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A reassessment of tribal affinities of *Cratystylis* and *Haegiela* (Asteraceae) based on three chloroplast DNA sequences

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Abstract. The tribal affinities of *Cratystylis* and *Haegiela* were assessed using three chloroplast DNA sequences, the *trn*L/F spacer, the *trn*L intron and the *mat*K coding region. The outgroup was represented by two species of the subfamily Barnadesioideae, whereas one to seven genera (45 species including *Cratystylis* and *Haegiela*) of the tribes of the Asteroideae [Anthemideae (6 genera), Astereae (7), Calenduleae (2), Gnaphalieae (7), Heliantheae s.l. (5), Inuleae s.str. (3), Plucheeae (3), Senecioneae (4)] and Cichorioideae, [Arctotideae (1), Cardueae (2), Lactuceae (2), Liabeae (1), Mutisieae (1) and Vernonieae (1)] comprise the ingroup. Phylogenetic analysis indicates that *Cratystylis* has strong support as a member of the tribe Plucheeae, whereas *Haegiela* is a member of Gnaphalieae. At some point in their taxonomic history, both genera have been placed in these tribes and there are good morphological and chemical characters that justify these placements. The monotypic *Haegiela* was once included in *Epaltes* (Plucheeae) and this study has confirmed the need for the separation of the two genera. The genus *Cratystylis* appears to be monophyletic.

Key words: Asteraceae, *Cratystylis*, *Haegiela*, Gnaphalieae, everlastings, molecular phylogeny, tribe, cpDNA, *mat*K, *trn*L/F region.

Our continuing studies on the phylogeny and systematics of the tribe Gnaphalieae (Asteraceae), have necessitated the reevaluation of the phylogenetic position of several Australian, South American, and South African genera. Recent treatments of the Gnaphalieae (Anderberg 1991a, Anderberg in Bremer 1994) have placed several enigmatic genera within this tribe, including *Haegiela* P.S. Short and Paul G. Wilson. On the other hand, while *Cratystylis* S. Moore has been in a broad Inuleae (sensu Bentham 1873) it has never been placed directly in Gnaphalieae. Bremer (1994) treated *Cratystylis* as a member of the subfamily Cichorioideae, but did not assign it to a tribe. These genera have suites of morphological and chemical traits, which offer conflicting clues as to their tribal affinities. Currently there is no molecular study to assist in providing corroborating evidence to the compelling question of their tribal alliance.

Taxonomic history of Cratystylis. *Cratystylis* is a genus of four functionally dioecious, sometimes spiny, shrubs with linear to spathulate, usually grey/blue leaves that occur in southern Australia, from Western Australia to Victoria and far western New South Wales. The capitula are solitary, homogamous, epaleate, with chartaceous involucral bracts in six to eight rows. The florets are deeply lobed, the anthers caudate, and the style branches have very short hairs dorsally and extending immediately below the bifurcation. Cypselae are glabrous with a pappus of scabrid-barbellate bristles and a substantial and undivided carpopodium.

The taxonomic history of *Cratystylis* is long and convoluted. *Eurybia* (Cass.) Cass. was originally erected by Cassini as a section of *Aster* L. In 1855, Ferdinand von Mueller described *Eurybia conocephala* F. Muell. Later, Mueller (1865) transferred the taxon into *Aster*, as *Aster conocephalus* (F. Muell.) F. Muell., and he gave as a basionym and a synonym respectively, *Eurybia conocephala* and *Olearia conocephala* F. Muell. *nomen nudum*. Bentham (1867) validated *Olearia conocephala* (F. Muell.) Benth. in *Flora Australiensis*; despite what Mueller may have intended. Therefore, this taxon was now in a third genus of the Astereae. Bentham and Hooker (1873) retained the taxon as *Olearia conocephala* in *Genera Plantarum*, and Mueller (1887) transferred it into *Pluchea* Cass., thereby changing its tribal position to the Inuleae, subtribe Plucheineae. It is difficult to determine tribal placement in *Die natürlichen Pflanzenfamilien* (Hoffmann 1890-1894) because the epithet "*conocephala*" was not specifically mentioned under any of the four genera in which it had been placed.

Moore (1905) transferred *Pluchea conocephala* (F. Muell.) F. Muell. to a new genus *Cratystylis,* as *Cratystylis conocephala* (F. Muell.) S. Moore, which he placed in Inuleae. He also validated two *nomina nuda* of Mueller and Tate (1896), *Pluchea conocephala* var. *microphylla* and var. *subspinescens*, as additional species of *Cratystylis*, *C. microphylla* S. Moore and *C. subspinescens* S. Moore. This brought the total number of species in the genus to three. The superfluous generic name *Stera* Ewart was proposed in Ewart et al. (1911), and as they were apparently unaware of Moore's (1905) paper on *Cratystylis*, they published the three species *Stera conocephala* (F. Muell.) Ewart and B. Rees, *S. microphylla* Ewart and B. Rees and *S. spinescens* Ewart and B. Rees. Hutchinson (in Ostenfeld 1921) described *Pteronia australiensis* Hutch. (= *Cratystylis conocephala*), as Ostenfeld had given the specimen to Hutchinson because he could not identify it. Subsequently, Hutchinson placed it in a genus with which he was very familiar from South Africa, *Pteronia* L. He was excited about this finding because it could be used as evidence of a former land connection between Australia and South Africa. The resemblance of *Pteronia* to *Cratystylis* is, however, merely superficial. Placing *Cratystylis conocephala* in *Pteronia* also leads us to conclude that Hutchinson (in Ostenfeld 1921) considered *Cratystylis* as a member of the Astereae. Black (1929, 1957), in the 'Flora of South Australia', and Willis, in the 'A Handbook to Plants of Victoria' (1973), left *Cratystylis* in the Inuleae sensu Bentham (1873). In their tribal revision of the Inuleae, Merxmüller et al. (1977) place *Cratystylis* in the Inuleae: Inulinae, but admit that the taxon is unusual in a number of characteristics, including its subdioecious breeding system and the size and position of the stigmatic surface.

