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On the taxonomic status and the phylogenetic relationships of some unispecific Mediterranean genera of *Compositae-Anthemideae*

I. *Brocchia*, *Endopappus* and *Heliocauta*

Abstract

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Sequences of the nrDNA internal transcribed spacer (ITS) region and the cpDNA *trnL/trnF* intergenic spacer (IGS) region were analysed for 67 representative species of 54 genera of *Compositae-Anthemideae* to ascertain the systematic position of the three unispecific Mediterranean genera *Brocchia*, *Endopappus* and *Heliocauta*. For *Brocchia cinerea*, which was considered to be closely related to *Cotula* due to its 4-lobed corolla of disc florets, a position among Mediterranean genera clearly distinct from *Cotula* but with unresolved sister group relationships is demonstrated. For *Endopappus macrocarpus* a close relationship with *Tripleurospermum* was suggested due to similarities in achene morphology; the molecular data, however, support its position distinctly distant from *Tripleurospermum* and among genera with a western Mediterranean centre of distribution characterized by a 5bp deletion in the *trnL/trnF* IGS. For *Heliocauta atlantica*, which was hitherto placed in the vicinity of *Hippolytia* from central Asia, the molecular data reveal its sister group relationship with the strictly Mediterranean genus *Anacyclus*. First efforts are made to date the diversification of members of the tribe based on sequence divergence rates (c. 1 % = 0.75 Myr) and assuming an origin of the tribe in the Middle Oligocene (c. 25 Myr). It is concluded that lineages within the clade of Mediterranean and Eurasian representatives characterized by a 17bp deletion in ITS2 diverged in the Middle Miocene (c. 15 Myr).

Introduction

According to the most recent conspectus of *Compositae-Anthemideae* in the Mediterranean and its adjacent European areas (Euro+Med project; Greuter & al. 2003) the tribe is represented in this region with 52 genera, corresponding to nearly the half of genera (111) assigned to this tribe by Oberprieler & al. (in press). The species number of around 620 for the above-mentioned area covered by Med-Checklist (Greuter & al. 1984-89) and Flora Europaea (Tutin & al. 1964-80) shows that this region plays a paramount role in the diversity of the whole tribe with its c. 1741 species

(Bremer & Humphries 1993), the two other centres of diversity being southern Africa and central and eastern Asia.

Recent molecular studies have shown that many southern African and central and eastern Asian genera form a basal grade in the phylogeny of the tribe while a majority of the mentioned 52 genera forms a monophyletic group (Watson & al. 2000). If southern hemisphere genera, which have only been naturalized in the Mediterranean and adjacent Eurasian regions (*Cotula*, *Eriocephalus*, *Soliva*) or reach this area with only single species (*Lasiospermum*, *Pentzia*), are excluded, this group is reduced to 47 genera. With the previous studies concentrated on the molecular phylogeny of Mediterranean genera (Francisco-Ortega & al. 1997, Oberprieler & Vogt 2000, Francisco-Ortega & al. 2001, Oberprieler 2001, 2002) sequence information for the internal transcribed spacer region of the nuclear ribosomal repeat (nrDNA ITS) is now available for representatives of all but the six unispecific genera *Brocchia*, *Daveaua*, *Endopappus*, *Heliocauta*, *Leucocyclus* and *Nananthea*. With the present publication on *Brocchia*, *Endopappus* and *Heliocauta*, and a forthcoming paper on the remaining genera (Oberprieler, in prep.) this gap will be filled and a comprehensive discussion on the taxonomy of the Mediterranean *Anthemideae* and their evolutionary history is envisaged.

The genus *Brocchia* Vis. was described by Visiani (1836) based on the annual herb *Cotula cinerea* Delile. The species occurs in N Africa and the Near East. It is characterised by a dense indumentum of basifixed hairs, alternate, pinnatipartite to pinnatisect, or sometimes even entire or lobed leaves, and distinctly pedunculate, discoid capitula with involucre bracts with narrow, pale membranous margins. The receptacle is hemispherical to conical and epaleate. The yellow disc florets are apically 4-lobed and the obovoid achenes provided with 4 inconspicuous lateral and adaxial ribs, a marginally rounded apical plate, and a rather thin pericarp with large, elongated myxogenic cells but devoid of any resin canals or sacs.

Endopappus Sch. Bip. was described by Schultz [Bipontinus] (1860) with the sole species *E. macrocarpus* Sch. Bip., an annual glabrous herb endemic to Morocco, Algeria, Tunisia and Libya. The species is characterised by alternate, pinnatisect leaves, solitary, radiate pedunculate capitula and involucre bracts with pale to brown scarious margins. The receptacle is flat to slightly convex and devoid of receptacular scales. Ray florets are female and fertile, the limb colour (white or yellow) is used to discriminate between two subspecies [subsp. *macrocarpum* and subsp. *maroccanus* (Jahand. & al.) Ibn Tattou]. The disc florets have a 5-lobed corolla, and the achenes are dorso-ventrally flattened, 3-angled with one adaxial and two lateral ribs, an apical corona, and the pericarp is furnished with myxogenic cells on the abaxial side and along the ribs.

Heliocauta Humphries was described comparatively recently by Humphries (1977) who based it on the Moroccan endemic *Anacyclus atlantica* Litard. & Maire, a creeping perennial herb with a premorse caudex, an indumentum of basifixed hairs and 3-pinnatisect leaves in a basal rosette. The capitula are solitary, pedunculate and discoid, the involucre bracts having pale to dark brown scarious margins, and the conical receptacle is furnished with narrowly elliptical and moderately canaliculate paleas. Disc florets are again 5-lobed, and achenes are narrowly obovoid, somewhat dorso-ventrally flattened, and 4-5-ribbed with 2 distinct lateral ribs. The achene apex possesses a minute corona formed by a dentate rim, while the thin pericarp lacks myxogenic cells but is furnished with elongated resin sacs.

Material and methods

Plant material. – Sixty-seven representatives from 54 genera of *Compositae-Anthemideae* were included in at least one of the present analyses. For the nrDNA ITS and the cpDNA *trnL/trnF* IGS data set (data sets 1 and 2), the main aim was a comprehensive sampling of the Mediterranean and Eurasian genera with an addition of sequence information for Asian and southern hemisphere centred genera. Sequence information for most of the taxa came from former publications (Oberprieler & Vogt 2000, Oberprieler 2001, 2002) and from published and unpublished EMBL/GenBank/

DDBJ accessions (Table 1), while sequences for *Broccchia cinerea*, *Endopappus macrocarpus*, *Heliocauta atlantica* and *Hippolytia dolichophylla* (data set 1 and 2) along with cpDNA *trnL/trnF* IGS sequences for *Anthemis rigescens*, *Gonospermum fruticosum*, *Hymenostemma pseudanthemis*, *Ismelia carinata*, *Leucanthemella serotina* and *Lugoa revoluta* (data set 2) are new to science and were obtained from herbarium specimens. Additionally, for the detailed analysis of the position of *Heliocauta atlantica* (data set 3) I included yet unpublished nrDNA ITS information for eight species of *Anacyclus* (Table 1). Following results of Watson & al. (2000), I used *Ursinia anthemoides* (L.) Poir. as outgroup in the analysis of data set 1, and – due to the lack of sequence information for *Ursinia* – *Pentzia flabelliformis* Willd. in the analysis of data set 2. Outgroup selection for the analysis of data set 3 followed the results of Oberprieler & Vogt (2000) and of the preceding analyses of data sets 1 and 2.

DNA isolation, PCR amplification, sequencing. – DNA was extracted from 30–40 mg dried and crushed leaf material according to Hellwig & al. (1999) and Oberprieler & Vogt (2000) using Qiagen tip-20 columns or Qiagen's DNeasy Plant Kit (Qiagen Inc., Hilden, Germany). Amplification of nrDNA ITS and *trnL/trnF* IGS was performed using primers designed by White & al. (1990) and Taberlet & al. (1991), respectively, and following the protocols given by Oberprieler & Vogt (2000). Amplified products were purified with a Qiaquick PCR cleaning column and filtration kit (Qiagen Inc.). Sequences were obtained using an automated sequencer (ABI 377; at the sequencing facility maintained by the DLMBC sequencing company, Rüdersdorf bei Berlin, Germany) and the ABI Prism BigDye Terminator technology. All new nrITS and cpDNA *trnL/trnF* IGS sequences were submitted to the EMBL sequence data bank (Table 1).

Sequence alignment. – Sequences were aligned using CLUSTAL W (Thompson & al. 1994) and alignments subsequently corrected manually. For the maximum parsimony analyses, gaps in aligned sequences were treated as missing data, but phylogenetically informative indels were coded as additional binary characters. The alignments are deposited as electronic supplement at <http://www.bgbm.org/bgbm/library/publikat/willd34/oberprieler-1.htm>.

