The taxonomic position of Matricaria macrotis (Compositae-Anthemideae)

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Abstract


Sequences of the nrDNA internal transcribed spacer (ITS) region were analysed for 116 representative species of 59 genera of *Compositae-Anthemideae* to ascertain the taxonomic position of *Matricaria macrotis*, a rare and enigmatic species from the E Aegean. The phylogenetic reconstruction indicates that *M. macrotis* is nested within the genus *Anthemis* s.str. next to members of *A.* sect. *Maruta*. This result is supported by morphological features. The previous inclusion of this species in the genus *Matricaria* was mainly based on the absence of receptacular scales (pales), whereas several characters of indumentum, achene morphology and achene anatomy support its transfer to *Anthemis*. The new combination *Anthemis macrotis* is validated and a lectotype for the basionym designated.

Key words: Asteraceae, Anthemis, systematics, ITS, Aegean, Greece, Turkey.

Introduction

*Matricaria macrotis* Rech. f. was described by Rechinger (1943) based on plant material collected by him on Mt Attaviros on the Greek island of Rhodes. The species is characterised (Rechinger 1943, Grierson in Davis 1975) as an annual appressed-pubescent herb with 5-25 cm long ascending stems bearing 2-pinnatisect leaves and solitary, radiate capitula with obovate involucral bracts furnished with pale brown, scarious margins. The receptacle is conical and devoid of receptacular scales (pales). While the white ray florets are sterile, the 5-lobed, yellow disc florets are hermaphrodite and produce 1.5-2 mm long achenes that are circular in cross-section, inconspicuously 10-ribbed and slightly verrucose. Achenes of the peripheral disc florets are found to bear a 2-2.5 mm long adaxial auricle, while those of the central disc florets show shorter auricles or are completely ecoronate.

The species was presumably observed already near Lindos on Rhodes by Boissieu (1896: 286), who mentioned it as a “*Matricaria* très curieux” because of its characteristic achenes. Referring to this first mention and presumably taking into account the diagnostic lack of pales,
Rechinger (1943) described it as a new species of *Matricaria* without further discussion on the generic classification. He only stated that the new species seems to be not closely related to any other known (*Matricaria*) species and gave some differential characters between the new species and *M. chamomilla* L. (= *M. recuita* L.). This statement was echoed by Grierson (in Davis 1975), who treated it also under the genus *Matricaria*.

In their generic revision of the *Compositae-Anthemideae*, Bremer & Humphries (1993), who had been unable to study specimens of the species, still list *Matricaria macrotis* under the genus *Matricaria* despite the fact that their generic description of the achenes (“slightly dorsiventrally compressed, with 5 mainly adaxially arranged thin ribs sometimes with resin canals, abaxially and on the ribs covered with myxogenic cells”, Bremer & Humphries 1993: 153) is in striking contrast to the details given by Rechinger (1943) and Grierson (in Davis 1975). A few years later Bremer & al. (1996: 263-264) when studying the delimitation between *Matricaria* and *Microcephala* found that *M. macrotis* “differs from other *Matricaria* species by its almost flat receptacles and radiosymmetrical, 10-ribbed fruits”. As a consequence, they regarded “the position of this species within *Matricaria*, and even within the *Matricariinae*, as doubtful” and excluded this species from their further analysis suggesting “a much larger study with sampling from several other genera”.

In the present contribution we provide an analysis based on a broader sampling. During the last eight years we were able to compile a comprehensive data set of nrDNA ITS sequence information for all genera of the Mediterranean and adjacent European regions, where the tribe is represented by 52 genera and around 620 species (Greuter & al. 2003). All unispecific genera are included and the large genera *Achillea*, *Anthemis*, *Cota*, *Tanacetum* and *Tripleurospermum* are represented by numerous species (Oberprieler 2004a-b, 2005). Additionally, the treatment of the tribe in the forthcoming asterid volume of Kubitzki’s *The Families and Genera of Vascular Plants* (Oberprieler & al., in press) led to an evenly intense morphological and anatomical survey of the tribe. Therefore, it is now possible to shed light on the generic position of the enigmatic *Matricaria macrotis*.