Zdero et al. (1988, 1991) investigated the phytochemical composition of *Cratystylis* in order to settle its tribal affinity. They determined that the secondary chemistry of both *Cratystylis conocephala* and *C. microphylla* were most similar to the Plucheoid *Sphaeranthus* L. and close to *Epaltes* Cass. and *Pluchea* Cass. These plants contain large amounts of 2α-hydroxycostic acid and eudesmanolide (cratystyolide triacetate).

In 1989, Anderberg suggested that *Cratystylis* lacked the diagnostic features of the Plucheeae and that the genus would best be placed in another tribe. Later, Anderberg (1991b) pointed out that the placement of *Cratystylis*, as a member of the *Pluchea* group of the Inuleae (Plucheeae sensu Anderberg), was aberrant because of its unusual characteristics, both morphological and chemical. Anderberg et al. (1992) further stated that *Cratystylis* was a member of neither Plucheeae nor Inuleae (subfamily Asteroideae), but more closely allied to tribes in the subfamily

Cichorioideae. They cited the 6-8 rows of phyllaries, the deeply lobed corolla, and style with minute hairs distributed below the bifurcation and stigmatic area as anomalous in Asteroideae. Detailed pollen studies revealed that *Cratystylis* has prominently spined, caveate, pollen grains, a thin foot layer, internal foramina and a complex 2–3 layered sexine. This differs from the Inuloid pollen type in that the pollen of *Cratystylis* possesses a double sexine and pronounced internal tectum and no singular baculate layer. This has also distinguished the pollen from many, but not all, Plucheeae, which have a single columellate sexine. Anderberg et al. (1992) did, however, note the similarity of *Cratystylis* pollen to that of *Stenachaenium* Benth. (Plucheeae). They concluded that the pollen type was like that of the Arctoteae (Cichorioideae), in spite of the rarity of caveate pollen in Cichorioideae and preponderance in Asteroideae. They additionally rejected the phytochemical assessment of Zdero et al. (1988) asserting that *Cratystylis* was more similar to the Cichorioid tribes, Arctotideae or Cardueae.

On the basis of a cladistic analysis of the morphology, Anderberg et al. (1992) concluded that *Cratystylis* is isolated within Cichorioideae and closely related to Vernonieae, Liabeae, and Lactuceae. Hunger (1997), in her revision of Australian *Pluchea*, accepted Anderberg's exclusion of *Cratystylis* from the Plucheeae/Inuleae. Lately, Wilson and Albrecht (2002) described a fourth species of *Cratystylis*, *C. centralis* Paul G. Wilson and Albr., and accepted Anderberg's placement of the genus as an isolated element in subfamily Cichorioideae.

Taxonomic History of Haegiela. Haegiela is a monotypic genus of annual herbs distributed in arid areas of southern Australia from New South Wales and Victoria west to Western Australia. Its leaves are small, basally opposite, sessile, ovate to lanceolate, and covered with a cobwebby indumentum. The capitula are small, axillary, heterogamous, epaleate and surrounded by 15 to 20 involucral bracts, the outer scarious and silvery translucent, the inner subcartilaginous. The florets are shallowly four-lobed, and papillose, the stamens strongly caudate, and the style branches are very short and truncate apically. The cypselae are minutely papillose, epappose, with a substantial and undivided carpopodium.

Haegiela tatei (F. Muell.) P.S. Short and Paul G. Wilson was originally described by Mueller (1883) as *Epaltes tatei* F. Muell., and for most of its taxonomic history it has resided in *Epaltes*. *Epaltes* (tribe Vernonieae *sensu* Cassini) was erected by Cassini in 1819 to accommodate *Ethulia divaricata* L., a clearly misplaced taxon. At the time of Lessing (1832), there were four species in *Epaltes,* which he placed in his subtribe Tarchonantheae (Tribe Asteroideae). Following Lessing, DeCandolle (1836) placed *Epaltes* in the subtribe Tarchonantheae (tribe Asteroideae), but recognized eight species. Both Lessing and de Candolle placed the subtribe Tarchonantheae near genera currently in the modern tribes Inuleae and Plucheeae. Bentham (1873) positioned *Epaltes* in the Inuleae, subtribe Plucheineae, and believed that the nine species of *Epaltes* formed a "very natural" group[1]. In 1883, Mueller described *Epaltes tatei* and stated that although he believed the taxon was somewhat anomalous in *Epaltes*, he did not have the conviction to transfer it to its own genus. The ten species of *Epaltes* remained a part of Inuleae: Plucheinae in Hoffmann's (1890–1894) monograph of the Compositae. Floristic works of South Australia (Black 1929, 1957) and Victoria (Willis 1973) placed *Epaltes* in the Inuleae.