Phylogenetic reconstructions

Maximum parsimony analysis. – Maximum parsimony (MP) analyses of the data sets were performed using the heuristic search algorithm of PAUP* version 4.0b10 (Swofford 2002) with ACCTRAN, MULPARS and TBR branch swapping in action. Character states were specified unordered and unweighted. 1000 (data set 1) and 10 000 (data sets 2 and 3) random addition sequence replicates were performed to locate potential islands of most parsimonious trees. Limitations of computer memory made it necessary to constrain the maximum number of most parsimonious trees saved in each replicate to five (MAXTREES = 5) in the analysis of data set 2. Support for clades of the strict consensus tree of the MP analysis was evaluated using bootstrap (Felsenstein 1985) and decay analyses (Bremer 1988). Bootstrap analyses were performed using the following settings: 100 bootstrap replicates, 1000 random addition sequence replicates per bootstrap replicate, ACCTRAN, TBR, and MULPARS. In data set 1 and 2, MAXTREES was set to 2 per replicate. Decay analyses were carried out using AutoDecay 3.0 (Eriksson & Wikström 1995). For each constraint analysis, 1000 random addition sequence replicates were performed (MAXTREES = 2 in data set 2).

Model-based phylogenetic reconstructions. – In addition to the above described cladistic (MP) analyses, data set 1 (with gap coding excluded) was also analysed with two model-based approaches to phylogenetic inference, the Maximum-Likelihood (ML) method (Felsenstein 1981, Kishino & Hasegawa 1989) and a Bayesian Inference (BI) approach (Lewis 2001). Since both methods are dependent on assumptions about the process of DNA substitution (a model of DNA evolution), the program MODELTEST version 3.06 (Posada & Crandall 1998) was used 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 (TrN + Γ), the base frequencies being freqA = 0.2670, freqC = 0.2019, freqG = 0.2155 and

Table 1. List of taxa and sources of plant material analysed. Unless otherwise stated, the cited vouchers are in the herbarium of the author.

Taxon	Accession	EMBL/GenBank/DBJ accession number		
		ITS1	ITS2	trnL/F
<i>Aaronsohnia pubescens</i> (Desf.) Bremer & Humphries	Oberprieler & Vogt (2000)	AJ3296408	AJ3296443	AJ3296483
<i>Achillea ptarmica</i> L.	Oberprieler & Vogt (2000)	AJ3296380	AJ3296415	AJ3296450
<i>Anacyclus clavatus</i> (Desf.) Pers.	Spain, Malaga, Coin-El Burgo, 3.6.1992, Oberprieler 3716 & Vogt	AJ748762	AJ748763	
<i>A. homogamos</i> (Maire) Humphries	Morocco, Ourika-Oukaïmeden, 13.7.1989, Oberprieler 3620	AJ748764	AJ748765	
<i>A. linearilobus</i> Boiss. & Reuter	Algeria, Bou-Sfer, 24.5.1980, <i>Dubuis 15595</i> (M)	AJ748766	AJ748767	
<i>A. maroccanus</i> (Ball) Ball	Morocco, Marrakech - Beni-Mellal, 25.4.1987, <i>Bayón, Oberprieler 2465 & Vogt</i>	AJ748768	AJ748769	
<i>A. monanthos</i> subsp. <i>cyrtolopidioides</i> (Pomel) Humphries	Tunisia, Thala - Kasserine, 7.5.1994, <i>Vogt 12562 & Oberprieler 6867</i>	AJ748770	AJ748771	
<i>A. pyrethrum</i> (L.) Link var. <i>pyrethrum</i>	Morocco, Ain-Leuh - Azrou, 13.5.1995, <i>Vogt 14954 & Oberprieler 9263</i>	AJ748772	AJ748773	
<i>A. radiatus</i> Loisel. subsp. <i>radiatus</i>	Oberprieler & Vogt (2000)	AJ3296381	AJ3296416	AJ3296451
<i>A. radiatus</i> subsp. <i>coronatus</i> (Murb.) Humphries	Morocco, Tizmit - Agadir, 19.5.1993, <i>Vogt 11907 & Oberprieler 6355</i>	AJ748774	AJ748775	
<i>A. valentinus</i> L.	Morocco, Selouane - Zaio, 12.4.1987, <i>Bayón, Oberprieler 2001 & Vogt</i>	AJ748776	AJ748777	
<i>Ajania pacifica</i> (Nakai) Bremer & Humphries	Francisco-Ortega & al. (1997)	L77787	L77787	
<i>Anthemis chia</i> L.	Oberprieler (2001)	AJ312785	AJ312814	
<i>A. cretica</i> L.	Oberprieler & Vogt (2000)	AJ3296388	AJ3296423	AJ3296458
<i>A. odontostephana</i> Boiss.	Oberprieler (2001)	AJ312797	AJ312826	
<i>Arctanthemum arcticum</i> (L.) Tzvelev	Francisco-Ortega & al. (1997)	L77756	L77756	
<i>Argyranthemum foeniculaceum</i> (Willd.) Sch. Bip.	Francisco-Ortega & al. (1997)	L77799	L77799	
<i>A. adauctum</i> (Link) Humphries	Oberprieler & Vogt (2000)			AJ3296460
<i>Artemisia vulgaris</i> L.	Oberprieler & Vogt (2000)	AJ3296389	AJ3296424	AJ3296459
<i>Athanasia crithmifolia</i> (L.) L.	Francisco-Ortega & al. (2001)	AF155241	AF155278	
<i>Brocchia cinerea</i> (Del.) Vis.	Tunisia, Tozeur, 30.3.1998, <i>Bauke & al. 509</i>	AJ748778		AJ748786
	Morocco, Erfoud - Merzouga, 3.4.1993, <i>Podlech 50884</i> (MSB)		AJ748779	
<i>Castrilanthemum debeauxii</i> (Degen & al.) Vogt & Oberprieler	Oberprieler & Vogt (2000)	AJ3296390	AJ3296425	AJ3296463
<i>Chamaemelum nobile</i> (L.) All.	Oberprieler & Vogt (2000)	AJ3296382	AJ3296417	AJ3296452
<i>Chlamydomorpha tridentata</i> (Del.) Less.	Oberprieler & Vogt (2000)	AJ3296391	AJ3296426	AJ3296464
<i>Chrysanthemum coreanum</i> (H. Lév. & Vaniot) Nakai	Francisco-Ortega & al. (1997)	L77802	L77802	
<i>C. indicum</i> L.	Zhao & al. (unpubl.)			AF314606
<i>Chrysanthoglossum deserticola</i> (Murb.) Wilcox & al.	Oberprieler & Vogt (2000)	AJ3296392	AJ3296427	AJ3296465
<i>Cladanthus arabicus</i> (L.) Cass.	Oberprieler & Vogt (2000)	AJ3296383	AJ3296418	AJ3296453
<i>Coleostephus myconis</i> (L.) Rchb.f.	Francisco-Ortega & al. (1997)	L77758	L77758	
	Oberprieler & Vogt (2000)			AJ3296466
<i>Cota rigescens</i> (Willd.) Holub	Oberprieler (2001)	AJ312801	AJ312830	AJ748787
<i>Cotula coronopifolia</i> L.	Wagstaff & Breitwieser (2002)	AF422118	AF422118	
<i>Cymbopappus adenosolen</i> (Harv.) B. Nord.	Francisco-Ortega & al. (1997)	L77759	L77759	
<i>Endopappus macrocarpus</i> Sch. Bip.	Algeria, Ghardaia - El Golea, 28.3.1981, <i>Podlech 35512</i> (MSB)	AJ748780	AJ748781	AJ748788