The present contribution is dedicated to Prof. Dr W. Greuter on the occasion of his 68th birthday and his retirement from the position as First Director of the Botanic Garden and Botanical Museum Berlin-Dahlem.

**Material and methods**

**Plant material.** – One hundred and sixteen representatives from 59 genera of *Compositae-Anthemideae* were included in the present analysis. Sequence information for most of these taxa came from former publications (see Oberprieler 2004a-b). For *Plagius flosculosus* (L.) Alavi & Heywood and *P. grandis* (L.) Alavi & Heywood see Oberprieler & Vogt (2000). The nrDNA ITS sequences for *Matricaria macrotis* are new to science and came from the specimen Raus 8360 (B): Greece, Saria, Hauptgipfel der Insel (“Monte Grosso”, Pachivouno), 22.5.1983.

**DNA isolation, PCR amplification, sequencing.** – DNA was extracted from the above mentioned herbarium specimen using Qiagen DNeasy Plant Kit (Qiagen). PCR amplification followed the protocols given in Oberprieler & Vogt (2000). Amplification products were purified with a Qiaquick PCR cleaning column and filtration kit (Qiagen) or with Montage PCR Centrifugal Filter Devices (Millipore). Cycle sequencing of purified PCR products was performed using the CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter) and sequences were analysed on a CEQ 8000 automated sequencer (Beckman Coulter). The new nrDNA ITS sequences (ITS1 and ITS2) for *Matricaria macrotis* were submitted to the EMBL sequence data bank (accession number AM176761).

**Sequence alignment.** – Sequences were aligned using CLUSTAL W (Thompson & al. 1994) and the alignment subsequently corrected manually. The alignment is deposited as electronic supplement at http://www.bgbm.org/willdenowia/willd36/oberprieler+vogt.pdf.
Phylogenetic reconstructions. – The data set was analysed with the Maximum Likelihood (ML) method (Felsenstein 1981, Kishino & Hasegawa 1989). Since this method is dependent on assumptions about the process of DNA substitution (a model of DNA evolution), we used the program Modeltest version 3.06 (Posada & Crandall 1998) to find the model (among the 56 models tested) that best fits the underlying sequence information. This resulted in the acceptance of the model of Tamura & Nei (1993) with a gamma distribution of substitution rates over the sites ($\text{TrN + } \Gamma$), the base frequencies being $\text{freqA} = 0.2722$, $\text{freqC} = 0.2014$, $\text{freqG} = 0.2075$ and $\text{freqT} = 0.3188$, a gamma distribution shape parameter $\alpha = 0.6591$ and a substitution rate matrix of $R[\text{A-C}] = R[\text{A-T}] = R[\text{C-G}] = R[\text{G-T}] = 1.0$, $R[\text{A-G}] = 2.3637$ and $R[\text{C-T}] = 4.9726$. Using these parameters, a ML search was performed with Treefinder (Jobb 2004) and bootstrapped (100 replicates) with the same software program.

Morphological studies. – For scanning electron microscopy (SEM) achenes and flowers were taken from herbarium specimens, mounted on preparation stubs and coated with a gold-palladium layer 2 nm thick in a Polaron sputter-coater. The observations and photography were performed with a Philips SEM 515 at the Botanical Museum Berlin-Dahlem.