Leins (1971), in his study of the systematic utility of pollen grain morphology in Asteraceae, was the first to strongly suggest that *Epaltes tatei* did not fit in *Epaltes*, nor in the subtribe Plucheinae. His conclusion was based both on the morphology of pollen grains, as

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[1] "The genus [*Epaltes*] as a whole is a very natural one, although the species of each region have been raised into separate genera upon characters which scarcely deserve more than specific rank."

well as that of the capitula (Leins 1971). He also maintained that *E. tatei* was very similar to *Stuartina*, of the Gnaphaliinae[2]. Skvarla et al. (1977), in evaluating pollen morphology, placed *Epaltes tatei* in subtribe Inulinae (Inuleae), and Merxmüller et al. (1977) suggested that all *Epaltes* species could be placed in the *Pluchea* group (Inuleae subtribe Inulinae). Anderberg (1989), accepting that *Epaltes tatei* was misplaced in the genus *Epaltes*, placed it in the *Filago* L. clade of his resurrected Gnaphalieae. Short and Wilson (1990) accepted Lein's suggestion[3] that *E. tatei* be moved into a new genus (Leins 1971) and transferred *E. tatei* to a new genus *Haegiela*. *Epaltes*, now excluding *Haegiela tatei*, expanded in the 200 years following Cassini's first species into a genus of 14 currently recognized species (Anderberg in Bremer 1994).

In the present study, we use DNA sequence data in an attempt to elucidate the tribal relationships of *Cratystylis* and *Haegiela*. We selected the *trn*L/F spacer, the *trn*L intron and the *mat*K coding region to resolve the phylogeny of the Asteraceae, as these regions have proven useful in resolving generic and tribal relationships in the Asteraceae (Bayer and Starr 1998, Bayer et al. 2002, Konishi et al. 2000).

Materials and methods

Source of material. Fieldwork was conducted in Australia. Fresh and/or herbarium material of *Cratystylis conocephala, C. subspinescens,* and *Haegiela tatei* was utilized for the purpose of DNA extraction and morphological study (Table 1). *Cratystylis conocephala* and *H. tatei* both represent the types of their respective genera. Material of the other ingroup and outgroup taxa were either collected in the wild or obtained from commercial sources (Table 1).

Taxon selection. The matrix consists of two outgroup taxa (Barnadesioideae) and 45 ingroup members (Asteroideae and Cichorioideae) and three members of the two genera, *Cratystylis* and *Haegiela,* of *incertae sedis*. Voucher specimens for all taxa are deposited in the herbaria cited in Table 1. Outgroup taxa were selected in accordance with the well supported placement of the Barnadesioideae as the earliest diverging lineage in the Compositae (Jansen and Palmer 1987, Bremer 1987, Kim and Jansen 1995, Bayer and Starr 1998), and are represented by two Barnadesioids, *Chuquiraga aurea* Skottsb. and *Doniophyton anomalum* (D. Don) Wedd. One to seven genera (45 species) represent all of the tribes of the Asteroideae [Anthemideae (6 genera), Astereae (7), Calenduleae (2), Gnaphalieae (7), Heliantheae s.l. (5), Inuleae s.str. (3), Plucheeae (3), Senecioneae (4)] and Cichorioideae, [Arctotideae (1), Cardueae (2), Lactuceae (2), Liabeae (1), Mutisieae (1) and Vernonieae (1)] and comprise the ingroup (see Table 1). Tribal circumscriptions and nomenclature in this work are based on the treatment of the Asteraceae by Bremer (1994).

DNA Isolation, amplification, sequencing and alignment. Ten new sequences were generated for this study (Table 1, which includes GenBank accessions numbers). The remaining DNA sequences are taken from our previous studies (Bayer and Starr 1998, Bayer and Cross 2002). Total DNA was isolated as outlined in Bayer et al. (1996), and DNA amplification and sequencing are exactly as given in Bayer and Cross (2002). Likewise the alignment of sequences proceeded by hand following the principles discussed in Bayer and Cross (2002).

Sequence data analysis. Sequence data were analyzed using PAUP 4.0610 (Swofford 2001). Phylogenetic reconstruction was performed on unweighted characters by heuristic searches with 1000 replicates of random addition of taxa to search for further islands of most parsimonious trees (Maddison 1991). Two data sets were

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[2] Innerhalb der Gnaphaliinae kommt sic der australischen monotypischen Gattung *Stuartina* am nächsten, mit der sie unter anderem die niedrige Wuchsform, die zusammengedrängten, kleinen, heterogamen Köpfchen, die wenigen, trockenhäutigen, glänzenden Hüllschuppen und die pappuslosen Blüten gemeinsam hat. (Translated into English by Randall Bayer: "Within the Gnaphaliinae, the Australian monotypic genus *Stuartina* comes closest [to *Epaltes tatei*], considering, among the things, the low stature and the small, heterogamous heads, which have a few, dry-membranous, shining, outer phyllaries and epappose florets".)