Taxon	Accession	EMBL/GenBank/DDDB accession number		
		ITS1	ITS2	trnL/F
<i>Glebionis coronaria</i> (L.) Spach	Francisco-Ortega & al. (1997) Oberprieler & Vogt (2000)	L77741	L77741	AJ3296461
<i>Glossopappus macrotus</i> subsp. <i>hesperius</i> (Maire) Maire	Oberprieler & Vogt (2000)	AJ3296394	AJ3296429	AJ3296468
<i>Gonospermum fruticosum</i> (C. Smith ex Link) Less.	Francisco-Ortega & al. (2001)	AF155245	AF155282	
	Spain, Canary Islands, Tenerife, Punta de Teno, 23.3.1999, Oberprieler 9877			AJ748789
<i>Heliocauta atlantica</i> (Litard. & Maire) Humphries	Morocco, Toubkal, 3850 m, 23.8.1992, <i>Kreisch</i> 920589 (herb. Kreisch)	AJ748782	AJ748783	AJ748790
<i>Heteranthemis viscidelirita</i> Schott	Francisco-Ortega & al. (1997) Oberprieler & Vogt (2000)	L77761	L77761	AJ3296462
<i>Heteromera fuscata</i> (Desf.) Pomel	Oberprieler & Vogt (2000)	AJ3296409	AJ3296444	AJ3296484
<i>Hippolytia dolichophylla</i> (Kitam.) Bremer & Humphries	Pakistan, Bulan-ridge, 4100 m, 28.7.1993, <i>Nüsser</i> 174 (B)	AJ748784	AJ748785	AJ748791
<i>Hymenostemma pseudanthemis</i> (G. Kunze) Willk.	Francisco-Ortega & al. (1997)	L77762	L77762	
	Spain, Cadiz, Bernoz, 2.4.1974, <i>Smithies</i> (B)			AJ748792
<i>Inulanthera calva</i> (Hutch.) Källersjö	Francisco-Ortega & al. (2001)	AF155251	AF155288	
<i>Ismelia carinata</i> (Schousboe) Sch. Bip.	Francisco-Ortega & al. (1997) Morocco, S Agadir, 26.4.1994, <i>Kilian</i> 3384 (B)	L77764	L77764	AJ748793
<i>Lepidophorum repandum</i> (L.) DC.	Oberprieler & Vogt (2000)	AJ3296395	AJ3296430	AJ3296469
<i>Leptinella serrulata</i> (D. G. Lloyd) D. G. Lloyd & C. J. Webb	Wagstaff & Breitwieser (2002)	AF422125	AF422125	
<i>Leucanthemella serotina</i> (L.) Tzvelev	Francisco-Ortega & al. (1997) 20.9.1967, <i>Moraria</i> & al. (B)	L77766	L77766	AJ748794
<i>Leucanthemopsis alpina</i> (L.) Heywood	Oberprieler & Vogt (2000)	AJ3296396	AJ3296431	AJ3296470
<i>Leucanthemum vulgare</i> subsp. <i>pujiulae</i> Sennen	Oberprieler & Vogt (2000)	AJ3296398	AJ3296433	AJ3296472
<i>Lonas annua</i> (L.) Vines & Druce	Oberprieler & Vogt (2000)	AJ3296411	AJ3296446	AJ3296486
<i>Lugoa revoluta</i> (C. Smith ex Link) DC.	Francisco-Ortega & al. (2001) Spain, Canary Islands, Tenerife, Benijo, 26.3.1999, Oberprieler 9919	AF155252	AF155289	AJ748795
<i>Matricaria discoidea</i> DC.	Oberprieler & Vogt (2000)	AJ3296412	AJ3296447	AJ3296487
<i>Mauranthemum gaetulum</i> (Batt.) Vogt & Oberprieler	Oberprieler & Vogt (2000)	AJ3296399	AJ3296434	AJ3296473
<i>Mecomischus halimifolius</i> (Munby) Hochreutiner	Oberprieler & Vogt (2000)	AJ3296384	AJ3296419	AJ3296454
<i>Nipponanthemum nipponicum</i> (Maxim.) Kitam.	Francisco-Ortega & al. (1997)	L77772	L77772	
<i>Nivellea nivellei</i> (Br.-Bl. & Maire) Wilcox & al.	Oberprieler & Vogt (2000)	AJ3296400	AJ3296435	AJ3296474
<i>Otanthus maritimus</i> (L.) Hoffmanns. & Link	Oberprieler & Vogt (2000)	AJ3296385	AJ3296420	AJ3296455
<i>Otospermum glabrum</i> (Lag.) Willk.	Oberprieler & Vogt (2000)	AJ3296413	AJ3296448	AJ3296488
<i>Pentzia flabelliformis</i> Willd.	Bayer & al. (2000)			AF100519
<i>Phalacrocarpum oppositifolium</i> (Brot.) Willk.	Oberprieler & Vogt (2000)	AJ3296401	AJ3296436	AJ3296475
<i>Plagiopus fontanesii</i> (Boiss. & Reut.) Vogt	Oberprieler & Vogt (2000)	AJ3296403	AJ3296438	AJ3296477
<i>Prolongoa hispanica</i> G. López & C. E. Jarvis	Francisco-Ortega & al. (1997) Oberprieler & Vogt (2000)	L77776	L77776	AJ3296479
<i>Rhetinolepis lonadioides</i> Coss.	Oberprieler & Vogt (2000)	AJ3296386	AJ3296421	AJ3296456
<i>Rhodanthemum hosmarianse</i> (Ball) Wilcox & al.	Oberprieler & Vogt (2000)	AJ3296407	AJ3296442	AJ3296482
<i>Santolina rosmarinifolia</i> L.	Oberprieler & Vogt (2000)	AJ3296387	AJ3296422	AJ3296457
<i>Tanacetum corymbosum</i> (L.) Sch. Bip.	Oberprieler & Vogt (2000)	AJ3296414	AJ3296449	AJ3296490
<i>Tripleurospermum inodorum</i> (L.) Sch. Bip.	Francisco-Ortega & al. (1997) Oberprieler & Vogt (2000)	L77782	L77782	AJ3296489
<i>Ursinia anthemoides</i> (L.) Poir.	Francisco-Ortega & al. (1997)	L77783	L77783	

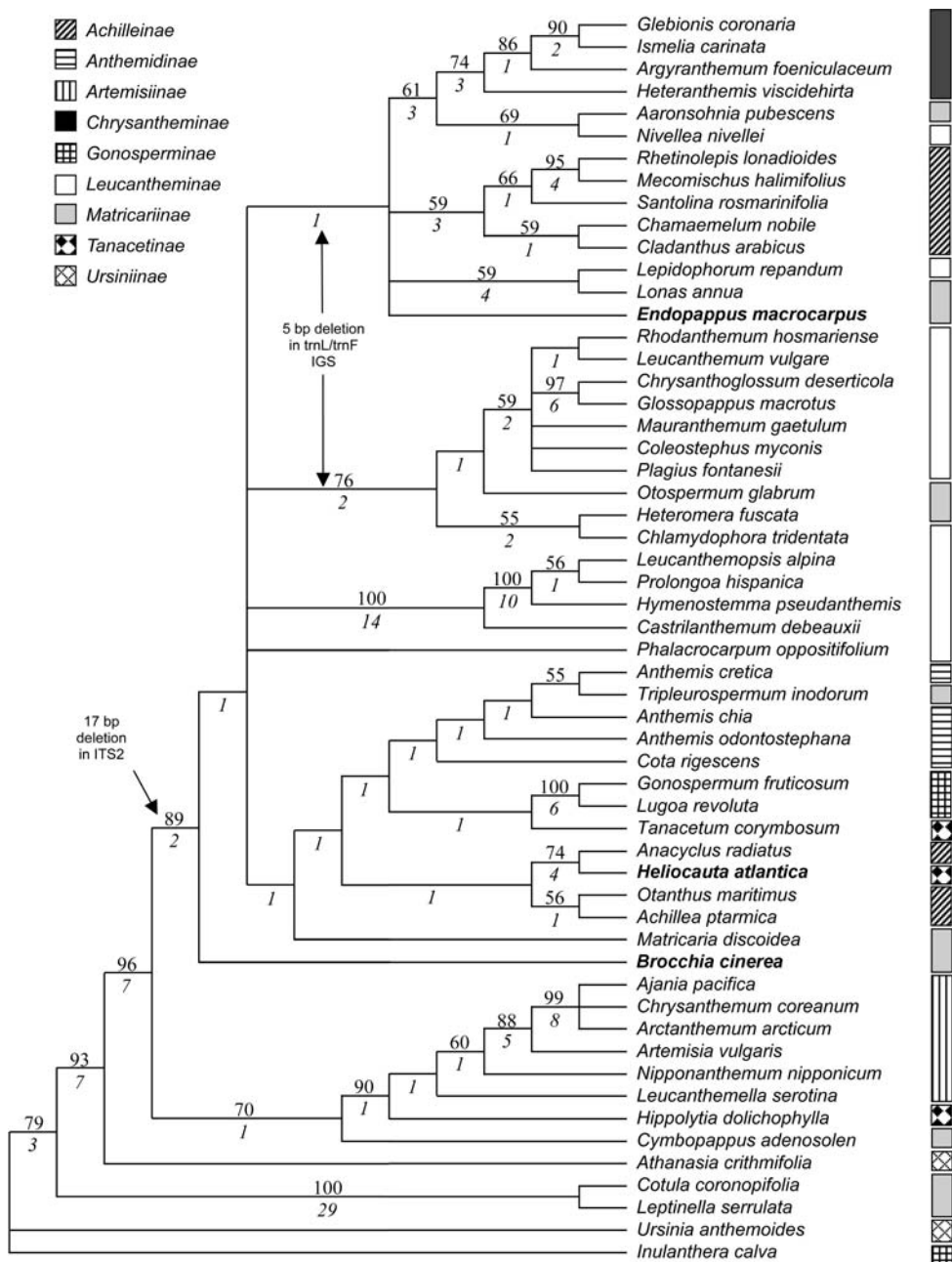


Fig. 1. Strict consensus tree of 337 equally most parsimonious trees (1248 steps; CI = 0.379, autapomorphies excluded; RI = 0.568), based on nrDNA ITS sequence information (data set 1). Numbers above the lines are bootstrap values, numbers below the lines are decay values. Subtribal affinities according to Bremer & Humphries (1993) are indicated by bar patterns.

freqT = 0.3155, a gamma distribution shape parameter of $\alpha = 0.6356$ and a substitution rate matrix of $R[A-C] = R[A-T] = R[C-G] = R[G-T] = 1.0$, $R[A-G] = 2.5916$ and $R[C-T] = 5.4212$.

