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A phylogenetic analysis of *Chamaemelum* Mill. (Compositae: Anthemideae) and related genera based upon nrDNA ITS and cpDNA *trn*L/*trn*F IGS sequence variation

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Sequences of the nrDNA internal transcribed spacer (ITS) region and the cpDNA *trn*L/*trn*F intergenic spacer (IGS) region were analysed for 11 species of *Chamaemelum* Mill. and its related genera *Cladanthus* Cass., *Mecomischus* Coss. ex Benth., *Rhetinolepis* Coss., and *Santolina* L. (Compositae, Anthemideae) to study the infrageneric taxonomy and phylogenetic relationships of *Chamaemelum* and clarify the proper taxonomic position of the enigmatic North African endemic *Santolina africana* Jord. & Fourr. The results suggest that *Chamaemelum* in its traditional circumscription is at best paraphyletic, with *Cladanthus arabicus* (L.) Cass. consistently nested within it. Additionally, the subdivision of *Chamaemelum* into two monophyletic entities [*Ch.* sect. *Chamaemelum* with *Ch. fuscatum* (Brot.) Vasc. and *Ch. nobile* (L.) All.*; Ch.* sect. *Santolinopsis* Benedí with *Ch. eriolepis* (Coss. ex Maire) Benedí, *Ch. flahaultii* (Emb.) Benedí, and *Ch. scariosum* (Ball) Benedí] based on floret and achene morphological features is corroborated by the phylogenetic analysis of molecular data. Finally, the correct classification of *Santolina africana* as a member of the genus *Santolina* is further demonstrated. *Botanical Journal of the Linnean Society*, 2002, **138**, 255–273.

ADDITIONAL KEYWORDS: Asteraceae – *Cladanthus* – *Mecomischus* – molecular phylogeny – *Rhetinolepis* – *Santolina*.

INTRODUCTION

Chamaemelum Mill. is a small Mediterranean genus of the Compositae–Anthemideae which comprises six species according to the most recent generic treatment of the tribe (Bremer & Humphries, 1993). While three of these species are geographically restricted endemics of north-west Africa [*Ch. eriolepis* (Coss. ex Maire) Benedí, *Ch. flahaultii* (Emb.) Benedí, and *Ch. scariosum* (Ball) Benedí], the other representatives are more widespread in the western Mediterranean and western European areas [*Ch. fuscatum* (Brot.) Vasc. and *Ch. nobile* (L.) All.] or throughout the whole Mediterranean and adjacent regions [*Ch. mixtum* (L.) All.].

Beginning with Linnaeus (1753), who treated all species of *Chamaemelum* known at that time as members of the genus *Anthemis* L., a close relationship between the two genera is described in most treatments of the Anthemideae throughout the 18th, 19th and 20th centuries. Shortly after *Species Plantarum* (Linnaeus, 1753), Miller (1754) validated the generic name *Chamaemelum* (Druce, 1914) for some *Anthemis* species cultivated in English gardens. In Miller (1768), the Linnean generic name *Anthemis* is adopted and *Chamaemelum* treated as a synonym. A few decades later, Necker (1790), Gaertner (1791), and Moench (1794) revived the name *Chamaemelum* to place some former *Anthemis* species with apically rounded achenes into a separate genus. Although Cassini (1817: 83) did not recognize these two entities on a level higher than subgenera, he later on contributed to the excessive dismemberment of *Anthemis* into a number of segregated genera (i.e. *Anthemis*, *Chamaemelum*, *Cladanthus Maruta*, *Ormenis*, *Lepidophorum*, Cassini 1823).

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Further generic names for taxa treated presently under *Chamaemelum* were added by Cassini [1825; *Marcelia* Cass. for the discoid *Anacyclus aureus* L. = *Ch. nobile* forma *discoideum* (Boiss. ex Willk. & Lange) Benedí] and Webb [1838; *Perideraea* Webb for *Anthemis fuscata* Brot. = *Ch. fuscatum* (Brot.) Vasc.]. Moris (1840–1843) was the first to note that the basally saccate florets observed by Cassini in *Ormenis mixta* (L.) Dumort. [= *Ch. mixtum* (L.) All.] are also found in *Maruta fuscata* (Brot.) DC. [= *Ch. fuscatum* (Brot.)Vasc.] and *Anthemis nobilis* L. [= *Ch. nobile* (L.) All.]. He united them all under the generic name *Maruta*, foreshadowing to some extent the present circumscription of the genus *Chamaemelum*, except that he also included *Anthemis cotula*, the type of *Maruta*, a proper *Anthemis* species in our present understanding (e.g. Oberprieler, 1998).

Problems relating to the correct demarcation of *Chamaemelum* against *Anthemis* owe their solution to Schultz's revised taxonomy of the Anthemideae (in Schnizlein, 1854: 69–70; Schultz, 1860) in which he proposed a subdivision of the tribe into six subtribes, mainly on carpological grounds, with members of *Anthemis* assigned to the subtribes Cotinae and Anthemidinae, and members of *Chamaemelum* (at that time called *Ormenis*) along with the unispecific *Cladanthus* forming subtribe Ormenidae. Despite attempts to re-establish the Linnean circumscription of *Anthemis* (i.e. including members of *Chamaemelum*) in the following decades (e.g. Bentham & Hooker, 1873; Hoffmann, 1891–1892), the generic independence of *Chamaemelum (Ormenis)* was confirmed by Briquet's (1916) meticulous achene anatomical studies on representatives of *Anthemis*, *Chamaemelum* and *Santolina.* He showed that achenes of *Chamaemelum* have a very thin and specialized pericarp consisting of large myxogenic cells in longitudinal rows, while pericarps of *Anthemis* and *Santolina* species were thick and sclerenchymatous (Briquet, 1916). Despite these carpological findings, Harling's (1960) embryological studies showed that *Anthemis* is characterized by a tetrasporic embryo sac while it is monosporic in *Chamaemelum*. Phytochemical differences between the two genera also supported the phylogenetic proximity of the two genera (Bohlmann *et al*., 1965; Bohlmann & Zdero, 1966; Heywood & Humphries, 1977). This view has been corroborated by crossing data of Mitsuoka & Ehrendorfer (1972) who found that some *Anthemis* species were much more easily crossed with a *Chamaemelum* representative than with congeners.

Conversely, Bremer & Humphries (1993) elaborated a subtribal classification of the Anthemideae using all morphological, embryological and phytochemical information available at that time in a cladistic study. This classification featured *Anthemis* together with the unispecific *Nananthea* DC. as the only members of the subtribe Anthemidinae, while *Chamaemelum* was considered to be a member of the subtribe Achilleinae. Molecular studies in the Anthemideae (Francisco-Ortega *et al*., 1997; Oberprieler & Vogt, 2000; Watson *et al*., 2000) revealed, however, that most of the subtribes proposed by Bremer & Humphries (1993) are not monophyletic. The most comprehensive molecular study relating to Mediterranean Anthemideae by Oberprieler & Vogt (2000) showed that the genera of Achilleinae *sensu* Bremer & Humphries (1993) fall into two unrelated groups: while *Achillea* L., *Anacyclus* L. and *Otanthus* Hoffmanns & Link (and presumably also the unispecific genus *Leucocyclus* Boiss. not included in the cited study, 'Achilleinae I') were members of a strongly supported monophyletic group together with *Anthemis*, *Matricaria* L., *Tanacetum* L. and *Tripleurospermum* Sch. Bip., the other five members of this subtribe (i.e. *Chamaemelum*, *Cladanthus*, *Mecomischus* Coss. ex Benth., *Rhetinolepis* Coss. and *Santolina*, 'Achilleinae II') formed an equally well supported monophyletic group within a clade comprising subtribe Chrysantheminae and parts of subtribes Leucantheminae and Matricariinae *sensu* Bremer & Humphries (1993).

While the above-mentioned molecular studies provide strong support for the placement of *Chamaemelum* within an as yet unelaborated new classification of Anthemideae, little is known either of its infrageneric taxonomy or of its phylogenetic relationships with other members of 'Achilleinae II'. The former is mainly due to the fact that a complete revision of the genus is still lacking. While the three widespread species *Ch. fuscatum*, *Ch. mixtum* and *Ch. nobile* were revised both for the treatment of *Chamaemelum* in *Flora Europaea* (Tutin *et al*., 1976) and for the Iberian Peninsula and the Balearic Islands (Benedí González, 1988a), the north African species *Ch. eriolepis*, *Ch. flahaultii* and *Ch. scariosum* were only transferred from *Ormenis* to *Chamaemelum* by Benedí González (1986, 1988b). However, in the latest publication, Benedí González (1988b) additionally proposed a subdivision of the genus into two sections, *Ch.* sect. *Chamaemelum* and sect. *Santolinopsis* Benedí, based on the orientation of the stylopodium (i.e. 'coronet' in Kynčlová, 1970; 'Nektarium' in Vogt, 1991; 'discus' in Benedí González, 1988b; Oberprieler, 1998) at the achene apex, the shape of pales and the dissection of leaves.