Results and discussion

The ML search yielded the tree depicted in Fig. 1 and 2. Its main topology (Fig. 1) is congruent with most of the previous phylogenetic reconstructions for Mediterranean Anthemideae based on nrDNA ITS sequence information (Oberprieler & Vogt 2000, Oberprieler 2004a-b, 2005) and shows a basal grade of S African representatives of the tribe (i.e., Athanasia, Cotula, Inulanthera, Leptinella, Soliva, Ursinia) and a strongly supported (92% bootstrap support) monophyletic group of S African, Asian and Eurasian genera. While the monophyletic group of circum-Mediterranean Anthemideae receives a quite low support from the analysis of sequence variation (64% bootstrap support), this clade is well characterised by an apomorphic, 17bp long deletion in ITS2 (Oberprieler 2004a-b, 2005). Within this clade, three presumably monophyletic groups were distinguished: (a) the Anthemis clade, which consists of the species-rich genera Anthemis, Cota, Tanacetum and Tripleurospermum along with the unispecific Nananthea, (b) the Achillea clade comprising Achillea, Anacyclus, Heliocauta, Leucocyclus, Matricaria and Onanthes and (c) the

Fig. 1. Basal part of the phylogenetic tree from a Maximum Likelihood analysis of nrDNA ITS sequence data based on the TrN + $\Gamma$ model of DNA substitution (Tamura & Nei 1993) with base frequencies, gamma distribution parameter $\alpha$ and substitution rate matrix given in the text. Numbers above branches denote bootstrap values based on 100 bootstrap replicates.
Fig. 2. Detailed representation of the *Anthemis* and *Achillea* clades (compare Fig. 1) of the phylogenetic tree from a Maximum Likelihood analysis of nrDNA ITS sequence data based on the TrN + Γ model of DNA substitution (Tamura & Nei 1993) with base frequencies, gamma distribution parameter α and substitution rate matrix given in the text. Numbers above branches denote bootstrap values based on 100 bootstrap replicates.
genus-rich *Leucanthemum* clade (for a detailed diagram of this clade see Vogt & Oberprieler 2006). Again, while bootstrap support values for these three clades are below 60 %, at least for the *Anthemis* clade there is good evidence that this constitutes a monophyletic group: all members surveyed so far (*Anthemis* spp., *Cota* spp., *Nananthea perpusilla*, *Tanacetum* spp. and *Tripleurospermum* spp.) share the apomorphic condition of a tetrasporic embryo sac development, while the plesiomorphic condition of a monosporic embryo sac development was found in members of the *Achillea* and *Leucanthemum* clades.

Within the *Anthemis* clade, there is an obvious separation between the representatives of *Tanacetum* and those of *Anthemis*. *Tanacetum* representatives form the base of this clade and are characterised by short terminal and internal branches. Due to these short and unsupported internal branches and the nesting of representatives of *Cota* among *Tanacetum* species, the monophyly of *Tanacetum* is highly questionable. In contrast, both the clade including *Tripleurospermum* and *Nananthea* (84 % bootstrap support) and the clade of *Anthemis* in the strict sense (i.e. with the exclusion of *Cota* and the two deviating species *A. chia* and *A. odontostephana*; 85 % bootstrap support) receive good statistical support and constitute monophyletic groups. Remarkably, in this part of the phylogenetic tree the branches are considerably longer. This may be the consequence of the shorter-lived or even annual habit of *Anthemis* and *Tripleurospermum* species in contrast to the perennial life forms in *Tanacetum*. This, together with transitions from self-incompatibility to self-compatibility in *Anthemis* and *Tripleurospermum* may have led to an acceleration of evolutionary change of ITS sequences in this branch of the tree.

The position of *Matricaria macrotis* in the present phylogenetic reconstruction is well settled. There is good support (85 %) for both its close relationship with representatives of *Anthemis* in the strict sense and its phylogenetic distance from *Matricaria discoidea* as representative of this genus, which is rather a member of the *Achillea* clade (monosporic embryo sac development!, Harling 1950, 1951) than of the *Anthemis* clade. *M. macrotis* is found to form a clade (with a low bootstrap support of 72 %) with three members of *Anthemis* sect. *Maruta*, i.e. *A. cotula*, *A. pseudocotula* and *A. tigreensis*.