Using these parameters, a heuristic ML search was performed with PAUP* version 4.0b10 (Swofford 2002) with TBR branch swapping, 100 random addition sequence replicates and the number of rearrangements tried limited to 1000 per replicate.

The same parameters of the TrN + Γ model were also used in the BI approach performed with the software programme MRBAYES version 2.01 (Huelsenbeck & Ronquist 2001a-b). Four Metropolis-coupled Markov chain Monte Carlo (MCMC) chains with incremental heating temperature of 0.2 were run for 201 000 generations and sampled every 10th generation. The burn-in period was determined graphically, and the first 200 of the 20 100 sampled trees were discarded. Estimation of tree topology and posterior probabilities of clades were based on the remaining 19 900 trees.

Results

Nuclear ribosomal DNA ITS (data set 1). – The ITS alignment is 513 bp long (278 bp for ITS1 and 235 bp for ITS2) with 325 variable positions including 228 phylogenetically informative substitutions, 44 phylogenetically informative indels of 1 to 5 bp length, and an additional, informative 17 bp-deletion in ITS2. The heuristic MP search yielded 337 equally most parsimonious trees (in 212 islands) with a length of 1248 steps, a consistency index (CI, with autapomorphies excluded) of 0.3789, and a retention index (RI) of 0.5679. The strict consensus tree of all equally most parsimonious trees is shown in Fig. 1. The three unispecific Mediterranean genera under study are consistently nested within a moderately supported (89 % bootstrap support, decay value of 2 steps) clade of genera with a Mediterranean and/or Eurasian centre of distribution. This clade is also supported by the above-mentioned 17bp deletion in ITS2. While sister group relationships are well supported below this node (e.g. 96 % bootstrap support and a decay value of 7 for the sister group relationship between the Mediterranean/Eurasian group and the *Artemisia* group of genera), the tree topology within the 17bp deletion clade is not well settled; thus leaving the phylogenetic relationships of *Brocchia* and *Endopappus* unresolved and the sister group relationship of *Heliocauta* and *Anacyclus radiatus* weakly supported (74 % / 4 steps).

The ML search yielded the tree depicted in Fig. 2 (56 896 rearrangements examined, $-\ln L = 6248.16037$). Its main topology is congruent with the consensus tree of the MP analysis. Main differences are the positions of *Matricaria discoidea*, *Lonas annua*, and *Lepidophorum repandum*, the latter two taxa consistently forming a monophyletic group in the MP analysis but with deviating positions and long branches in the ML tree. The positions of the three genera in question found in the MP analysis, however, are supported by this method of phylogenetic reconstruction.

Finally, the Bayesian analysis gave the tree topology in Fig. 3. Most of the monophyletic groups with bootstrap support values higher than 70 % from the MP analysis receive statistical support by a posterior probability of at least 95 % in this analysis. However, there are several exceptions to this observation: while the sister group relationships of *Rhynchospora / Mecomischus* and *Chrysanthoglossum / Glossopappus* are supported by high bootstrap values, the posterior probabilities for these clades are not significant. Conversely, two large assemblages of genera (i.e. the group of *Gleobionis* through *Phalacrocarpum* and the clade of *Anthemis* through *Matricaria* in Fig. 1) with bootstrap values below 50 % receive significant statistical support in the Bayesian analysis (posterior probabilities of 95 % and 96 %, respectively). Finally, the 17bp deletion clade with its moderate bootstrap support of 89 % in the MP analysis receives no support from the Bayesian analysis (posterior probability of 60%). Low support values for further subclades render the phylogenetic relationships of *Endopappus* and *Brocchia* unsettled, while the position *Heliocauta* receives further corroboration.

Chloroplast DNA trnL/trnF IGS (data set 2). – The *trnL/trnF* IGS alignment is 469 bp long with 135 variable positions, including 52 phylogenetically informative substitutions and 17 phylogenetically informative indels of one to 14 bp length. The restricted (see Material and Methods,

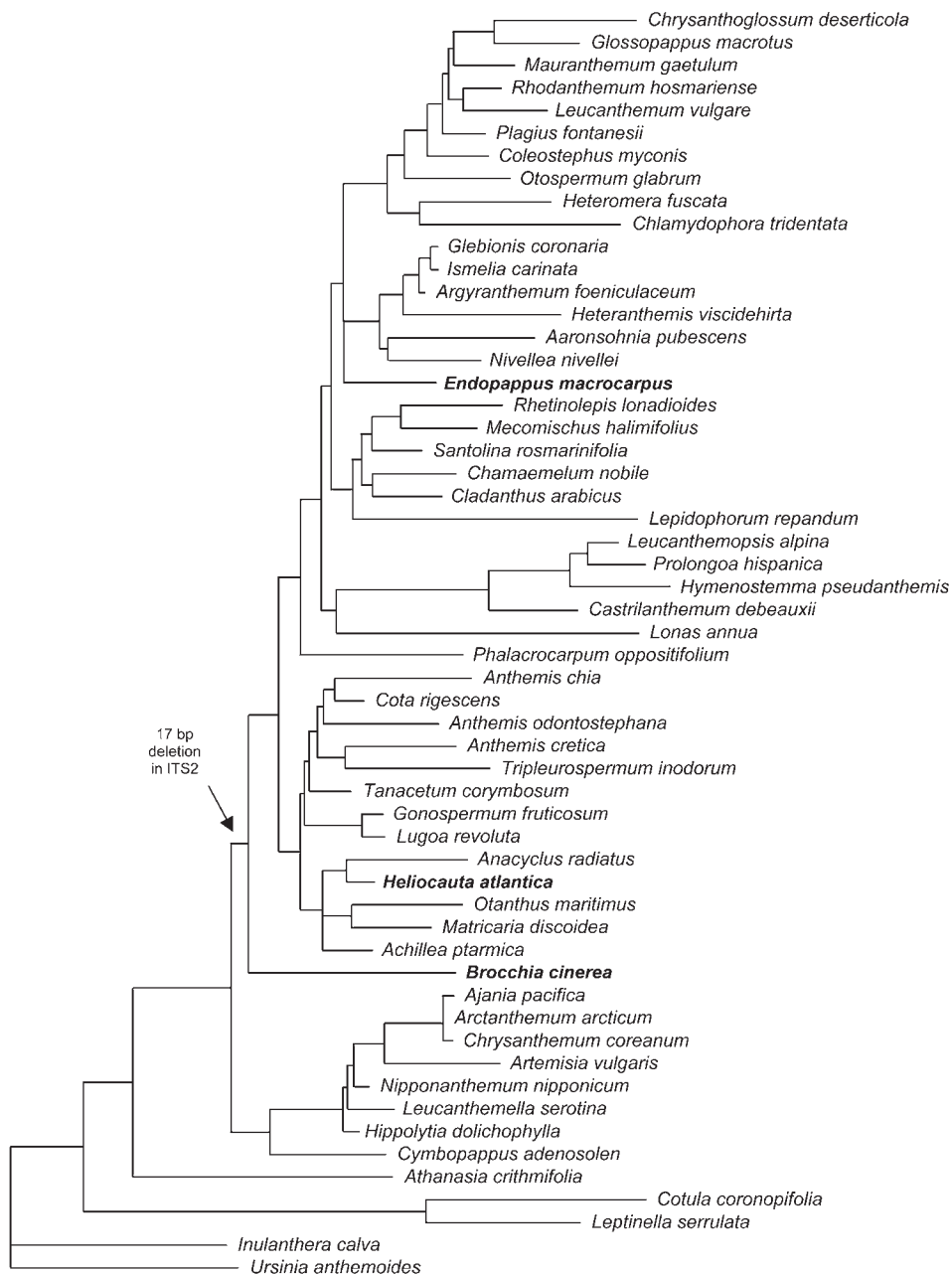


Fig. 2. Phylogenetic tree from a maximum likelihood analysis of nrDNA ITS sequence data (data set 1, indel information excluded) based on the TrN + Γ model of DNA substitution (Tamura & Nei 1993) with base frequencies, gamma distribution parameter α , and substitution rate matrix given in the text.