In regard to the phylogenetic relationships with other members of 'Achilleinae II', cladistic analyses of morphological data (Bremer & Humphries, 1993) suggest a sister-group relationship of *Chamaemelum* with a clade formed by *Cladanthus* + *Rhetinolepis.* On the other hand, molecular data (Oberprieler & Vogt, 2000) clearly indicate the distinction of *Chamaemelum*

+ *Cladanthus* from *Rhetinolepis*. Because *Chamaemelum* was only represented by the single species *Ch. nobile* in the mentioned study, little inference into the phylogenetic relationships of the genus was possible. Extensive field work in north Africa (Morocco, Tunisia), however, has yielded a complete sample of *Chamaemelum* species for us to address questions related to the infrageneric taxonomy of the genus and its phylogenetic relationships by molecular methods. Additionally, plant material of *Santolina africana* Jord. & Fourr. collected in Tunisia made it possible to extend the present study to questions related to the classification of this enigmatic species which had been originally described as a member of *Santolina* by Jordan & Fourreau (1868–1903), re-described as *Ormenis pseudosantolina* Maire by Maire (1926), combined as *Ormenis africana* (Jord. & Fourr.) Litard. & Maire in Jahandiez & Maire (1934) with *Ormenis pseudosantolina* in synonymy, and finally omitted from all further considerations of the two genera (e.g. Benedí González, 1986, 1988b; Bremer & Humphries, 1993). The problematic classification of this species results from combined characters typical for *Santolina* (suffruticose habit, discoid capitula) and *Chamaemelum* (achenes with pericarp formed of myxogenic cells in longitudinal rows).

The aim of the present molecular study was thus threefold: (1) the analysis of the infrageneric taxonomy of *Chamaemelum* (2) the clarification of phylogenetic relationships of *Chamaemelum* within 'Achilleinae II', and (3) the proper placement of *Santolina africana* within this group of genera.

MATERIAL AND METHODS

Plant material

Eleven species from the five genera of 'Achilleinae II' (*Chamaemelum*, *Cladanthus*, *Mecomischus*, *Rhetinolepis* and *Santolina*) were included as ingroup taxa. Plant material came from all presently accepted species of *Chamaemelum* (six species from seven populations for nrDNA analyses and from eight populations for cpDNA analyses, respectively), *Cladanthus* (one species), and *Rhetinolepis* (one species), while *Mecomischus* (two species) was represented by *M. halimifolius* (Munby) Hochreutiner and *Santolina* (eight species according to Bremer & Humphries, 1993) by *S. rosmarinifolia* L and *S. africana* (Table 1). A more comprehensive molecular study of Mediterranean and Eurasian genera of the Anthemideae showed uncertain sister group relationships of 'Achilleinae II' with other Mediterranean representatives of the tribe (Oberprieler & Vogt, 2000), and thus a broad sample of genera representing subtribes Chrysantheminae, Leucantheminae and Matricariinae *sensu* Bremer & Humphries (1993) in the Mediterranaean region were used as outgroups for the present analyses. For *Chrysanthemum coronarium*, nrDNA ITS sequence information was used from Francisco-Ortega *et al*. (1997), while sequences of *Aaronsohnia pubescens*, *Chamaemelum nobile*, *Chlamydophora tridentata*, *Chrysanthemum coronarium* (cpDNA *trn*L/*trn*F IGS), *Cladanthus arabicus*, *Lepidophorum repandum*, *Leucanthemum vulgare*, *Lonas annua*, *Mecomischus halimifolius*, *Nivellea nivellei*, *Otospermum glabrum*, *Phalacrocarpum oppositifolium*, *Rhetinolepis lonadioides* and *Santolina rosmarinifolia* were previously published by Oberprieler & Vogt (2000).

DNA isolation, PCR amplification, sequencing

DNA was extracted from 30–40 mg dried and crushed leaf material according to Hellwig *et al*. (1999) and Oberprieler & Vogt (2000) using Qiagen tip-20 columns (Qiagen Inc., Hilden, Germany). Amplification of both nrDNA ITS and cpDNA *trn*L/*trn*F IGS was performed using primers by White *et al*. (1990) and Taberlet *et al*. (1991) following the protocols of Oberprieler & Vogt (2000). Amplified products were purified with a Qiaquick PCR cleaning column and filtration kit (Qiagen Inc.). Cycle sequencing was performed using IRD-labelled primers (MWG-Biotech AG, Ebersberg, Germany) and the ThermoSequenase labelled primer cycle sequencing kit (Amersham Pharmacia) following the protocol of Facius *et al*. (1999). The prepared cycle sequencing products were analysed on a LI-COR DNA sequencer 4200. All new sequences were submitted to the EMBL sequence data bank (Table 1).

Sequence alignment and cladistic analyses

Sequences were aligned using CLUSTAL W (Thompson, *et al.,* 1994) and alignments subsequently corrected manually (Appendices 1 and 2). Gaps in aligned sequences were treated as missing data, but phylogenetically informative indels were coded as additional binary characters. Overlapping deletions which were not identical were treated as independent characters. In cases when a coded deletion enclosed other deletions, the latter were coded as missing data.

Maximum parsimony (MP) analyses of the data set were performed using the heuristic search algorithm of PAUP* version 4.0b2a (Swofford, 1999) with ACCTRAN, MULPARS, TBR branch swapping for 500 random addition sequence replicates. Character states were specified unordered and unweighted. Support for clades was evaluated using bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988). Bootstrap analyses were performed using the following settings: 100 bootstrap replicates, simple addition sequence, ACCTRAN, TBR branch swapping, MULPARS,

Table 1. List of taxa and sources of plant material analysed. The subtribal classification follows Bremer & Humphries (1993). With the exception of sequence accession numbers (GenBank) cited from Francisco-Ortega *et al*. (1997), all other accession numbers are for the EMBL data base

		EMBL/GenBank accession number		
Taxon	Accession	ITS1	ITS2	*trn*L/F
Achilleinae				
Chamaemelum Mill.				
Ch. eriolepis (Coss. ex Maire) Benedí	Morocco, Ouarzazate - Tazenakht, 12.v.1993, *Vogt 11713 & Oberprieler 6161* (B).	AJ420084	AJ420091	AJ420098
Ch. flahaultii (Emb.) Benedí	Morocco, Djebel Lekst, pass Tizi-n-Tagounit, 15.v.1993, *Vogt 11781 & Oberprieler 6229* (B).	AJ420085	AJ420092	AJ420099
Ch. fuscatum (Brot.) Vasc.	Spain, Cádiz, Rio Palmones near Los Barrios, 13.iii.1990, *Sundermeier A11* (Cult. in Hort. Bot. Berol. 166-21-92-10; B).	AJ420086	AJ420093	AJ420100
Ch. mixtum (L.) All.	Morocco, Ceuta – Tetouan, 7 km S Ceuta, 22.vi.1989, *Oberprieler 1746* (B).	AJ420087	AJ420094	–
Ch. mixtum (L.) All.	Italy, Reggio di Calabria, Gallico – Gambarrie, 27.v.1994, *Vogt 13979 & Oberprieler 8284* (B).	AJ420088	AJ420095	AJ420101
Ch. mixtum (L.) All.	Morocco, Ksar-es-Seghir – Tetouan, 17.iv.1993, *Vogt 9766 & Oberprieler 4214* (B).	–	–	AJ420102
Ch. mixtum (L.) All.	Tunisia, Aïn Draham – Tabarka, 20.v.1994, *Vogt 13721 & Oberprieler 8026* (B).	–	–	AJ420103
Ch. nobile (L.) All.	Oberprieler & Vogt (2000)	AJ3296382	AJ3296417	AJ3296452
Ch. scariosum (Ball) Benedí	Morocco, Taroudannt – Marrakech, Tizi-*n*-test, 12.vii.1989, *Oberprieler 3589* (B).	AJ420089	AJ420096	AJ420104
Cladanthus Cass.				
C. arabicus (L.) Cass.	Oberprieler & Vogt (2000)	AJ3296383	AJ3296418	AJ3296453
Mecomischus Coss. ex Benth.				
M. halimifolius (Munby) Hochr.	Oberprieler & Vogt (2000)	AJ3296384	AJ3296419	AL3296455
Rhetinolepis Coss.				
Rh. lonadioides Coss.	Oberprieler & Vogt (2000)	AJ3296386	AJ3296421	AJ3296456
Santolina L.				
S. rosmarinifolia L.	Oberprieler & Vogt (2000)	AJ3296387	AJ3296422	AJ3296457
S. africana Jord. & Fourr.	Tunisia, Siliana, *c*. 16 km S Makthar, 18.v.1994, *Vogt 13453 & Oberprieler 7758* (B).	AJ420090	AJ420097	AJ420105
Chrysantheminae				
Chrysanthemum L.				
Ch. coronarium L.	Francisco-Ortega *et al*. (1997).	L77741	L77741	
	Oberprieler & Vogt (2000)			AJ3296462
Leucantheminae				
Chlamydophora Ehrenb. ex Less.				
C. tridentata (Delile) Less.	Oberprieler & Vogt (2000)	AJ3296391	AJ3296426	AJ3296464

Table 1. *Continued*

Taxon	Accession	EMBL/GenBank accession number		
		ITS1	ITS2	*trn*L/F
Lepidophorum Cass.				
L. repandum (L.) DC.	Oberprieler & Vogt (2000)	AJ3296395	AJ3296430	AJ3296469
Leucanthemum Mill.				
L. vulgare ssp. *pujiulae* Sennen	Oberprieler & Vogt (2000)	AJ3296398	AJ3296433	AJ3296472
Nivellea B.H.Wilcox, K.Bremer & Humphries				
N. nivellei (Braun-Blanq. & Maire) B.H.Wilcox, K.Bremer & Humphries	Oberprieler & Vogt (2000)	AJ3296400	AJ3296435	AJ3296474
Phalacrocarpum (DC.) Willk.				
P. oppositifolium (Brot.) Willk.	Oberprieler & Vogt (2000)	AJ3296401	AJ3296436	AJ3296475
Matricariinae				
Aaronsohnia Warb. & Eig				
A. pubescens (Desf.) K.Bremer & Humphries	Oberprieler & Vogt (2000)	AJ3296408	AJ3296443	AJ3296483
Lonas Adans.				
L. annua (L.) Vines & Druce	Oberprieler & Vogt (2000)	AJ3296411	AJ3296446	AJ3296486
Otospermum Willk.				
O. glabrum (Lag.) Willk.	Oberprieler & Vogt (2000)	AJ3296413	AJ3296448	AJ3296488

MAXTREES = 1000 per replicate. Decay analyses were carried out using AutoDecay 3.0 (Eriksson & Wikström, 1995). For each constraint analysis, ten random addition sequence replicates were performed. Character evolution (perennial *vs* annual life cycle) was studied using MacClade version 4.0 (Maddison & Maddison, 2000).