Several features are used to discriminate between *Anthemis* and *Matricaria* and may help to shed further light on its generic placement. Unfortunately embryo sac development is still unknown for *M. macrotis*. Following Bremer & Humphries (1993), Bremer & al. (1996) and Oberprieler & al. (in press), members of *Matricaria* are characterised by conical to subulate, hollow and epaleate receptacles, while in *Anthemis* receptacles are usually hemispherical to narrowly conical, filled with pith and covered with pales. Though Bremer & al. (1996) describe the receptacles observed in *M. macrotis* as “almost flat” and considered this feature as an argument for an exclusion of this species from *Matricaria*, our observations (Fig. 4A) show that receptacles in *M. macrotis* also show conical shapes (cf. “receptaculum subacutum conicum, calvum”, Rechinger (1943)). Although this together with the lack of pales and the hollow receptacles in *M. macrotis* support its inclusion in *Matricaria*, there are a number of morphological features that elucidate both the phylogenetical distance to *Matricaria* and the proximity to *Anthemis* as seen in the molecular results.

Achene morphology and anatomy have been demonstrated to be of paramount importance for the genetic delimitation in *Compositae-Anthemideae* (e.g. Reitbrecht 1974; Källersjö 1985, 1988; Bremer & Humphries 1993; Vogt & Oberprieler 1996; Oberprieler & al., in press). Following Reitbrecht (1974), Bremer & Humphries (1993), Bremer & al. (1996) and Oberprieler & al. (in press), *Matricaria* is characterised by achenes that are slightly dorsiventrally flattened, have a rather thin pericarp and five, mainly adaxially arranged, thin ribs that are sometimes furnished with longitudinal resin canals and are covered with myxogenic cells mainly on their abaxial surface and on the adaxial ribs. In contrast, achenes of *Anthemis* s.str. (i.e. after the exclusion of members of subgenus *Cota* as an independent genus) are circular in cross-section, show a considerably thick pericarp, possess ten, equally dispersed ribs devoid of resin ducts and have myxogenic cells evenly dispersed over the whole pericarp (Oberprieler 1998). The achenes of *M. macrotis* show in all above-mentioned respects a pronounced similarity with *Anthemis* achenes: they are ± terete and 10-ribbed (Fig. 4B-D, Rechinger 1943, Grierson in Davis 1975), the rather
thick pericarp is devoid of resin ducts and mucilage cells are found scattered over the whole surface. This deviating achene morphology led Bremer & al. (1996) to exclude the species not only from *Matricaria* but also from the subtribe *Matricarioideae* sensu Bremer & Humphries (1993), which in the meantime has turned out, however, in molecular studies (Watson & al. 2000; Oberprieler & Vogt 2000; Francisco-Ortega & al. 1997, 2001; Oberprieler 2001, 2002, 2004a-b) to be an artificial group.

In addition to the achene characters, there are further micromorphological characters in favour of a position of *Matricaria macrotis* within *Anthemis*. While the filament collar of *Matricaria* species is slender (Bremer & al. 1996), *Anthemis* and *M. macrotis* share the swollen or baluster-form filament collars (Fig. 3C). The apical anther appendage is found to be obtuse in *Matricaria* (Bremer & al. 1996) but acute in *Anthemis* and *M. macrotis* (Fig. 3A-B). Finally, while *Matricaria* spe-
cies are either glabrous or have an indument of basifixed hairs (Oberprieler & al., in press) the indumentum of *Anthemis* species and of *M. macrotis* consists of medifixed hairs. All these characters support the molecular phylogenetic findings, so it is mainly the lack of pales that argues against the inclusion of *Matricaria macrotis* into *Anthemis*. However, since Greuter (1968) found that in species of the former *Ammanthus* presence or absence of pales “does not even necessarily suffice to distinguish species” (which led him to transfer these species to *Anthemis*), since Mitsuoka & Ehrendorfer (1972) demonstrated that the inheritance of pales is probably under simple oligogenic control and since Napp-Zinn & Eble (1978) observed sporadic paleate capitula in the usually epaleate species of *Glebionis* (sub *Chrysanthemum*), the restriction in the taxonomic value of this character has become evident. *Anthemis* species may even show variation in the presence or absence of pales, e.g. *A. cotula* where receptacles are furnished with pales only in their upper half.