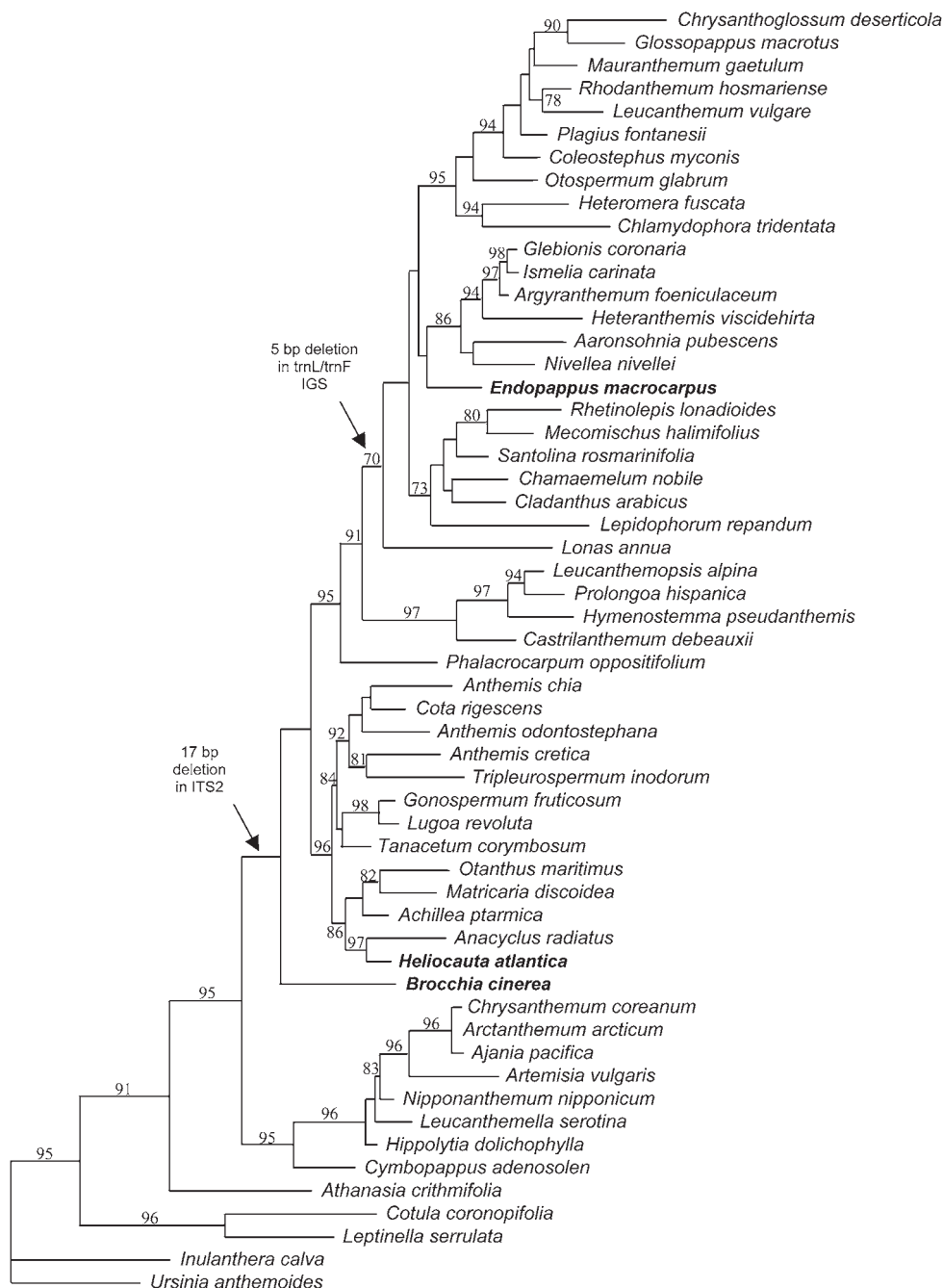


Fig. 3. Phylogenetic tree from a Bayesian analysis of nrDNA ITS sequence data (data set 1, indel information excluded) based on the TrN + Γ model of DNA substitution (Tamura & Nei 1993) with base frequencies, gamma distribution parameter α and substitution rate matrix given in the text. Tree topology and posterior probabilities ($\times 100$) of clades were based on 19 900 trees from a MCMC chain run for 201 000 generations.

above) heuristic MP search yielded 20 145 equally most parsimonious trees (in 4029 islands) with a length of 239 steps, a consistency index (CI, with autapomorphies excluded) of 0.6111, and a retention index (RI) of 0.7358. The strict consensus tree of all equally most parsimonious trees is shown in Fig. 4. Despite the relatively high CI and RI values of the trees found, most of the monophyletic groups of the consensus tree receive very low support, the exceptions being the clade of *Leucanthemopsis*, *Hymenostemma* and *Prolongoa* (100 % / 11), the clade of subtribe *Chrysantheminae* sensu Bremer & Humphries (1993; i.e. *Argyranthemum*, *Chrysanthemum*, *Heteranthemis* and *Ismelia*; 84 % / 13) and the sister group relationships of *Glossopappus* and *Mauranthemum* (97 % / 4), *Lepidophorum* and *Lonas* (73 % / 3), *Aaronsohnia* and *Nivellea* (99 % / 4) and *Mecomischus* and *Rhetinolepis* (75 % / 1). All taxa characterised by a 5bp-deletion at alignment positions 337-341 form a monophyletic, though scarcely supported group, the only exception being the representative of *Anacyclus*, which falls within this clade despite the lack of this deletion.

Nuclear ribosomal DNA ITS of Anacyclus species and Heliocauta (data set 3). – The ITS alignment of this data set is 467 bp long with 144 variable positions including 63 phylogenetically informative substitutions and eight informative indels of one to seven bp length. The heuristic MP search yielded 11 equally most parsimonious trees (in one island) with a length of 258 steps, a consistency index (CI, with autapomorphies excluded) of 0.5497 and a retention index (RI) of 0.6546. The strict consensus tree of all equally most parsimonious trees is shown in Fig. 5. It shows the consistent, but only weakly supported (74 % / 3 steps decay) placement of *Heliocauta atlantica* as the sister group of a monophyletic, moderately supported (88 % / 3 steps decay) genus *Anacyclus*.

Discussion

The present study adds nrDNA ITS and cpDNA *trnL/trnF* IGS sequence information for three unispecific Mediterranean *Anthemideae* genera to the data sets published by Francisco-Ortega & al. (1997) and Oberprieler & Vogt (2000). With the exception of *Daveaua* Willk. ex Mariz, *Leucocyclus* Boiss. and *Nananthea* DC. (Oberprieler, in prep.), sequence information for these two markers is now available for all Mediterranean genera of the tribe (when genera with distribution centres in the southern hemisphere that have been either naturalised in the Mediterranean, i.e. *Soliva*, *Eriocephalus*, or reach this area with only single species, i.e. *Lasiospermum*, *Pentzia*, are excluded from consideration).

In accordance with the results of previous studies by Francisco-Ortega & al. (1997) and Oberprieler & Vogt (2000) based on nrDNA ITS and on nrDNA ITS and cpDNA *trnL-trnF* IGS sequence information, respectively, and Watson & al. (2000) based on *ndhF* sequences, the present analysis adds further corroboration to the observation that most of the genera with a centre of distribution in western Eurasia and the Mediterranean region (including Macaronesia) form a monophyletic group. This clade receives a considerable bootstrap and decay index support in the MP analysis (89 % / 2) of the nrDNA data but lacks a significant posterior probability (PP) in the BI approach (PP = 0.6). This is considered to be mainly due to the inclusion of gap information in the MP analyses and the omission of this information in the ML and BI analyses.

In contrast to former, less comprehensive studies of cpDNA *trnL/trnF* IGS sequence variation in Mediterranean *Anthemideae* (Oberprieler & Vogt 2000), the MP tree received from the present sampling of genera is highly unresolved and contributes little to our understanding of the phylogeny of *Anthemideae* in the Mediterranean and Eurasian region. Nevertheless, this marker provides a highly informative character, a 5bp deletion, which characterises a monophyletic group also found in the BI analyses of the nrDNA ITS data set.