Testing for phylogenetic conflict among data sets

Testing for data set incompatibilities among nrDNA ITS and cpDNA IGS sequence information was done using nonparametric Wilcoxon signed ranks (WSR) tests as proposed by Templeton (Templeton, 1983; Larson, 1994). Following the test strategy of Mason-Gamer & Kellogg (1996), this was done by application of the WSR test to a comparison of the tree length of the most-parsimonious tree(s) of one of the two data sets with the number of changes required by the same data set on a constraint tree. Constraint trees were based on trees from the rival data set and included (1) the rival strict consensus tree, (2) the 70% majority rule bootstrap rival tree, representing the combination of all nodes with moderate to strong support ('strong nodes tree'), and (3) the strict consensus topology from the combined analysis of the two data sets. Like Larson (1994) and Mason-Gamer & Kellogg (1996), we used two-tailed probability values which give conservative estimates of significance values.

RESULTS

Nuclear ribosomal DNA ITS (Data set 1)

The nrDNA ITS alignment is 474 bp long (263 bp for ITS1 and 211 bp for ITS2) with 179 variable positions including 113 phylogenetically informative substitutions and 18 phylogenetically informative indels of 1 bp length (Appendix 1). The heuristic parsimony search yielded a single most-parsimonious tree (MPT) with a length of 392 steps, a consistency index (CI, with autapomorphies excluded) of 0.515, and a retention index (RI) of 0.558 (Fig. 1). Members of the ingroup (i.e. *Chamaemelum*, *Cladanthus*, *Mecomischus*, *Rhetinolepis* and *Santolina*) form a monophyletic group but the support for this group is very weak (decay value of 1 step, no bootstrap support). Within the ingroup, it is obvious that nrDNA ITS sequence

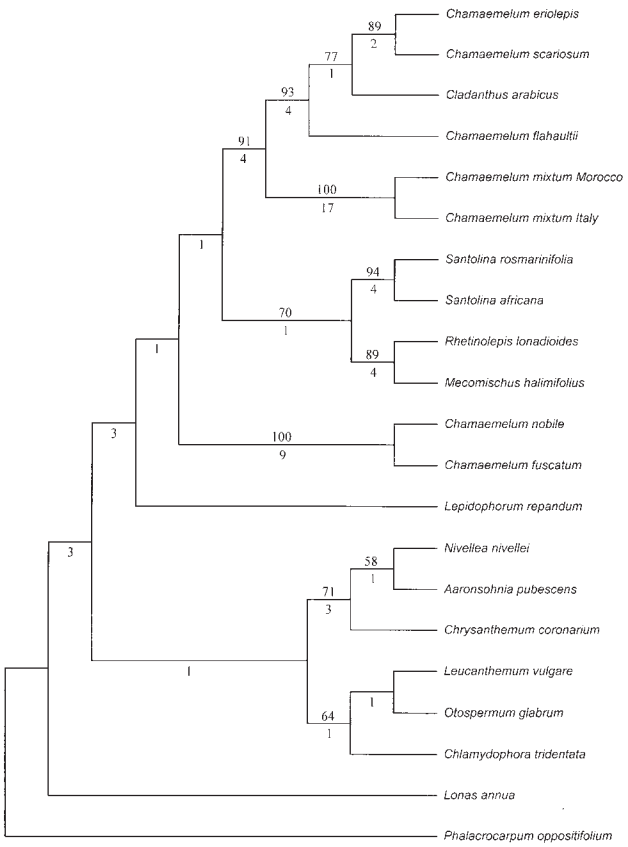

Figure 1. The single most-parsimonious tree (392 steps; CI = 0.515, autapomorphies excluded; RI = 0.558), based on nrDNA ITS sequence information (data set 1). Numbers above the lines are bootstrap values, numbers below the lines are decay values.

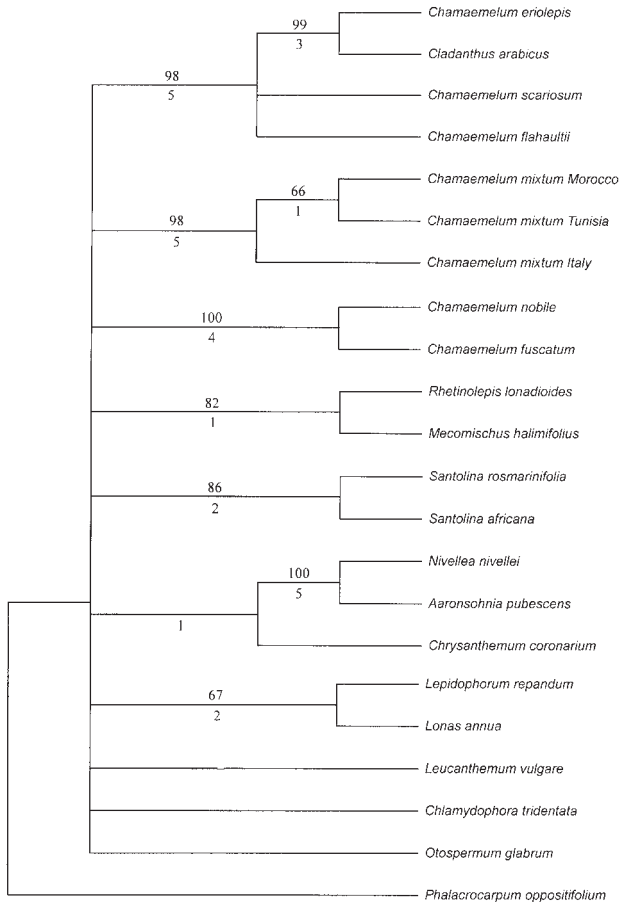

Figure 2. Strict consensus tree of 238 equally most-parsimonious trees (93 steps; CI = 0.768, autapomorphies excluded; RI = 0.838), based on sequence information from the cpDNA *trn*L/*trn*F IGS region (data set 2). Numbers above the lines are bootstrap values, numbers below the lines are decay values.

data do not support the monophyly of the genus *Chamaemelum* in its traditional circumscription: while *Ch. nobile* and *Ch. fuscatum* form a strongly supported monophyletic group (100% bootstrap, 9 steps decay) the other members of the genus form a comparatively well supported clade (91%, 4) which also includes *Cladanthus arabicus*. Further parsimony searches on the data set using constraint trees showed that a monophyletic genus *Chamaemelum* including *Cladanthus arabicus* would require only one extra step while a monophyletic genus *Chamaemelum* excluding the latter genus would lead to MPTs with a length of 404 steps (i.e. 12 extra steps). In relation to the other genera of the ingroup, the sister-group relationship of *Rhetinolepis* and *Mecomischus* (89%, 4) and the monophyly of the two *Santolina* representatives (94%, 4) is clearly demonstrated while the sister-group relationship of the former with the latter group (70%, 1) and the position relative to *Chamaemelum* remain unsettled.

Chloroplast DNA *trn*L/*trn*F IGS (data set 2)

The *trn*L/*trn*F IGS alignment is 404 bp long with 62 variable positions including 38 phylogenetically informative substitutions and 14 phylogenetically informative indels of one to 22 bp length (Appendix 2). The heuristic parsimony search yielded 238 equally most-parsimonious trees with a length of 93 steps, a consistency index (CI, with autapomorphies excluded) of 0.768, and a retention index (RI) of 0.838. The strict consensus tree of all MPTs is shown in Figure 2. While this marker fails to support the monophyly of the ingroup and the sister-group relationships within it, some terminal nodes receive considerably high bootstrap and decay values, most of them coinciding with monophyletic groups from the nrDNA ITS analysis. Both the sister-group relationship of *Chamaemelum*

nobile and *Ch. fuscatum* (100%, 4) and the monophyly of *Ch. eriolepis*, *Ch. flahaultii*, *Ch. scariosum* and *Cladanthus arabicus* (98%, 3) are strongly supported. However, in contrast to the ITS analysis, in which *C. arabicus* is sister to *Ch. scariosum* + *Ch. eriolepis*, *C. arabicus* is sister to *Ch. eriolepis* alone in the cpDNA analysis. Additionally, the sister-group relationship of the *Ch. eriolepis* clade with *Ch. mixtum* is not confirmed. Within *Ch. mixtum*, the north African accessions form a monophyletic group relative to the southern European accession, the result of an apomorphic 8 bp-deletion at alignment positions 204–211 (Appendix 2). Moderate bootstrap support and decay values characterize the sister-group relationships of *Rhetinolepis* and *Mecomischus* (82%, 1) and the monophyly of the two *Santolina* representatives (86%, 2). Constraint tree analyses indicate that a monophyletic genus *Chamaemelum* including *C. arabicus* would require three extra steps while a monophyletic genus *Chamaemelum* excluding *Cladanthus* would require nine extra steps.

Phylogenetic conflict among data sets

The Wilcoxon signed ranks (WSR) tests show that the nrDNA ITS data do not conflict with the cpDNA IGS consensus tree and the 'strong nodes tree' (see Material and Methods), nor with the consensus tree of the combined analysis of the two markers (Table 2). On the other hand, the cpDNA IGS data significantly reject the nrDNA ITS consensus tree ($P < 0.0075$). However, the borderline result ($0.0143 < P < 0.0578$) for the test of conflict between the same data set and the ITS 'strong nodes tree' indicates that this significant conflict is mainly due to the weakly (bootstrap values < 70%) supported nodes in the topology of the nrDNA ITS consensus tree. No significant conflict is observed between cpDNA IGS data and the consensus tree of the combined analysis of both markers. Therefore, these results argue for the legitimacy of a combined analysis.