[3] lch schlage deshalb vor, die als *Epaltes tatei* beschriebene Sippe in eine neue Gattung neben *Stuartina* zu stellen. (Translated into English by Randall Bayer: "I suggest, therefore, placing *Epaltes tatei* into a new genus beside *Stuartina*".)

Table 1. Collections of Asteraceae sequenced for this study. Presented are species, collectors (location of voucher), geographic origin, and GenBank accession numbers (new sequences generated for this study are in bold). Voucher specimens are deposited in AD, ALTA, CANB, F, MEL, MO, OS, NBG, RSA, and PERTH. GenBank accession numbers for the sequences (spacer, intron, *mat*K) are given

Species	Collectors and numbers (voucher location(s))	Geographic Origin	GenBank *trn*L/F spacer	Accession *trn*L intron	Numbers *mat*K
Ageratum houstonianum Mill.	Bayer GH-95011 (CANB)	Commercially grown plants; wild origin unknown, but native of Mexico	U82013	U82012	AF151434
Anthemis nobilis L.	Bayer GH-00001 and West (CANB)	Commercially grown plants; wild origin unknown, but native of Europe	AF452496	AF452496	AF456775
Antennaria luzuloides Torr. and Gray	Bayer et al. OR-91002 (ALTA)	U.S.A.: Oregon	U82015	U82014	AF456774
Artemisia tridentata Nutt.	Bayer et al. CO-90072 (ALTA) trnL/F Columbus 3874 (RSA) matK	U.S.A.: Colorado U.S.A.: California	U82017	U82016	AF456776
Aster novae-angliae L.	Bayer AB-95003 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82019	U82018	AF151441
Athanasia juncea D. Dietr.	Bayer and Puttock SAF-96286 (CANB)	South Africa: Western Cape Province	AF452497	AF452497	AF456777
Bedfordia arborescens Hochr.	Richardson 82 (CANB)	Australia: Australian Capital Territory	AF452498	AF452498	AF456778
Bedfordia salicina (Labill.) DC.	Stratham 10 (CANB)	Australia: Tasmania	AF452499	AF452499	AF456779
Blumea tenella DC.	Short 4869 and Dunlop (DNA, CANB)	Australia: Northern Territory	AF452500	AF452500	AF456780
Calendula officinalis L.	Bayer GH-95009 (CANB)	South Africa: Commercial source	U82021	U82020	AF151446
Chiliotrichum diffusum (G. Forst.) Kuntz	Story 8121 (CANB)	Argentina: Province of Santa Cruz	AF452501	AF452501	AF456781
Chionolaena lavandulifolia (Kunth) Benth. and Hook.f. ex B.D. Jacks.	R. and N. Soreng 3312b (CANB)	Mexico: State of Mexico	**AY14359**	**AY143593**	**AY143601**
Chuquiraga aurea Skottsb.	Stuessy et al. 12911 (OS)	Argentina	U82023	U82022	AF456782
Cirsium subniveum Rydb.	Bayer et al. WY-90044A (ALTA)	U.S.A.: Wyoming	U82025	U82024	AF456783

Table 1 (continued)

Species	Collectors and numbers (voucher location(s))	Geographic Origin	GenBank *trn*L/F spacer	Accession *trn*L intron	Numbers *mat*K
Cratystylis conocephala (F. Muell.) S. Moore	Chandler 317 (CANB)	Australia: Western Australia	**AY143595**	**AY143595**	**AY143603**
Cratystylis subspinescens S. Moore	Wilson 12098 (CANB)	Australia: Western Australia	**AY143596**	**AY143596**	**AY143604**
Crepis tectorum L.	Bayer et al. AB-95002 (CANB)	Canada: Alberta	U82027	U82026	AF456784
Doniophyton anomalum (D. Don) Wedd.	Stuessy et al. 12857 (OS)	Argentina	U82029	U82028	AF456785
Echinops exaltatus Schrad.	AB-95004 (CANB)	Commercially grown plants; wild origin unknown, but native of Siberia	U82031	U82030	AF456786
Epaltes australis Less.	Craven and Matarczyk 10018 (CANB)	Australia: Queensland	**AY143599**	**AY143599**	**AY143607**
Eriocephalus paniculatus Cass.	Bayer and Puttock SAF-96260 (CANB)	South Africa: Western Cape Province	AF452502	AF452502	AF456787
Euryops virgineus Less.	Bayer and Puttock SAF-96237 (CANB)	South Africa: Eastern Cape Province	AF100517	AF098854	AF318910
Felicia filifolia (DC.) Burtt-Davy subsp. *schaeferi* (Dinter) Grau	Bayer and Puttock SAF-96166 (CANB)	South Africa: Northern Cape Province	AF318929	AF318120	AF318911
Flaveria australasica Hook.	Brockway CB-167 (PERTH, CANB)	Australia: Western Australia	AF452503	AF452503	AF456788
Gaillardia aristata Pursh	Bayer GH-95006 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82033	U82032	AF318912
Gazania rigens (L.) Gaertn.	Bayer GH-95012 (CANB)	Commercially grown plants; wild origin unknown, but native of South Africa.	U82035	U82034	AF456789
Gerbera jamesonii Bolus ex Hook.	Bayer GH-95004 (CANB)	Commercially grown plants; wild origin unknown, but native of South Africa.	U82037	U82036	AF456790
Haegiela tatei (F. Muell.) P.S. Short and Paul G. Wilson	Davies, Cooke and Green 72 (AD)	Australia: South Australia	**AY143600**	**AY143600**	**AY143608**

