.g., Kolarčik & al., 2010) is a justified argument for not basing taxonomic decisions solely on molecular markers and for considering other sources of evidence like morphology, anatomy, cytology, etc.

With regards to these other sources of evidence, morphologically the close relationship of *T. funkii* and *Anthemis cotula* is especially supported by the presence of linear-subulate receptacular scales (fig. 2a, b) observed in the capitula of the type material of the former species. While receptacular scales are very rarely observed in *Tanacetum* species (Oberprieler & al., 2006), i.e., in *T. paleaceum* Podl. and the former members of genera *Gonospermum* Less. (4 spp.) and *Lugoa* DC. (1 sp.) nested within *Tanacetum* (Sonboli & al., 2012), they are the rule in *Anthemis* representatives (again with some exceptions, i.e., the four species of the genus *Ammanthus* Boiss. & Heldr., nowadays included in *Anthemis*; Lo Presti & al., 2010). However, crossing experiments carried out by Mitsuoka & Ehrendorfer (1972) have shown that the presence/absence of receptacular scales is under oligogenic control in the tribe and, therefore, may be of minor taxonomic significance. Nevertheless, the presence of receptacular scales in *T. funkii* together with its solitary, small, and shortly pedunculated capitula (fig. 3) with conical receptacle (fig. 2a), its short, female ray florets (observed by C. Benedí, pers. comm.), involucre bracts with hyaline margin (fig. 2d), and its finely dissected leaves (fig. 2c) argue for its proximity to *Anthemis cotula* rather than to any *Tanacetum* species. The lack of well-developed tubular

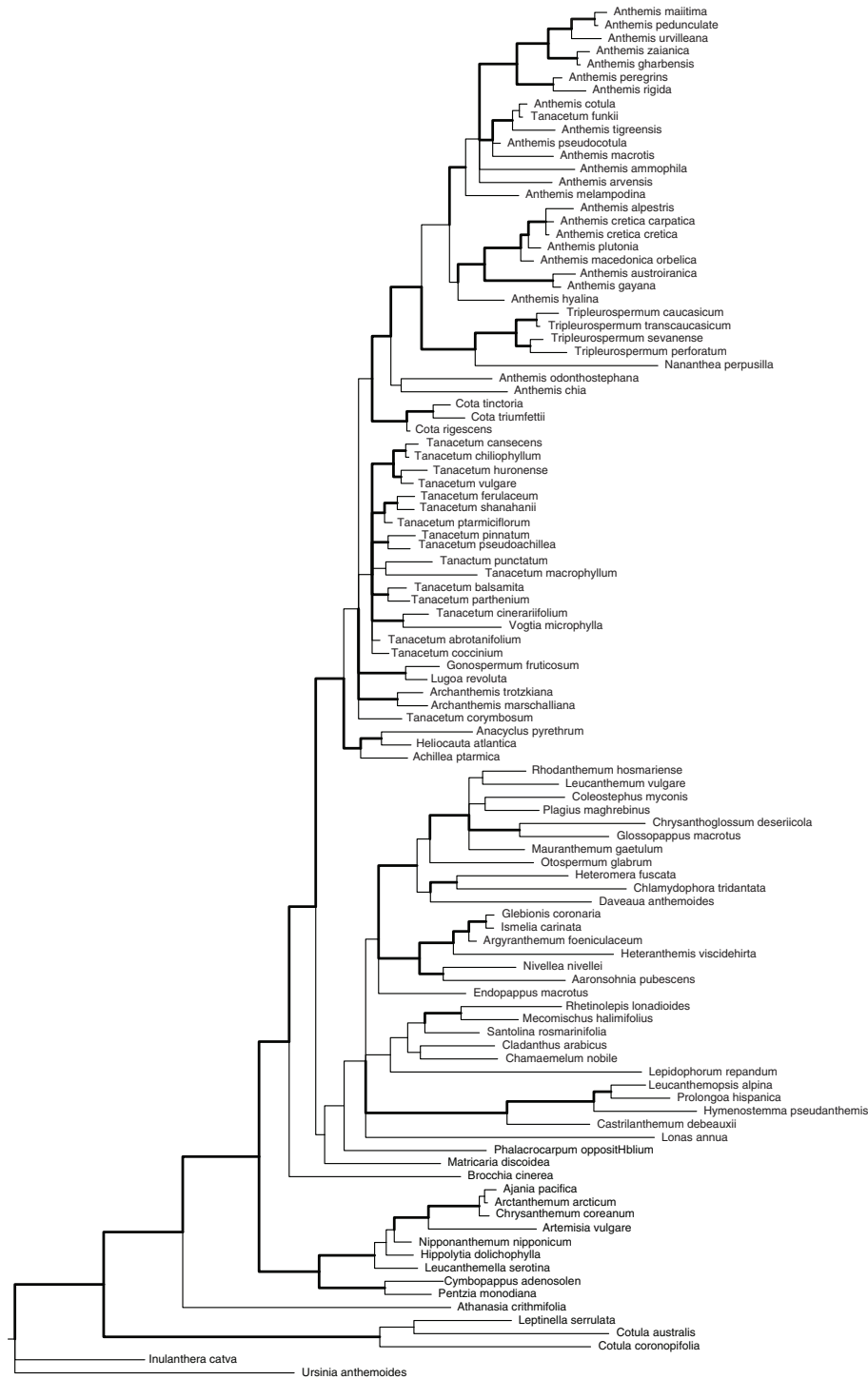


Fig. 1. Phylogenetic tree from a Bayesian analysis of nrDNA ITS sequence data based on the GTR+I+ Γ model of DNA substitution with base frequencies, gamma distribution parameter α , and substitution rate matrix given in the text. Tree topology and posterior probabilities of clades were based on 3,750 trees from a MCMC chain run for 5,000,000 generations. Clades receiving posterior probabilities larger than 0.95 are shown with bold lines.