Combined nrDNA ITS and cpDNA *trn*L/*trn*F IGS (Data set 3)

The heuristic parsimony search on this data set yielded 13 equally most-parsimonious trees with a length of 492 steps, a consistency index (CI, with autapomorphies excluded) of 0.5203, and a retention index (RI) of 0.5528. The strict consensus tree of all MPTs is shown in Figure 3. Its topology is very similar to the cladograms of the analyses based on the individual data sets. While the ingroup lacks support as a monphyletic group, most of the clades within this group are strongly supported, an exception being the clade of *Chamaemelum eriolepis* and *Cladanthus arabicus*. This reflects the controversial positions of these two annual species in the cladograms based on nrDNA ITS and cpDNA IGS data, respectively.

DISCUSSION

The two molecular markers used in the present analysis behave quite differently in the phylogenetic reconstructions: while the nrDNA ITS data set yields a single most-parsimonious tree (MPT) with relatively moderate support values (CI, with autapomorphies excluded = 0.515, RI = 0.558), the cpDNA *trn*L/*trn*F IGS data set appears to contain a weaker phylogenetic signal as reflected in the higher number of MPTs (238) and the low resolution in the consensus tree. On the other hand, this latter tree shows both a reasonably high CI and RI value (0.768 and 0.838, respectively). This agrees with observations made from the same two markers in a more extensive phylogenetic study

Table 2. Summary of Templeton (Wilcoxon signed ranks) test results for the two data sets and the constraint trees used in the analyses. The total gain, total loss and net gain of steps required by all characters on the most-parsimonious trees found given the indicated constraint tree relative to the steps required by the same set of characters on the most-parsimonious unconstrained trees are given, along with two-tailed probability values (P) for each test

		Number of steps			
Data set	Constraint	Gain	Loss	Net	P
nrDNA ITS	cpDNA IGS consensus tree	12–16	7–11	5	$0.2752 < P < 0.3718$
	cpDNA IGS strong nodes tree	2	0	2	0.1573
	Combined data consensus tree	2	0	2	0.1573
cpDNA IGS	nrDNA ITS consensus tree	10–11	0–1	10	$0.0016 < P < 0.0075$
	nrDNA ITS strong nodes tree	6–8	0–2	6	$0.0143 < P < 0.0578$
	Combined data consensus tree	1–4	0–3	1	$0.3173 < P < 0.7055$

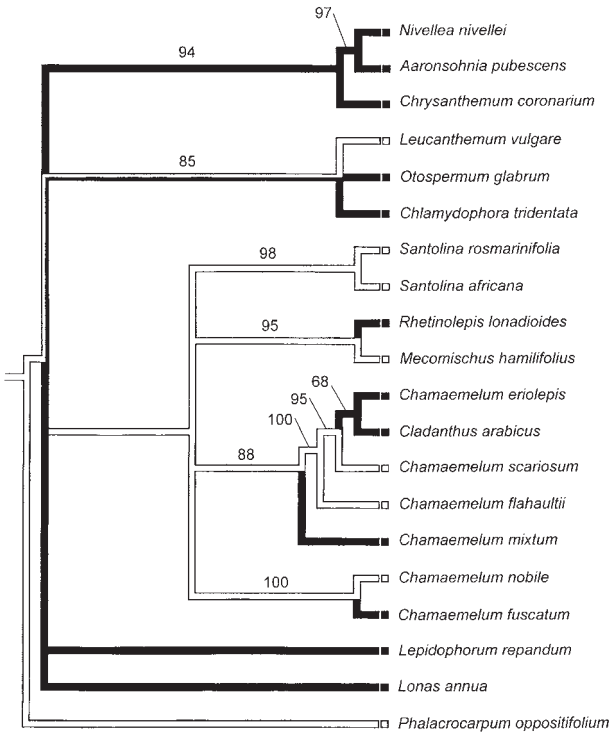

Figure 3. Strict consensus tree of 13 equally most-parsimonious trees (492 steps; CI = 0.6646, autapomorphies excluded; RI = 0.5528), based on the joint analysis of nrDNA ITS and cpDNA *trn*L/*trn*F IGS sequences (data set 3). Numbers above lines are bootstrap values. Branch shading depicts the inferred transitions in life cycle (open branches denote a perennial life history, solid branches an annual one) as reconstructed with MacClade 4.0 (Maddison & Maddison, 2000).

of Mediterranean Anthemideae (Oberprieler & Vogt, 2000) in which the authors suggested the different percentages of variable positions and phylogenetically informative characters in the data sets to be the possible reason. This seems also to be the case in the present data sets: the nrDNA ITS data set has 37.8% of the positions variable and 23.8% phylogenetically informative, and the cpDNA IGS data set has only 15.3% variable and 9.4% phylogenetically informative positions. In addition, there are differences between the two data sets with respect to the nature of informative indels: all indels are only one bp long in the nrDNA ITS data set, whereas indels in the cpDNA IGS are between one and 22 bp long (an uninformative deletion in *Otospermum glabrum* is even 89 bp). Overlapping gaps in the cpDNA alignment lead to their eventual coding as missing data. As a consequence of this gap coding, the phylogenetic power of the indels is partially reduced, without a corresponding increase in homoplasy. Therefore, differences in both the rate and nature of evolutionary change in the two DNA regions (nrDNA ITS: higher substitution rate, lower frequency of indel formation, smaller indels; cpDNA IGS: lower substitution rate, formation of more and larger indels) may be responsible for the observed differences in the phylogenetic reconstructions. With respect to the cpDNA IGS marker, these peculiarities of evolutionary change are the reason for its use both at the infraspecific level (e.g. phylogeographical studies of Fuji *et al*., 1997, 1999; differences between accessions of *Chamaemelum mixtum*, present study) where mainly indels are observed, and at higher taxonomic levels (e.g. Bayer & Starr, 1998, tribal interrelationships in Compositae; Bayer *et al*., 2000, phylogeny of South African Gnaphalieae) where nucleotide substitutions become relatively more relevant. A critical examination of the alignment of cpDNA IGS sequences in the present study group (see Appendix 2) reveals that a number of indels are connected with repetitive motives (e.g. alignment positions 54–64 *vs* 65–75; 124–131 *vs* 146–153; 127–131 *vs* 132–136; 288–291 *vs* 292–295). As Kelchner (2000) has pointed out when reviewing the evolution of non-coding chloroplast DNA and its consequences for phylogenetic reconstructions, this mechanism of sequence evolution may considerably decrease the quality of homology assessments. As a consequence, and despite the fact that the homology assessments in the present alignment appear to be trustworthy and in accordance with suggestions made by Kelchner & Clark (1997), Bayer *et al.* (2000) and Kelchner (2000) to improve their accuracy, it appears necessary to deal with the outcome of the phylogenetic reconstruction based on cpDNA IGS sequences more warily than with the nrDNA ITS phylogeny.

Despite the described differences between the two markers, the conflict between the cladograms of Figures 1 and 2 is minimal; most of the clades consistently found in all MPT from the analysis of the cpDNA IGS data set (Fig. 2) correspond to monophyletic groups found in the single MPT of the nrDNA ITS analysis. Additionally, most of these monophyletic groups are supported by moderate-to-high bootstrap and decay values in both analyses. Concerning the ingroup made up of *Chamaemelum* and related genera, the following clades are supported by both data sets: the group of *Chamaemelum eriolepis*, *Ch. flahaultii*, *Ch. scariosum* and *Cladanthus arabicus*; the sister-group relationships between the two *Santolina* species; between *Rhetinolepis* and *Mecomischus*; between *Chamaemelum nobile* and *Ch. fuscatum*; and the monophyly of the different accessions of *Chamaemelum mixtum*.

On the other hand, neither analysis resolves relationships between these monphyletic groups, thus limiting our ability to answer some of the questions

raised earlier, i.e. the monophyly of the ingroup ('Achilleinae II', i.e. the group of *Chamaemelum*, *Cladanthus*, *Mecomischus*, *Rhetinolepis* and *Santolina*) is not supported by either data set. This is in contrast to results of Oberprieler & Vogt (2000) in which the five taxa representing these genera were found to form a monophyletic group with high bootstrap support both in the analysis of nrDNA ITS sequence information (92%) and in the joint analysis of nrDNA ITS and cpDNA IGS data (93%). However, *Chamaemelum* was only represented by *Ch. nobile* which was found to be the sister-taxon to *Cladanthus arabicus*, while the other three genera formed another monophyletic group (Oberprieler & Vogt, 2000). It seems that the addition of other species of *Chamaemelum* not only changed sister-group relationships of *Ch. nobile* (now with *Ch. fuscatum*) and *Cladanthus arabicus* (now as a member of the clade around *Ch. eriolepis*), but also lowered support for the monophyly of the whole group of genera. There are good reasons, however, to prefer the placement of *Ch. nobile* and *Cladanthus arabicus* over that found in our previous study (Oberprieler & Vogt, 2000). First, bootstrap support for the placement of the two taxa is higher in the present analysis, both in the nrDNA ITS (100% and 93%, respectively, *vs* 82% in Oberprieler & Vogt 2000) and in the cpDNA IGS analysis (100% and 98%, respectively, no support in Oberprieler & Vogt, 2000). Second, there is morphological evidence for these groupings: ray florets are white in *Ch. nobile* and *Ch. fuscatum*, while those of *Cladanthus arabicus*, *Ch. eriolepis*, *Ch. flahaultii* and *Ch. scariosum* are orange or golden yellow. Third, *Ch. fuscatum* and *Ch. nobile* have achenes with a horizontal apical plate and a central stylopodium, flat to slightly carinate pales, and 2–3-pinnatisect leaves, while the achenes of *Cladanthus arabicus*, *Ch. eriolepis*, *Ch. flahaultii* and *Ch. scariosum* have a slanted apical plate with a sublateral stylopodium, strongly carinate pales, and dentate to 2-pinnatisect leaves. These two groups coincide largely with the two subgenera of *Chamaemelum* as proposed by Benedí González (1988b), with *Ch. fuscatum* and *Ch. nobile* on the one hand *(Chamaemelum* sect. *Chamaemelum)* and the yellow-rayed species of *Chamaemelum* together with *Ch. mixtum* on the other *(Chamaemelum* sect. *Santolinopsis* Benedí*)*. According to our present analysis, however, *Cladanthus arabicus* has to be included in the latter section, which makes *Chamaemelum* in its present circumscription at least paraphyletic.