Evolutionary history of Mediterranean Compositae-Anthemideae. – Recently published, calibrated nrDNA ITS rates in different families of flowering plants were found to be around 1 % = 1 Myr (*Gossypium*; Wendel & al. 1995), 1 % = 0.6-1.1 Myr (*Gentianella*; Von Hagen & Kadereit 2001), 1 % = 0.6-1.3 Myr (*Soldanella*; Zhang & al. 2001), 1 % = 0.6 Myr (*Robinsonia*; Sang & al. 1995)

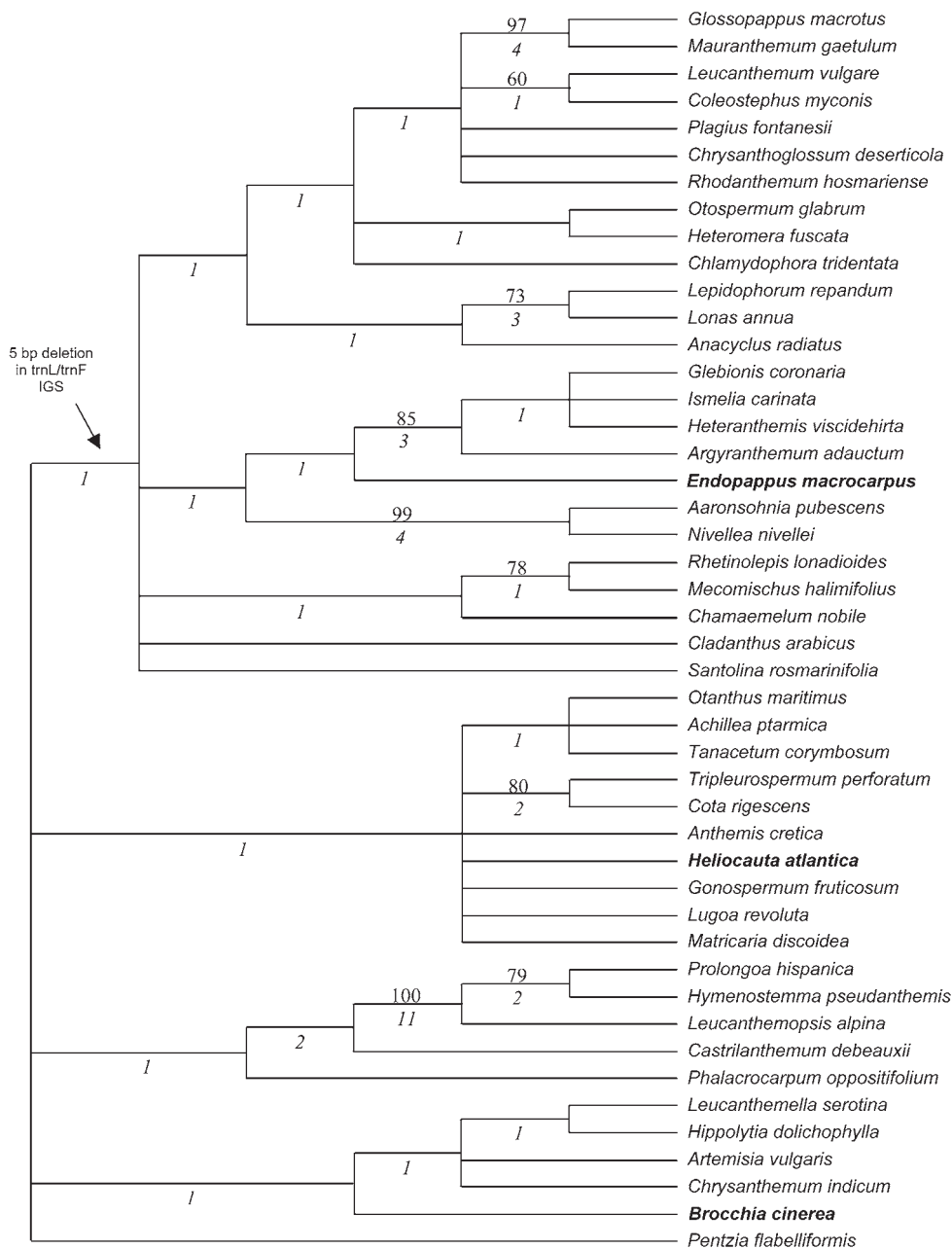


Fig. 4. Strict consensus tree of 20 145 equally most parsimonious trees (239 steps; CI = 0.611, autapomorphies excluded; RI = 0.736), based on sequence information from the cpDNA *trnL/trnF* IGS region (data set 2). Numbers above the lines are bootstrap values, numbers below the lines are decay values.

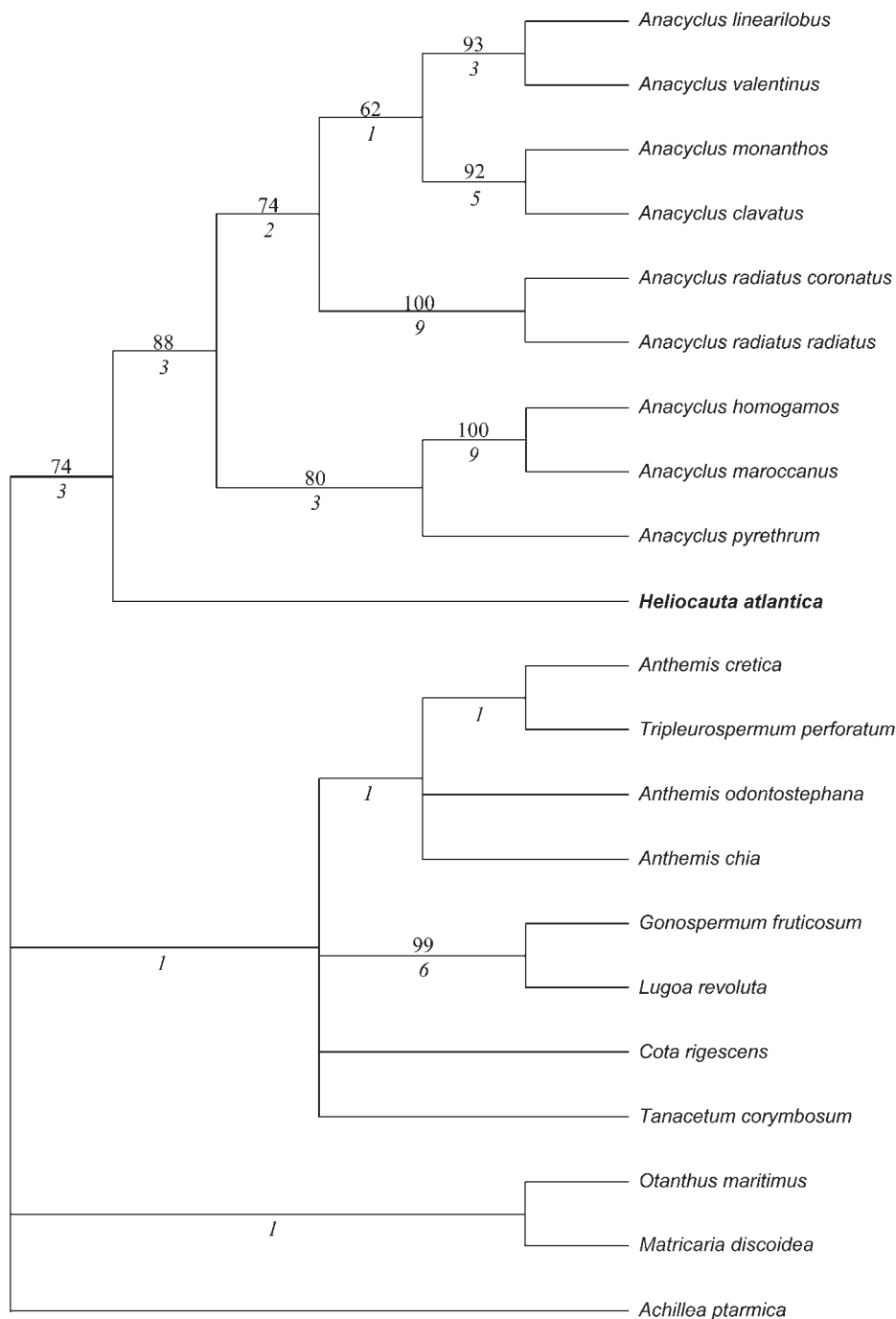


Fig. 5. Strict consensus tree of 11 equally most parsimonious trees (258 steps; CI = 0.55, autapomorphies excluded; RI = 0.6546), based on sequence information from nrDNA ITS of *Anacyclus* spp. and *Heliocauta* (data set 3). Numbers above the lines are bootstrap values, numbers below the lines are decay values.