Table 1 (continued)

Helianthus annuus L.	Bayer GH-95007 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82039	U82038	AF151469
Inula helenium L.	Bayer GH-95013 (ALTA)	Commercially grown plants; wild origin unknown, but native of Eurasia	U82041	U82040	AF151473
Isoetopsis graminifolia Turcz.	Bayer et al. SA-97017 (CANB)	Australia: South Australia	AY069925	AY069925	AF456791
Lactuca sativa L.	Bayer AB-95007 (CANB)	Commercially grown plants; wild origin uncertain.	U82043	U82042	AF456792
Leysera gnaphalodes (L.) L.	Bayer and Puttock SAF-96021 (CANB, F, MO)	South Africa: Western Cape Province	AF100473	AF098810	AF456793
Liabum solidagineum (Kunth) Less.	Dillon and Sánchez 6253 (F)	Peru: Prov. Huancabamba	U82045	U82044	AF456794
Matricaria matricarioides (Less.) Port.	Bayer AB-95005 (CANB)	Canada: Alberta	U82047	U82046	AF151481
Oedera squarrosa (L.) Anderb. and K. Bremer	Bayer and Puttock SAF-96112 (CANB, F, MO)	South Africa: Western Cape Province	AF100475	AF098812	AF456795
Olearia covenyi Lander	Lyne, A.M. 2218 et al. (CANB)	Australia: New South Wales	AF452504	AF452504	AF456796
Olearia tomentosa (Wendl.) DC.	Makinson 1234 and Butler (CANB)	Australia: New South Wales	AF452505	AF452505	AF456797
Osteospermum clandestinum (Less.) Norl.	Bayer WA-94070 (CANB)	Australia: Western Australia	U82049	U82048	AF151488
Pegolettia oxydonta DC.	Bayer and Puttock SAF-96161 (CANB)	South Africa: Northern Cape Province	AF452506	AF452506	AF456798
Pluchea dentex R.Br. ex Benth.	Short, Watanabe, Kosuge and Denda 4405 (AD, CANB, MEL, PERTH)	Australia: Western Australia	AF100521	AF098858	AF151495
Printzia polifolia (L.) Hutch.	Bayer and Puttock SAF-96284 (CANB)	South Africa: Western Cape Province	AF098854	AF098816	AF456799
Relhania fruticosa (L.) K. Bremer	Bayer and Puttock SAF-96294 (CANB, F, MO)	South Africa: Western Cape Province	AF100476	AF098813	AF456800
Rosenia glandulosa Thunb.	Goldblatt and Manning 10528 (MO, NBG)	South Africa: Western Cape Province	AF100478	AF098815	AF456801
Senecio vulgaris L.	Bayer AB-95006 (CANB)	Canada: Alberta	U82053	U82052	AF151509

Table 1 (continued)

Species	Collectors and numbers (voucher location(s))	Geographic Origin	GenBank	Accession	Numbers
			*trn*L/F spacer	*trn*L intron	*mat*K
Stokesia laevis Greene	Bayer GH-95014 (ALTA)	Commercially grown plants; wild origin unknown, but native of North America	U82055	U82054	AF456802
Streptoglossa cylindripes (J.M. Black) Dunlop	Bayer WA-94049 (ALTA)	Australia: Western Australia	U82057	U82056	AF151513
Stuartina muelleri Sond.	Bayer, Breitwieser, Puttock and Ward SA-97010 (CANB)	Australia: South Australia	U82059	U82058	AF151514
Tagetes patula L.	Bayer s.n. (CANB)	Commercially grown plants; wild origin unknown, but native of Mexico	U82061	U82060	AF151515
Ursinia trifida (Thunb.) N.E.Br.	Bayer and Puttock SAF-96283 (CANB)	South Africa: Western Cape Province	AF452507	AF452507	AF456803

analysed; the first excluded all the coded indels, and the second included all indels and nucleotide characters. Forty-three coded indels were included in the final analysis, which improved resolution and strengthened support in the resulting trees. Indels were scored as binary characters for use in analyses, following the recommendations of Wojciechowski et al. (1993), van Ham et al. (1994) and Lloyd and Calder (1991) with gaps treated as missing data. The robustness of clades was assessed using a resampling protocol, jackknife analysis (Felsenstein 1988) with 10,000 replicates and 33% character deletion.

Results

Phylogenetic analysis yielded one island of 56 most parsimonious trees of length 1231 steps. The strict consensus of these trees (Fig. 1) and a phylogram (Fig. 2) detailing branch length, indicate that the Asteroideae is a strong monophyletic lineage (Figs. 1–2, synapomorphies (SYN) = 4; jackknife value (JKV) = 89%). They are sister to an evolutionary grade of tribes from the Cichorioideae with the two outgroup taxa from the Barnadesioideae. Thus, we have found confirmation that the Cichorioideae is paraphyletic, as most other studies have reported (see Bayer and Starr, 1998 for a complete discussion of the Cichoriodieae paraphyly issue).