The long-standing independence of *Cladanthus* from *Chamaemelum* is mainly attributable to the unique growth form of the former, which is characterized by an acrotonic ramification where lateral branches originate in the axils of foliage leaves immediately below the capitulum (Weberling & Reese, 1988), while the capitula of *Chamaemelum* species appear to be long pedunculate. This led Bremer & Humphries (1993) to describe the capitula of *Cladanthus* as 'sessile', and furthermore synapomorphic for the sister-group of *Cladanthus* and *Rhetinolepis*. However, if morphological data are scrutinized more intensively, the alleged sister-group relationship of these two genera becomes invalid: the capitula of *Rhetinolepis lonadioides* are not sessile but shortly pedunculate and arranged solitary or in few-headed corymbs, lateral branches (paracladia) originate at the bases of stems or along the stems but not immediately below capitula as in *Cladanthus*. The indumentum of *Rhetinolepis* consists of medifixed hairs, while hairs are basifixed in *Cladanthus* (as they are in all *Chamaemelum* species). Capitula of *Rhetinolepis* are discoid, while those of *Cladanthus* are radiate. Finally, both molecular markers used in the present study point to the remoteness of the two entities. Therefore, the unique growth form of *Cladanthus arabicus* is merely an autapomorphy of this species which has obscured the true phylogenetic relationships with the yellow-rayed *Chamaemelum* species since the establishment of this unispecific genus by Cassini (1816).

While the monophyly of the different geographical accessions of *Chamaemelum mixtum* is strongly supported by both markers, the position of this taxon relative to the other *Chamaemelum* species is controversial. In the nrDNA ITS analysis, *Ch. mixtum* forms the sister-group of the *Ch. eriolepis* clade with considerable support, while in the cpDNA IGS analysis, there is no consistently supported sister-group relationship with other groups. There are morphological characters which support the nrDNA ITS scenario: as a member of Benedí González's (1988b) *Ch.* sect. *Santolinopsis*, *Ch. mixtum* is also characterized by achenes with a slanted apical plate and a sublateral stylopodium, strongly carinate pales, and only dentate to 1–2-pinnatisect leaves. Additionally, it is true that ray florets in *Ch. mixtum* over large parts of its geographical range are white instead of orange or golden yellow, but these white ray florets always have a yellow base. Some populations in north Africa (Morocco, Algeria), however, even have completely yellow ray florets and were recognized as an independent variety, *Ch. mixtum* var. *aureum* (Durieu) Benedí. Therefore, speculations on the hybrid origin of *Ch. mixtum*, as one might deduce from the usually bicoloured ray florets in this species, are not supported by either morphological or molecular data.

Hybridization may have played a role within the just mentioned group of *Chamaemelum* sect. *Santolinopsis* and *Cladanthus arabicus*, as there is some conflict in the phylogenetic analyses of nrDNA ITS and cpDNA IGS. While in the former, *Ch. eriolepis*

is sister to *Ch. scariosum*, the latter analysis places it in a monophyletic group with *Cladanthus arabicus*. There is insufficient morphological evidence to differentiate between the two scenarios, but the shared annual life history in *Ch. eriolepis* and *C. arabicus* may argue for the second grouping if perennial life history is considered to be plesiomorphic (see reconstruction of character evolution in Fig. 3). Further studies using other molecular markers are needed to help clarify phylogenetic relationships in this monophyletic group of four closely related species, and may lead to the establishment of a model group for further research into interesting aspects of speciation in the Anthemideae, e.g. changes of life history, mating behaviour, or structural changes of chromosomes.

Connected with the failure of the present analyses to elucidate the monophyly of *Chamaemelum* (including *Cladanthus*) is the failure to provide evidence for the phylogenetic relationships with other members of 'Achilleinae II'. The sister-group relationships of the two *Santolina* species and of *Rhetinolepis* and *Mecomischus* receive considerable support from both molecular markers. On the other hand, the monophyly of *Mecomischus*, *Rhetinolepis* and *Santolina* as found in the single MPT based on nrDNA ITS sequence information is not supported, although there is support for this group based on morphology. While hairs (when present) are always basifixed in *Chamaemelum* and *Cladanthus*, *Santolina* and *Rhetinolepis* have indu-ments of medifixed hairs and *Mecomischus* is characterized by stellate hairs (the latter being some sort of special medifixed hairs). However, without any clear phylogenetic relationship of the 'Achilleinae II' group, it is impossible to polarize this character. In their comprehensive study on the phylogeny of Mediterranean Anthemideae, Oberprieler & Vogt (2000) found only marginal support for a sister-group relationship of 'Achilleinae II' with other members of a large, mainly western Mediterranaen centred monophyletic group characterized by a 5 bp-deletion in cpDNA *trn*L/*trn*F IGS (positions 274–278 in the present alignment). The representatives of this group used in the present analysis as outgroup taxa (all except *Phalacrocarpum* which is more basal due to the mentioned deletion being missing) are glabrous or furnished with basifixed hairs. This would tentatively argue for the derived nature of medifixed or stellate hairs in the ingroup and the monophyly of the group of *Mecomischus*, *Rhetinolepis* and *Santolina*. The occurrence of basifixed hairs in the *Chamaemelum* species (and *Cladanthus*) may thus be considered to be symplesiomorphic and unsuitable for the demonstration of the monophyly of this genus.

In contrast to the difficulties concerning the monophyly of *Chamaemelum* and its phylogenetic position within 'Achilleinae II', evidence for the proper placement of *Santolina africana* is quite strong. Both molecular markers point to the sister-group relationship of this species with the other representative of *Santolina* with considerable support. Since all members of 'Achilleinae II' along with *Santolina africana* are characterized by achenes with a thin pericarp completely covered by large myxogenic cells in longitudinal rows (pers. obs.), while other members of *Santolina* have achenes with a thick pericarp devoid of myxogenic cells (Briquet, 1916; pers. observ.), the achene anatomical similarity of *Santolina africana* and *Chamaemelum*, *Cladanthus*, *Mecomischus* and *Rhetinolepis* is best interpreted as a symplesiomorphy and thus not phylogenetically informative. Additionally, our data on two genomes do not provide any evidence for hybrid origin of *Santolina africana* as initially hypothesized based upon mixed features typical for *Santolina* and *Chamaemelum*. Eventually, sampling of more nuclear and plastid markers and inclusion of more species of *Santolina* (presently consisting of around 13 species) may further elucidate the proper classification of this enigmatic species.

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APPENDIX 1

Aligned nrDNA ITS sequence data of 21 representatives of *Chamaemelum,* its closely related genera *Santolina*, *Rhetinolepis*, *Mecomischus* and *Cladanthus*, and nine outgroup taxa. The border between ITS1 and ITS2 is indicated above the sequences. Gap positions and resulting gap codings are indicated at the end of the sequences. The data have been deposited in the EMBL data base (for accession numbers see Table 1).