and $1\% = 0.6\text{--}1.1$ Myr (*Lepidium*; Mummenhoff & al. 2004). Maximum divergence [based on the Kimura two parameter (K2P) model of DNA evolution] in the present *Anthemideae* data set was found to be 33.6% (between *Cotula coronopifolia* and *Lepidophorum repandum*), thus arguing for a minimum age of the tribe of 20.2 to 37 Myr (Tertiary, Late Eocene to Early Miocene), since it is hypothesized that the *Compositae* diverged from their sister family (*Calyceraceae*) in South America in the Late Eocene to Early Oligocene (c. 38 Myr; DeVore & Stuessy 1995, Bohm & Stuessy 2001) and that from these early origins in South America have rapidly dispersed into the rest of the world giving rise to the present subfamilies and tribes until the Middle Miocene (c. 10 Myr; Graham 1996), a minimum age of *Anthemideae* of around 20 Myr appears more plausible than the upper boundary of 37 Myr. Taking into account, however, that the earliest fossil pollen record of representatives of this tribe is based on an *Artemisia* type from Late Oligocene (Europe) to Early (western North America) or Middle Miocene (eastern North America) as given in Graham (1996), and following molecular phylogenies (Watson & al. 2000) that point to a S African origin of the tribe (from where data on fossil pollen are lacking) and a relatively derived position of *Artemisiinae* within the tribe, we have to assume that the divergence of the southern hemisphere genera around *Cotula* and the rest of the tribe dates to at least the Middle Oligocene (c. 25 Myr), thus resulting in a nrDNA ITS divergence rate of $1\% = 0.75$ Myr for the *Anthemideae*.

Nuclear ribosomal DNA ITS sequence divergence within the monophyletic group of Mediterranean and Eurasian genera (characterized by the 17bp deletion in ITS2) ranges from 0.4 to 20.4% (10.8% mean; K2P sequence divergences). Using the rate calculated above as a rough estimate of the age of the Mediterranean and Eurasian lineage under study, we could assume that this monophyletic group dates back to around 15 Myr; hence to a Tertiary (Middle Miocene) origin.

Sequence divergence within the monophyletic group of genera with a western Mediterranean centre of distribution was found to range from 0.4 to 19.7% (11.47% mean), indicating that this clade characterized by the 5bp deletion in cpDNA *trnL/trnF* IGS has its origin around 14.8 Myr ago (Tertiary, Middle Miocene). This may show that the deep split between genera with a predominantly eastern Mediterranean and Eurasian distribution (the species-rich genera *Achillea*, *Anthemis/Cota*, *Tanacetum*) and those genera with a predominantly or exclusively western Mediterranean distribution (e.g. *Lepidophorum*, *Leucanthemum*, *Lonas*, *Santolina*) emerged soon after the diversification of the whole monophyletic group of Mediterranean and western Eurasian *Anthemideae* (see above). Further, it is tempting to speculate on the Late Miocene (Messinian, i.e. 5.3–5.6 Myr) desiccation of the Mediterranean basin (“Messinian salinity crisis”, Hsü 1972) and its following flooding as a driving force of diversification in both lineages (species-richness in *Achillea*, *Anthemis/Cota*, *Tanacetum*; genera-richness in the western Mediterranean clade): some arguments for this hypothesis may be the mean sequence divergence among the representatives of *Anthemis* and *Cota* being 5.84 and 4.13%, respectively (Oberprieler 2001, Lo Presti & Oberprieler, unpubl.) corresponding to mean ages of 4.4 and 3.1 Myr), the sequence divergence between *Tanacetum corymbosum* and *Gonospermum/Lugoa* (the latter two genera being endemics of the Canary Islands) of around 4.1% (~3.1 Myr), and the separation of *Leucanthemum* (Iberian peninsula, Central Europe) and *Rhodanthemum* (N Africa) at around 3.4 Myr (4.53% sequence divergence).

However, as may be seen from the different branch lengths in the phylograms from the ML and BI analyses, it appears reasonable that there is a considerable amount of rate heterogeneity in the molecular marker (nrDNA ITS) which may be attributable to the wealth of different life forms (annuals vs. perennials) and different breeding systems (selfing vs. outcrossing; cf. Uitz 1970) in the Mediterranean representatives of the *Anthemideae*. Therefore, the above-mentioned dating must be considered highly speculative until sound calibrations of a molecular clock for the ITS marker are made for this group.

Suprageneric classification. – The present results based on an extended data set of nrDNA ITS sequence information for nearly all genera of *Compositae-Anthemideae* found in the Mediterranean and adjacent Eurasian regions corroborate findings of former molecular studies (Francisco-Ortega & al. 1997, Oberprieler & Vogt 2000, Watson & al. 2000, Francisco-Ortega & al.

2001) showing that most of the subtribes erected or accepted by Bremer & Humphries (1993) based on morphological, anatomical, cytological and phytochemical evidence are far from being monophyletic assemblages. This is especially obvious for the subtribe *Matricariinae* to which two of the three genera of special interest of the present paper (i.e. *Brocchia* sub *Cotula*, *Endopappus*) were assigned by Bremer & Humphries (1993). Members of this subtribe are found scattered all over the phylogenetic trees based on nrDNA ITS sequences, with the southern hemisphere representatives (*Cotula*, *Leptinella* and *Cymbopappus*) close to the base of the tree and the northern hemisphere genera as members of the clade characterized by a 17bp deletion in ITS2. This shows that the subtribe is considerably polyphyletic and that the characters used by Bremer & Humphries (1993) to circumscribe this entity (achenes with myxogenic cells on abaxial surface and on the ribs of the adaxial surface, corona adaxially long) are highly homoplasious.

The same holds true for the subtribe *Tanacetinae*, which was provisionally adopted by Bremer & Humphries (1993) to comprise *Tanacetum* and some of its alleged close relatives, being aware, however, of the probable paraphyly of this entity. According to our present results it appears obvious that the *Tanacetinae* sensu Bremer & Humphries are even a polyphyletic assemblage of genera, with at least one of them (*Hippolytia*) being more closely related to members of the subtribe *Artemisiinae*. Following the phylogenetic reconstructions of Watson & al. (2000) based on ndhF sequence information, the genus *Lepidolopha* may be also closer related to this subtribe with its centre of distribution in central and eastern Asia. As noted by Bremer & Humphries (1993), both the genus *Tanacetum* and the generic complex around it is in need of detailed investigations since several other genera and even subtribes may have their sister group within *Tanacetum*. Molecular and morphological studies to solve this “key question” in the *Anthemideae* are on the way (Oberprieler, unpubl.) and may contribute to a more natural infratribal classification.

Brocchia Vis. – The phylogenetic position of *Brocchia cinerea* in the present analyses is unequivocal. In all trees based on nrDNA ITS sequence variation (MP, ML, BI analyses) the species is member of the monophyletic group of Mediterranean and western Eurasian genera (89 % bootstrap support in MP, PP = 0.6 in BI, see discussion above), but appears to be the first split-off at the base of this clade. While the latter receives little support from the MP analysis (bootstrap support < 50 %, 1 step decay), it is significantly backed by the BI analysis (PP = 0.95). On the other hand, due to the unresolved and badly supported topology received from the phylogenetic analysis (MP) of cpDNA *trnL/trnF* IGS sequence variation (Fig. 4), this marker contributes little to the further understanding of the relationships of *Brocchia*.

All nrDNA ITS analyses definitively argue against a close relationship of *Brocchia cinerea* with *Cotula* to which the species was ascribed by Delile (1813), but also in the most recent generic revision of the tribe by Bremer & Humphries (1993). The latter authors, however, already considered the species as “aberrant within *Cotula*” (Bremer & Humphries 1993: 158) and noted that “the matter requires further studies.” Support for the generic independence of *Brocchia* from *Cotula* comes from cytological evidence: while *Brocchia cinerea* was found to have a diploid chromosome number based on $x = 9$ (Reese 1957, Oberprieler & Vogt 1993), *Cotula* is characterized by an extensive ploidy series with a basic chromosome numbers of $x = 8, 9$, and 10. Additionally, the occurrence of 4-lobed corollas of tubular florets that are found in *Brocchia* and in *Cotula* does not point to a close relationship of these two genera since similar corollas are observed both in the southern hemisphere genera *Foveolina*, *Hillardia*, *Inezia*, *Leptinella*, *Lidbeckia*, *Myxopappus*, *Schistostephium*, *Oncosiphon*, *Soliva* and *Thaminophyllum* and in the Eurasian genera *Artemisiella*, *Filifolium*, *Lepidolopsis*, *Matricaria*, *Nananthea* and *Phaeostigma* (Bremer & Humphries 1993, Oberprieler & al., in press). Finally, achene morphological and anatomical features add further evidence to the distinction and the distant relationship of *Brocchia* and *Cotula*. Published (Bruhl & Quinn 1990) and unpublished (pers. obs.) studies show that all surveyed species of *Cotula* and its relative *Soliva*, along with most species of the equally closely related genus *Leptinella*, have dorso-ventrally flattened achenes with two distinct, lateral wing-like ribs, while