florets (fig. 2b) and achenes (presumably due to either the late collection date in August and September 1848 or some developmental irregularities) hampers evaluation of further and even more diagnostic characters.

As noted above, *Anthemis cotula* is an annual species of *Anthemis* sect. *Maruta* (Cass.) Griseb. that is wide-spread in the whole Mediterranean and Eurasian region (and

introduced into other continents due to human activities). The species was studied morphologically quite extensively in its distribution range on the Iberian Peninsula by Benedí (1987) who described it as being an annual, foetid *Anthemis* species, with (10)20-35(45) cm long, apically ramified shoots, with 2-3-pinnatisect leaves (1.5)2.5-5.5(6) cm long and (0.5)1-3 cm wide, and having 3-5 mm long and mucronate



Fig. 2. *Tanacetum funkii*: a, receptacle; b, disc floret with receptacular scale; c, leaf; d, involucral bracts [*M. Funk* s.n. (M-0032588)].

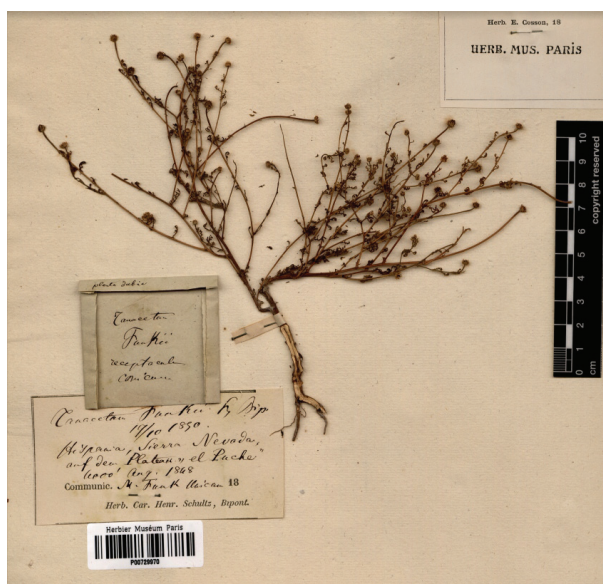


Fig. 3. *Tanacetum funkii*: lectotype (P 00729970).

lobes, and with capitula furnished with white, sterile ray-florets and a conical receptacle equipped with 3.5 mm long and 0.5 mm wide, subulate receptacular scales either in its apical half or throughout (but then caducous in the lower half). In

all these morphological characters, the Iberian populations correspond both to species circumscriptions given in other parts of the distribution range of the species (e.g., Fedorov, 1961; Yavin, 1970; Grierson & Yavin, 1975; Fernandes, 1976) and to the type specimens of *T. funkii*. The lack of well-developed florets and achenes on the type specimens is regrettable but not an impediment to the assumption of conspecificity of *Anthemis cotula* and *T. funkii*, because it appears more conservative and parsimonious, and therefore scientifically sound, to us to base this decision on observable characters than on mere speculations about alleged conditions of missing ones. Therefore, together with the above-mentioned proximity in molecular respects, we feel justified to propose the treatment of *T. funkii* as a synonym of *Anthemis cotula*:

***Anthemis cotula* L., Sp. Pl.: 894 (1753)**

Tanacetum funkii Sch. Bip. ex Willk. in Willk. & Lange, Prodr. Fl. Hispan. 2: 102 (1865), **syn. nov.** *Ind. loc.*: “In Sierra Nevada in jugo argilloso el Puche et prope Cortijo de mimbres ad alt. circ. 3000', FK.! – o Sept. (v.s.)”. TYPE: [Spain. Granada:] Sierra Nevada, auf dem Plateau “el Puche” [37°8'19"N, 3°29'30"W], 4000' [feet], *M. Funk* s.n., Aug. 1848 (lectotype, here designated: P 00729970!, fig. 3; isolectotypes: M 0032589!, COI 00037339 *icon!*).

Additional material: [Spain. Granada:] Sierra Nevada, Cortijo de mimbres [37°7'27"N, 3°28'8"W], *M. Funk* s.n., “Anf. Septbr.”, 1848 (syntype: M0032588!).

The specimens from the locality El Purche (“el Puche”) kept in the herbaria COI and P, and the one from the locality Cortijo de las Mimbres (“Cortijo de mimbres”) in M, bear determination slips in Schultz’s handwriting with the indication “*Tanacetum Funkii*, C. H. Schultz Bip. 19.10.[18]50”. The specimen from the herbarium of Schultz kept in P is selected as lectotype (fig. 3).

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