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                                10        20        30        40        50        60        70        80        90       100
                                .........|.........|.........|.........|.........|.........|.........|.........|.........|.........|
Santolina rosmarinifolia        TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAACAACCGAGCATCGAGTGGATTGAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATGA
Santolina africana              TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAACAACCGAGCGTCGAGTGGATTGAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATGA
Rhetinolepis lonadioides        TCGAACCCTGCAAAGCAGAACGACCCGTGAACAAGTAAAA-CAATCGAGTGTCGAGAAGATCAAGCACTT-GTTTGATCCTTTCGATGCTTTGTTGATGA
Mecomischus halimifolius        TCGAACCCTGCAAAGCAGAACGACCCGTGAACAAGTAAAAACAACCGAGCATTAAGTAGATCAAGCACTT-GTTTGATTCTCTCGATGCTTTGTCGATGA
Chamaemelum eriolepis           TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAATAACCGAGC-TCGAGTGGGTTAAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATG-
Chamaemelum scariosum           TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAACAACCGAGC-TCGAGTGGGTTAAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATG-
Cladanthus arabicus             TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAACAACCGAGC-TCGAGTGGGTTAAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATG-
Chamaemelum flahaultii          TCGAACCCTGCAAAGCAGAACGACCCGTGGACACGTAAAAACAACCGAGC-TCGAGTGGGTTAAGCACTT-GTTTGATCCTCTCGATGCTTTGTCGATG-
Chamaemelum mixtum (Morocco)    TCGAACCCTGCAAAGCAGAACGACCCGTGAACATGTAAAAACAACCGAGCGTCGAGTGGGTTAAGCTCTT-GCTTGATCCTCTTGATGCTTTGTCGATG-
Chamaemelum mixtum (Italy)      TCGAACCCTGCAAAGCAGAACGACCCGTGAACATGTAAAAACAACCGAGCGTCGAGTGGGTTAAGCTCTT-GCTTGATCCTCTTGATGCTTTGTCGATG-
Chamaemelum nobile              TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAATAA-CGAGCGTCGAGTGGATTAAGCGCTT-GTTTGATCCTCTCGATGCTTTGTCGATG-
Chamaemelum fuscatum            TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAAAAATAA-CGAGCGTCGAGTGGATTAAGCGCTT-GTTTGATCCTCTTGATGCTTTGTCGATG-
Lepidophorum repandum           TCGAACCCTGCAAAGCAGAACGACCCGTGAACAAGTAAAA-CAAACGAGCGTCGAGCGGATTAGGCACTT-GTTTGGTCCTCTCGATGCTTTGTCGATG-
Phalacrocarpum oppositifolium   TCGAACCCTGCAAAGCATAACGACCCGTGAACATGTAAAAACAATCGGGCGTCAATTGGATTAAGCACTT-GTTTGATCCTCTTGGTGCTTTGTCGATG-
Lonas annua                     TCGAATCCTGCAAAGCAGAAACATCTGTGAACATGTAAAAATGA-CGAGCGTTGAGTGGATTAAGCACTA-GATTGATCCTCTCGGCGCTTTGTCGATGA
Nivellea nivellei               TCGAACCCTGCACAGCAGAACGACCCGTGAACACGTAACAATAACTGAGCGCTGAGTGGGTTAAGCAATTTGTTTGATTCTCTCGGTGCTTTGTCGATG-
Chrysanthemum coronarium        TCGAACCCTGCAAAGCAGAASGACCCGTGAACACGTAATAATAACCGAGCGCCGAGTGGGTTAAGCGYTTTGTTTGATCCTCTCGGTGCTTTGTCGATG-
Aaronsohnia pubescens           TCGAACCCTGCAAAGCAGAACGACCCGCGAACACGTAATTATAAATGAGCGCCGAGTGGGTTAAGCATTTTGTTTGATCCTCTCGGTGCTTTGTCGATG-
Leucanthemum vulgare            TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAATA-CAACCGAGCGTCGAGCGGGTTAAGCGCTA-GTTTGATCCTCTCGATGCTTTGTCGATG-
Otospermum glabrum              TCGAACCCTGCAAAGCAGAACGACCCGTGAACACGTAATA-CAACTGTGTGTCGAGTTGGTTAAGCACTT-GTTTGATCATCTCGATGCTTTGTCGATG-
Chlamydophora tridentata        TCGAACCCTGCAAAGCAGAACGACCTGTGAACACGTAATA-CAACCGGGCGTCGAGTGGGTTAAGCATAT-GTTTGATCCTCTTGATGCTTTGTCGACG-
                                       110       120       130       140       150       160       170       180       190       200
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Santolina rosmarinifolia        T-GCA-TTTACTTGAGTCCTTTGGGGC-CTTTGTGAGTGCGTCATTGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Santolina africana              TTGCA-TTTACTCGAGTCCTTTTGGGG-CYTTGGGAGTGCGTCATCGGCKCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Rhetinolepis lonadioides        T-GCA-TTTACCTGACTCTTTTAGGGC-CTT-GTGAGTGCGTCGTTGGCGCAATAACAACCCCCGGCACAACGTGTGCCAAGGAAAACTAAA-AGTAAGA
Mecomischus halimifolius        T-GCA-TTTACTCGAGTCCTTTAGGGC-CTT-GTGGGTGCGTCATTGGCGCAATXXCAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Chamaemelum eriolepis           T-GCACTT-ACTCGACTCTTTGGGAGT-CTT-GTGAGTGTGTCGTTGGCGCAACAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Chamaemelum scariosum           T-GCACTT-ACTCGACTCCTTTGGAGT-CTT-GTGAGTGTGTCGTTGGCGCAACAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Cladanthus arabicus             T-GCACTT-ACTCGACTCCTTTGGAGT-CTT-GTGAGTGTGTCGTTGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA--GTAAGA
Chamaemelum flahaultii          T-GCACTT-ACTCGAGTCCTTTGGAGT-CTT-GTGAGTGTGTCGTTGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Chamaemelum mixtum (Morocco)    T-GCACTT-ACTTGTCTCCATTGGGGA-CTT-GCGAGTGCGTCGTCGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
Chamaemelum mixtum (Italy)      T-GCACTT-ACTTGTCTCCATTGGGGA-CTT-GCGAGTGCGTCGTCGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACTAAA-CGTAAGA
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Chamaemelum nobile                T-GCA-TTCACTTGAGTCCTTTGGGGT-CAT-GTGAGTGTGTCATTGGCGCAATAACAACCCCCGGCACAACGTGTGCCAAGGAATACTAAA-CGTAAGA
Chamaemelum fuscatum              T-GCA-TTCACTTGAGTCCTTTGGGTC-CAT-GTGAGTGTG-CATTGGCGCAATAACAACCCCCGGCACAACGTGTGCCAAGGAATACTAAA-CGTAAGA
Lepidophorum repandum             T-GCA-TTTACTTG-GTCCTTTTGGGC-TGT-GTGAGTGTGTCATTGGCGCAATAACAACCCCCGGCACAATGCGTGCCAAGGAAAACAAAA-CGAAAGA
Phalacrocarpum oppositifolium     T-GCACTTTACTAGAGTTTTTTTTGGAC-CTT-GTGAGTGTTCCATTGGCGCAATAACAACCCCCAGCACAATGTGTGCCAAGGAAAACTAAA-CTTAAGA
Lonas annua                       --GCACTG-ACTTGGGTCCCTTTGGTCCCTT-GTGAGTGT-CATTGGCGCAATAACAACCCCCGGCACAATGCGTGCCAAGGAAAACAAAAACTTAAGA
Nivellea nivellei                 T-GCA-TTTACTCGAGTCCTTTTGGGC-CTT-GTGACTGTGTCATTGGCGCAATAACAACCCCCGGCACAATGCGTGCCAAGGAAAACTAAA-CTTAAGA
Chrysanthemum coronarium          T-GCA-TTAACTCGAGTCCTTTTGGGC-CTT-GTGAGTGTGTCATTGGCGCAATAACAACCCCCGGCACAATGCGTGCCAAGGAAAACTAAA-CTTAAGA
Aaronsohnia pubescens             T-GCA-TTTACTTGAGTCCTCT-GGGC-CTT-GTGAATGCGTCTTCGGCGCAATAACAACCCCCGGCACAATGCGTGCCAAGGAAAACTAAA-CTTAAGA
Leucanthemum vulgare              T-GCA-TTCACTTGAGTCCTCTTGGGC-CTT-GTGAATGTGTCGTTGGCGCAATAACAACCCCCGGCACAACGTGTGCCAAGGAAAACTAAA-CTTGAGA
Otospermum glabrum                T-GCA-TTCACTCGAGTCCTAAGTGACGCTT-GTGAATGTGTCATCGGCGCAATAACAACCCCCGGCACAATGTGTGCCAAGGAAAACAAAA-CTTGAGA
Chlamydophora tridentata          T-GCA-TTTGCTTGAGGCCTCTTGGTC-CTT-GTGAATGTGTTGTTGGCGCAATAACAACCCTCGGCACAATGCGTGCCAAGGAAAACTAAA-CTTTAGA

                                                                                                ITS1→||←ITS2
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Santolina africana                AGGCTTGTTTCATGTTTGCCCC-GTTCGCGGTGTGCTCATGGGATGKGGCTTMTTTATAATCAATCGCGTCGCCCCCCGC-AAATCTATGTC-GGGGG-C
Rhetinolepis lonadioides          AGGCTTCTTTCATGTT-GCCCCCGTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCTC-AAATCTTTGTT-GGGGG-C
Mecomischus halimifolius          AGGCTTATTTCATGTT-GCCCCCGTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATATTTGTT-GGGGG-C
Chamaemelum eriolepis             AGGCTTGTTTCATGTA-GCCCC-GTTCGCGGTGTGCTCGTGGGATGTGGCTTCTTTATAATCTATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Chamaemelum scariosum             AGGCTTGTTTCATGTA-GCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Cladanthus arabicus               TGGCTTGTCTCATGTA-GTCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATTAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Chamaemelum flahaultii            AGGCTTGTTTCATGTA-GCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAACCTTTGTT-GGGGG-C
Chamaemelum mixtum (Morocco)      AGGCTTGTTTCATGTT-GCCCC-GTTCGCGGTGCGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Chamaemelum mixtum (Italy)        AGGCTTGTTTCATGTT-GCCCC-GTTCGCGGTGCGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Chamaemelum nobile                AGGCTTGTTTCATGTT-GCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCTGC-AAATCTTTGTT-GGGGG-C
Chamaemelum fuscatum              AGGCTTGTTTCATGTA-GCCTC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Lepidophorum repandum             AGGCTTGTTTCATGTC-GCCCC-GTTCGCGGTGTGCGCGTGGAATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCGC-AAATATATGTG-GGGGG-C
Phalacrocarpum oppositifolium     AGGCTTGTTTCATGTT--CCTC-GTTTGCGGTGTGCTCATGAGACTTGGCTTCTTTATAATCAATCGCGTCGCCCCCGC-AAATCTTTGTT-GGGGG-C
Lonas annua                       AGGCTTGTTCCATGTT-GCCCC-GTTCGCGGTGTGCTCATGAGACGCGACTTCTTTTTTAATCAATCGCGTCGCCCCCACCAAATCTTTGTT-GGGGGGC
Nivellea nivellei                 AGGCTTGTTTCATGTTTGCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AAATCTTTGTT-GGGGG-C
Chrysanthemum coronarium          AGGCTTGTTTCATGTTTGCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAA??ATCGC-TCGCCCCCCGC-AAATCTATGTT-GGGGG-C
Aaronsohnia pubescens             AGGCTTGTTTCATGTTTGCCTC-GTTCGCGGGGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCATCGCCCCCCGC-AAATCTTTGTT-GGGGG-C
Leucanthemum vulgare              AGGCTTGTCTCATGTT-GCCCC-GTTCGCGGTGTGCTCATGGGATGTGGCTTCTTTATAATCAATCGCGTCGCCCCCAC-AATTATTTGTTAGGGGG-C
Otospermum glabrum                AGGCTTGTTTCATGTTTGCCCC-GTTCGCGGTGTGCTCATGGGACGTGGCTTCTTTATAATCAATCGCGTCGCCCCC-AC-AAGTATTTGTC--GGGG-C
Chlamydophora tridentata          AGGCTTGTTTCATGTT-GCCCC-GTTCGCGGTGTGCTCATGGAATATGGTTTCTTTATAATTAATCAGCTCACCCCCCAC-AAATCATTGTTA-GGGG-C