achenes of *Brocchia* are circular in cross-section and have around four inconspicuous lateral and adaxial ribs (Giroux 1930, Oberprieler, unpubl.). Furthermore, specialized (myxogenic) epicarpic cells are completely different in the two groups. While in *Cotula*, *Leptinella* and *Soliva* these cells contain a lumen that stains dark-blue with toluidine blue and that is surrounded by thickened outer and anticlinal walls with a gelatinous appearance, mucilaginous cells in *Brocchia* have a strongly reduced lumen and extremely thick outer walls, which readily swell and burst during the preparation of microtome sections. In their appearance and the described behaviour they appear to be very similar to mucilage cells found in many northern hemisphere genera of *Anthemideae*, such as in the so-called *Leucanthemum* group of genera (i.e. *Chlamydophora*, *Chrysanthoglossum*, *Coleostephus*, *Glossopappus*, *Leucanthemum*, *Mauranthemum*, *Plagiis* and *Rhodanthemum*). Similar mucilage cells, however, are also observed in *Matricaria* and the occurrence of 4-lobed corollas of tubular florets in some members of this genus (i.e. *M. aurea*, *M. dioscoidea* and *M. occidentalis*, Bremer & al. 1996) may further argue for a closer relationship with *Brocchia*. On the other hand, our present molecular data along with preliminary achene anatomical comparisons of other *Brocchia* (pericarp with vascular bundles in two of the four inconspicuous ribs) with *Matricaria recutita* (vascular bundles in all of the two lateral and three adaxial ribs) point to the independence of the two entities from each other.

Endopappus Sch. Bip. – In their cladistic analysis of *Compositae-Anthemideae* based on morphological, anatomical, phytochemical and cytological information, Bremer & Humphries (1993: 154) found “no obvious close relatives” of *Endopappus*, because sister group relationships of this unispecific genus were unresolved in the strict consensus tree of their analysis of subtribe *Matricariinae*. Based on the external shape of the achenes with two lateral and one adaxial thick and protruding rib they considered some closer relationship to *Tripleurospermum* (Bremer & Humphries 1993). There is, however, no support of this interpretation from our present molecular data. While *Tripleurospermum perforatum* is found among members of genera with a more eastern Mediterranean and/or Eurasian distribution (e.g. *Anthemis*, *Cota*, *Tanacetum*) and a tetrasporic embryo sac development (Harling 1950, 1951, 1960), *Endopappus macrocarpus* appears to be a member of the monophyletic group of genera characterized by the diagnostic 5bp-deletion in cpDNA *trnL/trnF* IGS and by a distinctly western-centred distribution range (e.g. *Argyranthemum* group, *Santolina* group, *Leucanthemum* group). The position of *Endopappus* within this monophyletic group, however, is highly unstable because high bootstrap and PP support values are only found for the surrounding monophyletic assemblages of the *Leucanthemum* group and the *Argyranthemum* group of genera. This uncertainty it shares with two other unispecific genera of this monophyletic group, i.e. *Lepidophorum* and *Lonas*, both being also annuals and having their centre of distribution in the western Mediterranean region. Further investigations of molecular, morphological and anatomical characters are needed to solve these problems of generic relationships.

Heliocauta Humphries – Originally described as a member of the genus *Anacyclus* by Litardière & Maire (1924), *A. atlantica* Litard. & Maire was moved to an independent genus by Humphries (1977). In this publication, Humphries presents also a comprehensive discussion of the generic relationship of *H. atlantica*, mainly using morphological and anatomical characters of the achene and phytochemical data provided by Greger (1977). According to this discussion, the closest relationships of *Heliocauta* should be with *Achillea*, mainly based on the joint occurrence of only moderately flattened achenes with two vascular bundles in the pericarp, the lack of wing-like ribs, and an identical flavonoid profile (Greger 1977). On the other hand, Humphries (1977) refrained from inclusion of *Heliocauta* in *Achillea* because of the position of vascular bundles in the ribs of achenes, the scalariform thickenings of epicarpic cells, and the occurrence of additional pericarpic ribs and of a small, erose apical corona in achenes of *Heliocauta*. Additionally, Humphries (1977) found that the epicarpic mucilage cells of *Heliocauta* were rather filled with dark resin instead of being colourless as in *Anacyclus*, *Matricaria*, *Tanacetum* or *Achillea*; in this respect he suggested also a closer relationship with the genus *Sclerorhachis* from Iran and Afghanistan where similar specialized epicarpic cells are observed.

In contrast to the findings of Humphries (1977), Bremer & Humphries (1993) in their generic monograph of the *Compositae-Anthemideae* placed *Heliocauta* neither in their subtribe *Achilleinae* (including *Achillea* and *Anacyclus*) nor in their subtribe *Handeliinae* (including *Sclerorhachis*) but in the highly artificial subtribe *Tanacetinae* for which they assumed a paraphyletic nature. Within this subtribe, the authors discuss the genus *Hippolytia* (central Asia, Mongolia, China, Himalaya) as another possible relative of *Heliocauta* and/or a joint close relationship of the two genera with some representatives of *Tanacetum* (Bremer & Humphries 1993).

According to the present results based on nrDNA ITS and cpDNA *trnL/trnF* IGS sequence variation the situation appears to be quite clear. Neither the alleged close relationship of *Heliocauta* with *Achillea* nor with *Tanacetum* or *Hippolytia* is found. In the case of the latter genus, the occurrence of a 17bp deletion in ITS2 in *Heliocauta* places it in the monophyletic group of strictly Mediterranean and Eurasian genera and rules out a close relationship with *Hippolytia*, which is consistently placed among the genera of subtribe *Artemisiinae* with rather eastern and central Asian centres of distribution. Following unpublished results (Oberprieler, in prep.), the genus *Sclerorhachis* is also characterized by the lack of the mentioned deletion and is therefore not available as candidate for a close relative, accordingly. Conversely, the analyses suggest a (moderately supported) sister group relationship of *Heliocauta* with *Anacyclus*, which was ruled out by both Humphries (1977) and Bremer & Humphries (1993). Indeed, there are a number of differences between the two genera. Especially the strongly dorso-ventrally flattened achenes of *Anacyclus* species contrast conspicuously with the only moderately flattened achenes in *Heliocauta*. Furthermore, the formation of stolons and the premorse caudex of the latter are also not observed in *Anacyclus* representatives, which are either characterized by an annual life form or by solitary, woody rootstocks in the sole perennial species *A. pyrethrum*. Humphries (1977) mentions also differences in the shape and texture of receptacular scales (paleas), in the shape of corolla lobes of tubular florets, and in achene anatomical features. However, there are also characters that may serve as synapomorphies for the clade of *Heliocauta* and *Anacyclus* seen in the molecular phylogenetic reconstructions. First, in both genera the number of vascular strands in the pericarp is reduced to two (sometimes three in *Anacyclus*; Khandijan 1992, pers. obs.) while in *Anthemis*, *Cota*, *Tanacetum* and *Tripleurospermum* we observe five bundles in the first three genera (Oberprieler 1998) and three in the last (Kynčlová 1970, Reitbrecht 1974). Since the reduction to two (or sometimes three) vascular bundles also appears in some representatives of *Achillea* (Briquet 1916, Reitbrecht 1974, Khandijan 1992) but not in *Otanthus* and *Matricaria* (4-5 vascular bundles; Kynčlová 1970, Reitbrecht 1974, Khandijan 1992) this monophyletic group may also comprise the former genus. Second, while cotyledons are dorso-ventrally orientated in *Anacyclus* and *Heliocauta* (together with *Otanthus* and *Cota*; Reitbrecht 1974, Humphries 1977, pers. obs.), laterally (transversely) orientated ones are observed in *Achillea* (Reitbrecht 1974) and *Matricaria* (Kynčlová 1970, Reitbrecht 1974) while the character is variable within *Anthemis* and *Tripleurospermum* (Reitbrecht 1974; pers. obs.). Again, a comprehensive morphological and anatomical study together with further information from additional molecular markers is needed in the case of *Heliocauta* to have stronger evidence for its taxonomic placement. For the time being, the position as sister to *Anacyclus* seen in the present molecular study seems plausible and supported by some achene anatomical features. Additionally, inclusion of genera with hitherto unknown nrDNA ITS sequence information (especially of *Leucocyclus*; Oberprieler, in prep.) may further clarify the picture considerably.

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