The following tribes, Astereae (SYN = 8; JKV = 87%), Anthemideae (SYN = 35; JKV = 100%), Calenduleae (SYN = 61; JKV = 100%), Gnaphalieae (SYN = 8; JKV = 82%), Heliantheae s.l. (SYN = 6; JKV = 100%), Inuleae + Plucheeae (SYN = 19; JKV = 67%), Plucheeae (SYN = 12; JKV = 96%); Lactuceae (SYN = 11; JKV = 100%), and Senecioneae (SYN = 40; JKV = 100%) have strong support for monophyly. On the whole, support for the tribes throughout the topologies is high (Figs. 1–2), with most branches being supported by multiple synapomorphies.

With respect to *Cratystylis* and *Haegiela*, both genera are placed in what were once tribes that were included in the Inuleae (Figs. 1–2). Two species of *Cratystylis*, *C. conocephala* and *C. subspinescens*, are sister taxa in Plucheeae

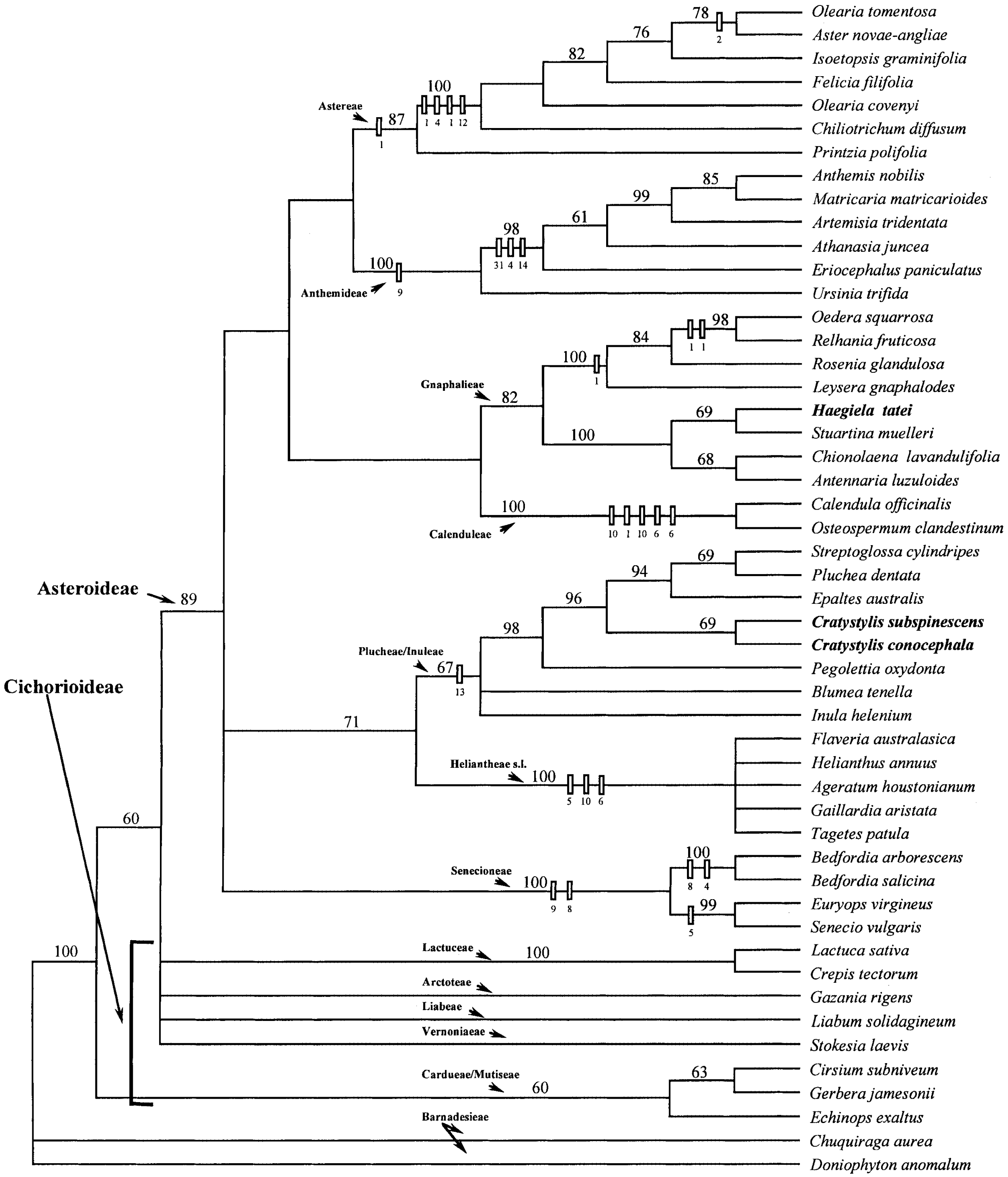

Fig. 1. Strict consensus of 56 trees of length 1231 in one island found from an heuristic search of the combined molecular data sets. CI = 0.6954, RI = 0.7658, RCI = 0.5325. Jackknife values > 50% are shown on the branches. Informative indels are mapped on the tree as broad bars. The length of the indels is indicated below the bars