                                      310       320       330       340       350       360       370       380       390       400
                                  ....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Santolina rosmarinifolia          GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Santolina africana                GGATATTGGTCTCCCGTGCTCACGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Rhetinolepis lonadioides          GGATATTGGTCTCCCGTGTTCACGACGTGGTTGGCCGAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Mecomischus halimifolius          GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTTCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
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Chamaemelum eriolepis                GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum scariosum                GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Cladanthus arabicus                  GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum flahaultii               GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum mixtum (Morocco)         GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTACGTCGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum mixtum (Italy)           GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTACGTCGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum nobile                   GGATATTGGTCTCCCGTGCTAATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGGCGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chamaemelum fuscatum                 GGATATTGGTCTCCCGTGCTAATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Lepidophorum repandum                GGATATTGGTCTCCCGTGATATTGTCGTGGTTGGCCAAAATATGGTTTCCTCTGATGGATGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Phalacrocarpum oppositifolium        GGATATTGGTCTCCCGTGCTCATGGTGTGGTTGGCCAAAATAAGAGTCCCTTCGACGGACGCACTAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Lonas annua                          GGATATTGGTCTCCCGTGCTATTGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACAAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Nivellea nivellei                    GGATATTGGTCTCCCGTGCTCATAGCGTGGTTGGCCAAAATAGGAGTCCCTTTGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chrysanthemum coronarium             GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Aaronsohnia pubescens                GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTAGT
Leucanthemum vulgare                 GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCGAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Otospermum glabrum                   GGATATTGGTCTCCCGTGCTCATGGCGTGGTTGGCCAAAATAGGAGTCCCTTCGATGGACGCACAAACTAGTGGTGGTCGTAAAAACCCTCGTTCTTTGT
Chlamydophora tridentata             GGATATTGGTCTCCTGTGCTCATCGTGTGGTTGGCCAAAATATGAGTCCCTTCGTTGGACGCACAAACTAGTGGTGGTCGAAAAAACCCTCGTTCTTTGT

                                           410       420       430       440       450       460       470
                                     ..........|.........|.........|.........|.........|.........|.........|....
Santolina rosmarinifolia             TTTGTGTCGTACGTCGA--TAAGGAAACACTCTCTAAAAACCCCAACGTGTTGTCTTATGATGACGCTTCGACC
Santolina africana                   TTTGTGTCGTACGTCGC--TA-GGAAACACTCTCTAAATACCCCAACGTGTTGTCTTAGGATGACGCTTCGACC
Rhetinolepis lonadioides             TTTGTGTCGTACGTCGC--TA-GGAAACACTCTCTAAATACCCCAACGTGTTGTCTTCGGATGACGCTTCGACC
Mecomischus halimifolius             TTTGTGTCGTACTTCGC--TC-GGAAACACTCTTTAAATACCCCAACGTGTTGTCTTTGGATGACGCTTCGACC
Chamaemelum eriolepis                TT-GTGTCGCACGTCGA--TA-GGAAACACTCTCTAAATACCCCAACGTGTTGTCTTAGGATGACGCTTCGACC
Chamaemelum scariosum                TT-GTGTCGCACGTCGA--TA-GGAAACACTCTCTAAATACCCCAACGTGTTGTCTTAGGATGACGCTTCGACC
Cladanthus arabicus                  TT-GTGTCGCACGTCGA--TA-GGAAACACTCTCTAAATACCCCAATGTGTTGTCTTAGGATGACGCTTCGACC
Chamaemelum flahaultii               TT-GTGTCGCACGTCGC-TT--GGAAACACTCTCTAAATACCCCAATGTGTTGTCTTAGGATGACGCTTCGACC
Chamaemelum mixtum (Morocco)         TT-GTGTCGTACGTCGT--TA-GGATACGCTCTCTAAAAACCCCAGTGTGTTGTCTTAGGATGAXGCTTCGACC
Chamaemelum mixtum (Italy)           TT-GTGTCGTACGTCGT--TA-GGATACGCTCTCTAAAAACCCTAGTGTGTTGTCTTAGGATGACGCTTCGACC
Chamaemelum nobile                   TTTGTGTCGCACGTTGC--TA-GGAAGCACTCTCTAAATAACCCATTGTGTTGTCTTAGGATGACGCTTCGACC
Chamaemelum fuscatum                 TTTGTGTCGCACGTCGC--TA-GGAAGCACTCTCTAAATAACCCATTGTGTTGTCTTAGGATGACGCTTCGACC
Lepidophorum repandum                TTTGTGCTGTATCTCTCATTA-GGAAACTCT-----AATA-CCCAATGTGTTGTTTTAGGATGACGCTTCGACC
Phalacrocarpum oppositifolium        TATGTGTTGTTAGTTGC---AAGGAAACACTCTTCAAATACCCCAACGTGTTGTCTTAGGATGATGCTTCGACC
Lonas annua                          TTTGTGCCGTCTCTCGC---AAGGGAACACTCTTCAAGTAACCCAGCGCATCGTCTAAGGATGATGCTTCGACC
Nivellea nivellei                    TTTGTGTCGTCCGTTAT---AAGGAAACGCTCTCCAAAAACCCTAATGTGTTGTCTAAGGATGACGCTTCGACC
Chrysanthemum coronarium             TTTGTGTCGTTCGTTGC---AATGGAACACTCTATAAAAAACCCAATGTGTTGTCTTAGGATGACGCTTCGACC
Aaronsohnia pubescens                GTTGTGTCGTATGTCGC---AAGGAAATGCTCTTGAAAAACCCCAACGCATTGTCTAAGGATGATGCTTCGACC
Leucanthemum vulgare                 TTTGTGCCGACATTTGC---AAGGAAACACTCTTTCTTTACCTAATGCGTTGTCTTAGGACGATGCTTCGACC
Otospermum glabrum                   TTTGTGCCGATAGTCGC---AAGGAAACACTCTTCAAATAACCCAATGTGTTGTCTTAGGATGACGCTTCGACC
Chlamydophora tridentata             TTTGTGCCGATAA---C---AAGGAGTCACTCTTCAAAAAACCCAACGTGTTGTCTTAGGATGACGCTTCGACC
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Santolina africana  11101010111100111010  02  45
Rhetinolepis lonadioides  01101010010101011010  03  51
Mecomischus halimifolius  11101010010101011010  04  71
Chamaemelum eriolepis  11000100010000100010  05  100
Chamaemelum scariosum  11000100010000100010  06  106
Cladanthus arabicus  11000100010000100010  07  109
Chamaemelum flahaultii  11000100010000100110  08  128
Chamaemelum mixtum (Morocco)  11100100010000100010  09  132
Chamaemelum mixtum (Italy)  11100100010000100010  10  142
Chamaemelum nobile  10100010010000111010  11  217
Chamaemelum fuscatum  10100010000000111010  12  223
Lepidophorum repandum  01100010010000111110  13  281
Phalacrocarpum oppositifolium  11100110010000111001  14  282
Lonas annua  10101101000000111001  15  403
Nivellea nivellei  11110010011000111001  16  419
Chrysanthemum coronarium  11110010011000111001  17  420
Aaronsohnia pubescens  11110010011000111001  18  422
Leucanthemum vulgare  01100010010001111001
Otospermum glabrum  01100011011000101001
Chlamydophora tridentata  01100010010010101001
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APPENDIX 2

Aligned cpDNA *trn*L/*trn*F IGS sequence data of 22 representatives of *Chamaemelum,* its closely related genera *Santolina*, *Rhetinolepis*, *Mecomischus* and *Cladanthus*, and nine outgroup taxa. Gap positions and resulting gap codings are indicated at the end of the sequences. The data have been deposited in the EMBL data base (for accession numbers see Table 1).