Fig. 4. *Anthemis macrotis* – A: receptacle; B-D: achenes. – Scale bar in A-D = 1 mm; from Raus 8360, B.
The close phylogenetic relationship of *Matricaria macrotis* to *Anthemis* receives further support by morphological similarities with members of *Anthemis* sect. *Maruta*, indicated as closest relatives by the ITS analysis. Members of this section are prone to exhibit partially epaleate receptacles, not only *A. cotula*. Yavin (1970) found receptacles lacking pales in their lower part in *A. adonidifolia* Kotschy ex Boiss., *A. corymbulosa* Boiss. & Hausskn., *A. fungosa* Boiss. & Hausskn., *A. lithuanica* (DC.) Trautv., *A. parvifolia* Eig, *A. patensissima* Eig, *A. pseudocotula* Boiss. and *A. tripolitana* Boiss. & Blanche. Therefore, the completely epaleate receptacles of *M. macrotis* may just represent the extreme state of this tendency among members of *A.* sect. *Maruta*. A further character of *M. macrotis* shared with some representatives of *A.* sect. *Maruta* is the occurrence of sterile ray florets, which are also observed in *A. corymbulosa*, *A. cotula*, *A. lithuanica* and *A. patensissima* (Yavin 1970). Additionally, the peripheral achenes of disc florets of *M. macrotis* with their long, solid adaxial auricle (Fig. 4B) resemble very nicely achenes known from other members of this section, i.e. *A. bornmuelleri* Stoj. & Acht. (Yavin 1970: t. III, fig. 1 sub *A. galilaea* var. *galilaea*) or *A. tripolitana* (Yavin 1970: t. III, fig. 15).

In conclusion, we found considerable evidence both from morphological and molecular studies that *Matricaria macrotis* is nested within the genus *Anthemis* s.str. with close relationship to members of *A.* sect. *Maruta*. It is shown that the previous classification of this species in *Matricaria* was mainly based on the character of absence of pales, which is increasingly found to be prone of parallelisms in unrelated taxa and therefore of limited phylogenetical value. As a consequence, we here transfer *M. macrotis* to *Anthemis* and validate the required new combination.

Additionally, a lectotype for the basionym is designated here.

**Taxonomy**


**Note.** – The type collection is cited in the protologue as “S: Rhodos, M. Attaio, Karstterrain, zirka 1000 m (R. 7383).” Rechinger did not select a holotype from his collection no. 7383, containing at least two specimens, of which one was later distributed to Berlin. We therefore designate the specimen at W, which bears the plant illustrated in the original publication, as lectotype, and the duplicate specimen at B as isolecotype.

**Ic.** – Fig. 1-3; Rechinger 1943: t. XIII, fig. 2.

**Distribution.** – Turkey (SW Anatolia and islands) and Greece (Rhodes, Saria). The report from Saria near Karpathos is the first for Europe. Grierson (in Davis 1975: 294) reports the species from Simi in Turkey (Yavalides, Islet of Plati, *Runemark & Nordenstam 16753*).

**Specimens seen.** – **Greece:** Dodekanes, Saria, Hauptgipfel der Insel, “Monte Grosso”, Pachivouno, 35°50’50”N, 27°13’40”E, offene Kleinstrauchflur des Gipfels, Kalkgestein, 630 m, 22.5.1983, Th. Raus 8360 (E); Insula Rhodos (Rodi), in saxosis calc. Montis Attairo, c. 1000 m, 16.5.1935, K. H. & F. Rechinger (B, W). – Turkey: C1 Muğla, d. Bodrum, Musgebi to Karatoprak, 50-100 m, igneous sandy slope, in maquis, 12.4.1965, P. H. Davis 40984 (E)

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