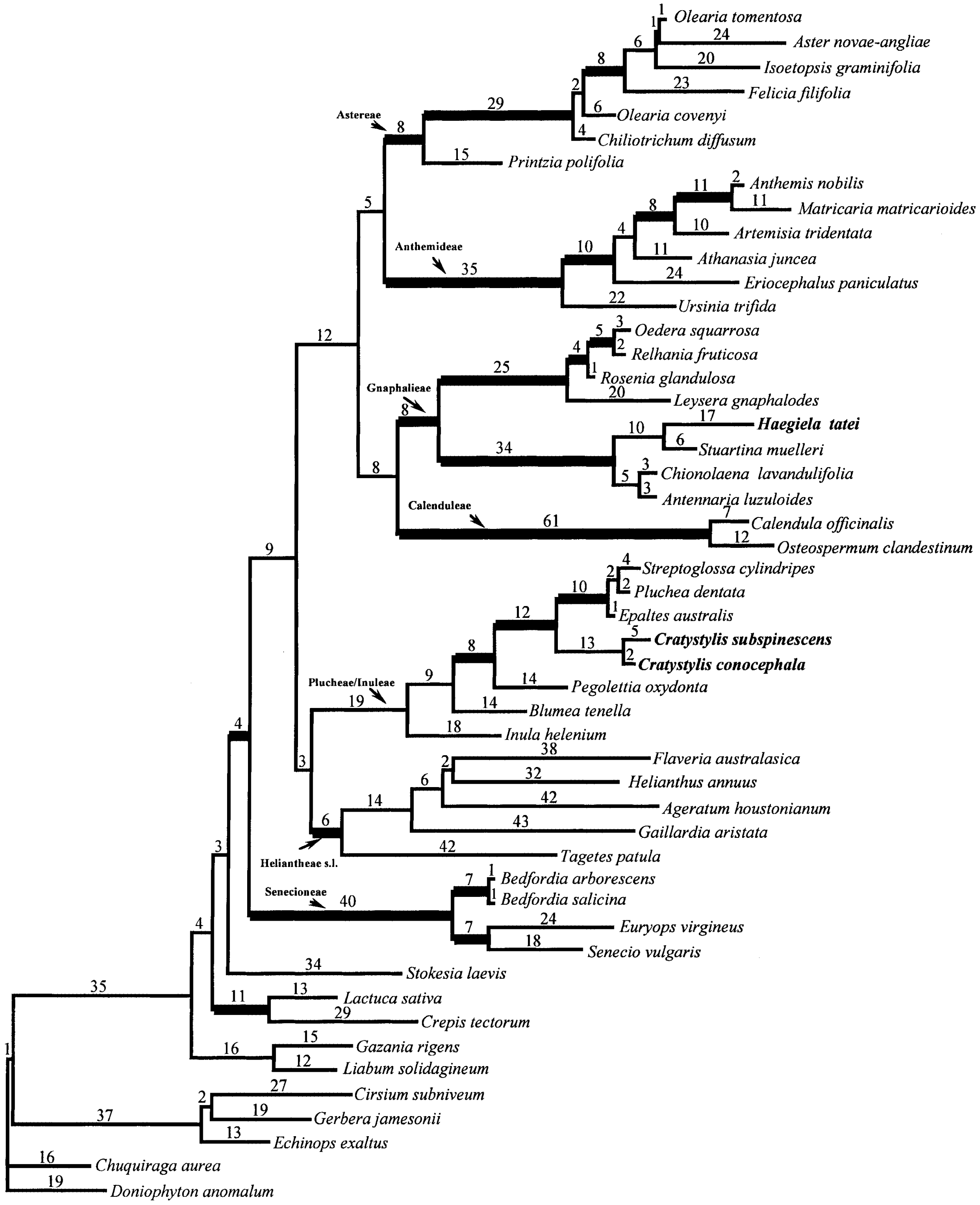

Fig. 2. A phylogram derived from one of the 56 most parsimonius trees. Branch lengths, and the numbers above the branches, are the number of changes. Bold branches receive >80% jackknife support as in Fig. 2

(SYN = 12, JKV = 96%) and sister to the remaining species of Plucheeae. *Haegiela* is sister to *Stuartina muelleri* (SYN = 10; JKV = 69%) in the Gnaphalieae (SYN = 8; JKV of 82%).

Discussion

Throughout their taxonomic histories, both *Cratystylis* and *Haegiela* have been placed variously within several tribes of the Asteraceae. Here we discuss their tribal placement based on the results of our molecular analysis and in light of morphology.