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                                10        20        30        40        50        60        70        80        90       100
                                .........|.........|.........|.........|.........|.........|.........|.........|.........|
Chamaemelum nobile              TCCCCAAAAAGACCGTTTGACTACCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCAGTTCCAAATTCCTTATC
Chamaemelum fuscatum            TCCCCAAAAAGACCGTTTGACTACCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCAGTTCCAAATTCCTTATC
Santolina rosmarinifolia        TCCCCAAAAAGACCATTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Santolina africana              TCCCCAAAAAGACCATTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Rhetinolepis lonadioides        TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCAGTTCCAAATTCCTTATC
Mecomischus halimifolius        TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCAGTTCCAAATTCCTTATC
Chamaemelum mixtum (Morocco)    TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTCTTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Chamaemelum mixtum (Tunisia)    TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTCTTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Chamaemelum mixtum (Italy)      TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTCTTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Cladanthus arabicus             TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Chamaemelum eriolepis           TCACCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Chamaemelum flahaultii          TCCCCNNNAAGACNGTTTGACTCCCTAATTCNTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Chamaemelum scariosum           TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Chrysanthemum coronarium        TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Lepidophorum repandum           TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTAATCCTTTTGATTTATCTTGTATTTATCTTGTTTTTCATTAGCGGTTAAAAATTCCTTATC
Lonas annua                     TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTTATTTATCTTTTTTTT------TTTTTCATTATCGGGTCAAAATTCCTTATC
Nivellea nivellei               TCCCCAAAAAGGCCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATTTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Aaronsohnia pubescens           TCCCCAAAAAGGCCGTTTTATTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CATTAGCGGTTCAAAATTCCTTATC
Phalacrocarpum oppositifolium   TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
Otospermum glabrum              TCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTT-----------CGTTA-------------------
Leucanthemum vulgare            TCCCCAAAAAGACCGTTTGACTCCATAATTCTTTATTGTA-TCCTTTTGATTTATCTTGTTTTG-----------CGTTAGTGGTTCAAAATTCCTTATC
Chlamydophora tridentata        TCCCCAAAAAGACCGTTTGACTTCCTAATTCTTTATTGTA-TCCTTTTTATTTATCTTGTTTTT-----------CGTTAGCGGTTCAAAATTCCTTATC
                                       110       120       130       140       150       160       170       180       190       200
                                .........|.........|.........|.........|.........|.........|.........|.........|.........|.........|
Chamaemelum nobile              TTTCC-----CATTCACTACTCTTTATACAA--------------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACACGGGAT
Chamaemelum fuscatum            TTTCC-----CATTCACTACTCTTTATACAA--------------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACACGGGAT
Santolina rosmarinifolia        TTTCC-----CATTCACTACTCTTTATACAA--------------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACACGGGAT
Santolina africana              TTTCC-----CATTCACTACTCTTTATACAA--------------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACACGGGAT
Rhetinolepis lonadioides        TTTCC-----CATTCACTACTCTTTATACAATACAA---------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACAGGG-AT
Mecomischus halimifolius        TTTCC-----CATTCACTACTCTTTATACAATACAA---------TTATACAAAAGGA-TCTGAGCGGAAAAGCTGTTCTCTTATCACATCACAGGG--T
Chamaemelum mixtum (Morocco)    TTTAC-----ATTCACTACTCTTTATACAA----------------------AAGGA-TCTGAGCGGAAAAGCTGTTCTCT-ATCACATCACAGGG-AT
Chamaemelum mixtum (Tunisia)    TTTAC-----ATTCACTACTCTTTATACAA----------------------AAGGA-TCTGAGCGGAAAAGCTGTTCTCT-ATCACATCACAGGG-AT
Chamaemelum mixtum (Italy)      TTTAC-----ATTCACTACTCTTTATACAA----------------------AAGGA-TCTGAGCGGAAAAGCTGTTCTCT-ATCACATCACAGGG-AT
Cladanthus arabicus             TTTCCTTTCCCATTCACTACTCTTTATACA-TACAATTATACAAATTATACAAAA----TCTGAGCGTAAAAGCGGTTCTCTTATCACATCACAGGGGAT
Chamaemelum eriolepis           TTTCCTTTCCCATTCACTACTCTTTATACA-TACAATTATACAAATTATACAAAAGGA-TCTGAGCGTAAAAGCTGTTCTCTTATCACATCACAGGGGAT
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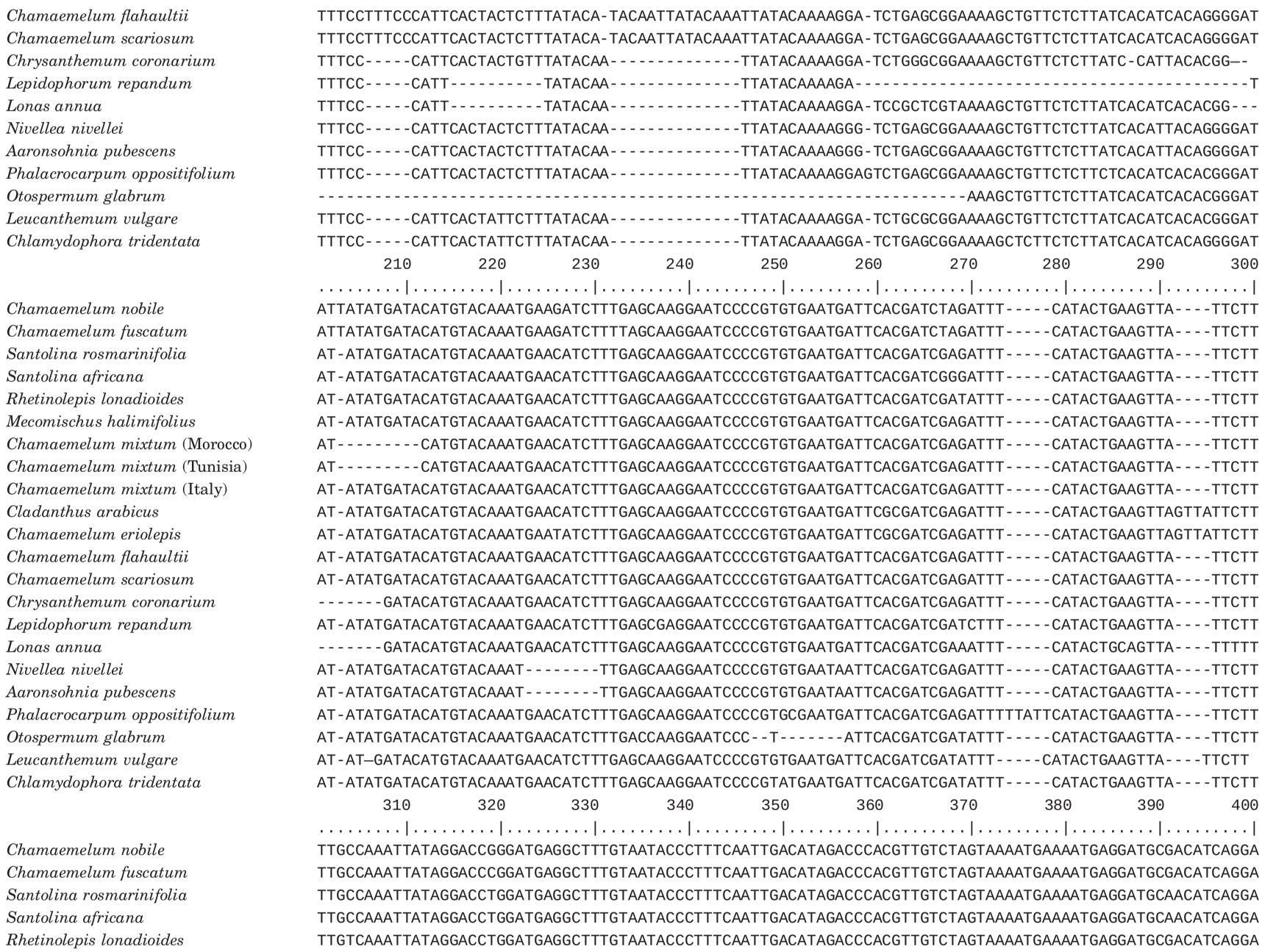

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Mecomischus halimifolius          TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum mixtum (Morocco)      TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum mixtum (Tunisia)      TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum mixtum (Italy)        TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Cladanthus arabicus               TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGGTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum eriolepis             TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGGTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum flahaultii            TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGGTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chamaemelum scariosum             TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGGTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chrysanthemum coronarium          TTGCCAAATTATAGGACCTGGAGGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGAATGCGACATCAGGA
Lepidophorum repandum             TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Lonas annua                       TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Nivellea nivellei                 TTGCCAAATTATAGGACCCGAATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Aaronsohnia pubescens             TTGCCAAATTAGAGGACCCGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Phalacrocarpum oppositifolium     TTGCCAAATTATAGGACCTGGATGAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Otospermum glabrum                TTGACAAATTATCGGACCCGGATGAGGCTTTGTAATACCCTTTCAATTGACATATACCCACGTTGTCTAGTAAAATAAAAATGAGGATGCGACATCAGGA
Leucanthemum vulgare              TTGCCAAATTATCGGACCTGGATAAGGCTTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
Chlamydophora tridentata          TTGCCAAATTATCGGACCTGGA----------ATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA

                                        Gap coding
                                  00000000011111
                                  1234567890123 4   Gap  Alignment position
                                  .... ..........   ...  ..................
Chamaemelum nobile                ATAG 0011110?111110  01   71-75
Chamaemelum fuscatum              ATAG 0011110?111110  02   106-110
Santolina rosmarinifolia          ATAG 0011110?111010  03   111
Santolina africana                ATAG 0011110?111010  04   115-125
Rhetinolepis lonadioides          ATAG 0011111010101010  05   131
Mecomischus halimifolius          ATAG 0011111010101010  06   132-153
Chamaemelum mixtum (Morocco)      ATAG 0001107?100?10  07   132-145
Chamaemelum mixtum (Tunisia)      ATAG 0001107?100?10  08   137-145
Chamaemelum mixtum (Italy)        ATAG 0001107?101010  09   198-207
Cladanthus arabicus               ATAG 0111011111111011  10   198
Chamaemelum eriolepis             ATAG 0111011111111011  11   203-211
Chamaemelum flahaultii            ATAG 0111011111111010  12   203
Chamaemelum scariosum             ATAG 0111011111111010  13   213-230
Chrysanthemum coronarium          ATAG 0011110?0?1?10  14   292-295
Lepidophorum repandum             ATAG 1010110?1?1010
Lonas annua                       ATAG 1010110?0?1?10
Nivellea nivellei                 ATAG 0011110?111000
Aaronsohnia pubescens             ATAG 0011110?111000
Phalacrocarpum oppositifolium     ATAG 0011110?111010
Otospermum glabrum                ATAG 0?????111010
Leucanthemum vulgare              ATAG 0011110?111010
Chlamydophora tridentata          ATAG 0011110?111010
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