***Cratystylis* as a member of the Plucheeae.** For the first 30 years of its history, *Cratystylis* was regarded as a member of the tribe Astereae based on its perceived resemblance to the large Australasian genus *Olearia* Moench. In 1887, when Mueller transferred the taxon to *Pluchea*, by implication, he transferred it to another tribe (Plucheeae). He noted that the narrow style branches and the sagittate anther tails allied the taxon to *Pluchea* rather than *Olearia* or *Aster*. Although somewhat anomalous in *Pluchea*, Mueller (1887) argued that the generic circumscription of *Pluchea* should be broadened to include *Cratystylis*. When Moore (1905) formally described *Cratystylis* as a separate genus, he remarked that he could ‘‘only marvel why’’ Bentham didn’t place them in their own genus rather than retaining the three species in *Olearia*. He argued for a placement of *Cratystylis* in Inuleae (sensu Bentham 1873), because of the tailed anthers and thickened style branches. As *Cratystylis* differed from *Pluchea* in capitulum morphology, he rejected it being subsumed into an enlarged *Pluchea*. When Merxmüller et al. (1977) placed *Cratystylis* in the *Pluchea* group of their subtribe Inulinae, they claimed it was anomalous in being dioecious and having stigmatic rows that converge near the base and cover nearly the whole surface. Interestingly, there are some species of *Pluchea* (*P. tetranthera* F. Muell. and *P. baccharoides* (F. Muell.) Benth.) which are imperfectly dioecious (Mueller 1887).

Further, Zdero et al. (1988, 1991), present compelling chemical evidence for placement of *Cratystylis* in the Plucheeae, and this taken together with historical morphological data concurs with our placement of *Cratystylis* in a group of genera (Figs. 1 and 2), related to *Pluchea*. Additional carpopodial characters also support this placement; Haque and Godward (1984) surveyed carpopodia throughout the Asteraceae and found that members of subfamily Cichoriodieae had narrow carpopodia usually formed in sectors instead of complete rings, whereas Asteroideae usually have substantial carpopodia that form complete rings at the proximal end of the cypsela. Here we report that *Cratystylis* has the Asteroideae type of carpopodium, forming a complete and substantial ring.

In placing *Cratystylis* in Cichorioideae, close to Arctoteae, Anderberg et al. (1992) considered that both the morphological and palynological traits of *Cratystylis* were inconsistent with the traditional view of a Plucheoid/Inuloid affinity; we find this conclusion untenable. The multiple rows of involucral bracts found in *Cratystylis*, though common in Barnadesioideae and Cichorioideae (Anderberg et al. 1992), do occur in many Asteroideae such as the Australian endemic *Pluchea baccharoides* which has 6–7 (8) rows of involucral bracts (Hunger 1997). Likewise, caveate pollen is almost exclusive to the Asteroideae and very rare in Cichorioideae (Bolick 1978). The external structure of the pollen with a double tectum between the spines is very similar to the Plucheoid *Stenachaenium* (Anderberg et al. 1992), while the pollen diameter/exine thickness ratio (Bolick 1991) fall within the expected ranges for members of the Asteroideae.

Given the position of *Cratystylis* in the molecular phylogeny, coupled with its morphological similarities to members of the Plucheeae, we see no reason why *Cratystylis* should not be reinstated in the tribe Plucheeae, as first proposed by Ferdinand von Mueller (1887) over one hundred years ago.

***Haegiela* as a distinct genus and a member of the Gnaphalieae.** The molecular data robustly indicate that *Haegiela* is distinct from *Epaltes* and is a well supported member of the tribe Gnaphalieae. This concurs with Mueller (1883) who discussed the anomalous placement of *Haegiela* in *Epaltes*, Leins (1971) who considered the detailed palynology of both groups, and Short and Wilson (1990) who transferred *Epaltes tatei* into a new monotypic genus *Haegiela*. *Haegiela* shares many morphological features with Gnaphalieae including heterogamous discoid heads, epaleate involucre, the shortly caudate anthers, and truncate style branches with long sweeping stigmatic hairs.

Although the placement of *Haegiela* in Gnaphalieae is very robust, the generic relationships are difficult to ascertain given the limited sampling of only 7 species from within the tribe. Preliminary molecular data of both *mat*K and *trn*L/F sequences (unpubl. matrix) including a wide range of Australian genera (see Bayer et al. 2002), shows that *Haegiela* is in a clade that it is topologically distant from *Stuartina*, and belongs to the same major clade as both *Waitzia* and *Triptilodiscus*. This is broadly concordant with the treatments of both Wilson (1992) and Anderberg (1991a). The molecular data (see Figs. 1 and 2), also support the placement of *Epaltes* in Plucheae, as suggested by Bentham (1873). It is clear, however, that further representatives of *Epaltes* are required to substantiate this claim.

In recent molecular and morphological studies (Anderberg 1991c, Eldenas et al. 1999), the Plucheeae remains a strong monophyletic lineage. Our recent molecular findings (Bayer and Starr 1998, Bayer et al. 2000, Bayer et al. 2002, Bayer and Cross, 2002) support a strong monophyletic Gnaphalieae that is topologically distant from the Inuleae/Plucheeae clades in all molecular phylogenies. The morphological similarities of *Haegiela* to other members of the Gnaphalieae and its location in the molecular phylogeny suggests that *Haegiela* should remain in the Gnaphalieae. This was first implied by Leins (1971) when he studied the pollen morphology of the species.

In summary, there is now ample molecular and morphological evidence to establish the tribal position of *Cratystylis* in the tribe Plucheeae and *Haegiela* in Gnaphalieae.

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