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A reassessment of tribal affinities of the enigmatic genera *Printzia* and *Isoetopsis* (Asteraceae), based on three chloroplast sequences

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Abstract. The tribal affinities of two dubiously placed genera of the Asteraceae, *Printzia* and *Isoetopsis*, were assessed by using three chloroplast DNA sequences, the *trnL/F* spacer, the *trnL* intron and the *matK* coding region. The outgroup was represented by two species of the tribe Barnadesieae, whereas one to six genera (43 species including *Printzia* and *Isoetopsis*) of the tribes of the Asteroideae [Anthemideae (six genera), Astereae (five) Calenduleae (two), Gnaphalieae (six), Heliantheae *s.l.* (five), Inuleae *s.str.* (three), Plucheeae (two), Senecioneae (four)] and Cichorioideae [Arctotideae (one), Cardueae (two), Lactuceae (two), Liabeae (one), Mutisieae (one), Vernonieae (one)] were chosen as the ingroup. Phylogenetic analysis indicates that both *Printzia* and *Isoetopsis* have a strong affinity with members of the tribe Astereae. At some point in their taxonomic history, both genera had been placed in this tribe and there are good morphological and chemical characters that justify this placement.

Introduction

Our continuing studies on the phylogeny and systematics of the tribe Gnaphalieae (Asteraceae), have necessitated the re-evaluation 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 the tribe, including *Isoetopsis* Turcz. and *Printzia* Cass. These genera have suites of morphological and chemical traits, which offer conflicting clues as to their tribal affinities. Currently, there is no authoritative and convincing answer to the question of tribal alliance.

In this study, we use a DNA sequence data set in an attempt to elucidate the tribal relationships of *Printzia* and *Isoetopsis*. We selected the *trnL/F* spacer, the *trnL* intron and the *matK* coding region to resolve the phylogeny of the Asteraceae as the *trnL* intron and *trnL/F* intergenic spacer regions have proven useful in resolving generic and tribal relationships in the Asteraceae (Bayer and Starr 1998; Bayer *et al.* 2000, 2002). The *matK* coding region has been used widely in a number of plant groups (see Hilu and Liang 1997, for review), but its use has been limited in the Asteraceae (Konishi *et al.* 2000; Bayer *et al.* 2002). We have found it to be especially useful for reconstructing higher-level phylogenies in the Asteraceae (Bayer *et al.* 2002) and thus have incorporated it into this analysis.

Materials and methods

Fieldwork

Fieldwork was conducted in Australia, North America and the Republic of South Africa. Fresh material of the monotypic genus *Isoetopsis*, *Isoetopsis graminifolia* Turcz. and *Printzia polifolia* (L.) Hutch. was collected for the purpose of DNA extraction and morphological study (Table 1). Both of these species represent the types of their respective genera. Material of the other ingroup and outgroup taxa was either collected in the wild or obtained from commercial sources (Table 1).

Outgroup Selection. Tribal circumscriptions and nomenclature in this work are based on the treatment of the Asteraceae by Bremer (1994). Outgroup taxa were selected in accordance with the well-supported placement of the Barnadesioideae as the earliest diverging lineage in the Asteraceae (Bremer 1987; Jansen and Palmer 1987; Kim and Jansen 1995; Bayer and Starr 1998). Therefore, the outgroup was represented by two Barnadesioids, *Chuquiraga aurea* Skottsbo. and *Doniophyton anomalum* (D. Don) Wedd.

Ingroup sampling of Asteroideae

One to six genera (43 species) of all the tribes of the Asteroideae [Anthemideae (six genera), Astereae (five), Calenduleae (two), Gnaphalieae (six), Heliantheae *s.l.* (five), Inuleae *s.str.* (three), Plucheeae (two), Senecioneae (four)] and Cichorioideae [Arctotideae (one), Cardueae (two), Lactuceae (two), Liabeae (one), Mutisieae (one), Vernonieae (one)] were chosen as the ingroup (see Table 1).

The final matrix consists of two outgroup taxa (Barnadesioideae) and 41 ingroup members (Asteroideae and Cichorioideae) and the two genera, *Isoetopsis* and *Printzia*, of *insertae sedis*. Voucher specimens for all taxa are deposited in the herbaria cited in Table 1.

Table 1. List of species studied, their origin and voucher location, and GenBank accession number for sequences

Species	Collectors and numbers [voucher location(s)]	Geographic origin	<i>trmL/F</i> spacer	GenBank accession number	<i>trmL</i> intron	matK
<i>Ageratum houstonianum</i> Mill.	Bayer GH-95011 (CANB)	Commercially grown plants; wild origin unknown, but native of Mexico	U82013	U82012	AF151434	
<i>Anthemis nobilis</i> L.	Bayer GH-00001 and West (CANB)	Commercially grown plants; wild origin unknown, but native of Europe	AF452496	AF452496	AF456775	
<i>Antennaria luzuloides</i> Torr. & Gray	Bayer <i>et al.</i> OR-91002 (ALTA)	USA: Oregon	U82015	U82014	AF456774	
<i>Artemisia tridentata</i> Nutt.	Bayer <i>et al.</i> CO-90072 (ALTA) <i>trmL/F</i> Columbus 3874 (RSA) matK	USA: Colorado USA: California	U82017	U82016	AF456776	
<i>Aster novae-angliae</i> L.	Bayer AB-95003 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82019	U82018	AF151441	
<i>Athanasia juncea</i> D.Dietr.	Bayer and Puttock SAF-96286 (CANB)	South Africa: Western Cape Province	AF452497	AF452497	AF456777	
<i>Bedfordia arborescens</i> Hochr.	Richardson 82 (CANB)	Australia: Australian Capital Territory	AF452498	AF452498	AF456778	
<i>Bedfordia salicina</i> (Labill.) DC.	Stratham 10 (CANB)	Australia: Tasmania	AF452499	AF452499	AF456779	
<i>Blumea tenella</i> DC.	Short 4869 & Dunlop (DNA, CANB)	Australia: Northern Territory	AF452500	AF452500	AF456780	
<i>Calendula officinalis</i> L.	Bayer GH-95009 (CANB)	Commercially grown plants; wild origin unknown, but native of South Africa	U82021	U82020	AF151446	
<i>Chilotrimum diffusum</i> (G.Forst.) Kuntz	Story 8121 (CANB)	Argentina: province of Santa Cruz	AF452501	AF452501	AF456781	
<i>Chuiraga aurea</i> Skottsb.	Stuessy <i>et al.</i> 12911 (OS)	Argentina	U82023	U82022	AF456782	
<i>Cirsium subnivium</i> Rydb.	Bayer <i>et al.</i> WY-90044A (ALTA)	USA: Wyoming	U82025	U82024	AF456783	
<i>Crepis tectorum</i> L.	Bayer <i>et al.</i> AB-95002 (CANB)	Canada: Alberta	U82027	U82026	AF456784	
<i>Dontophyton anomalum</i> (D.Dom) Wedd.	Stuessy <i>et al.</i> 12857 (OS)	Argentina	U82029	U82028	AF456785	
<i>Echinops exaltatus</i> Schrad.	AB-95004 (CANB)	Commercially grown plants; wild origin unknown, but native of Siberia	U82031	U82030	AF456786	
<i>Eriocephalus paniculatus</i> Cass.	Bayer and Puttock SAF-96260 (CANB)	South Africa: Western Cape Province	AF452502	AF452502	AF456787	
<i>Euryops virgineus</i> Less.	Bayer and Puttock SAF-96237 (CANB)	South Africa: Eastern Cape Province	AF100517	AF098854	AF318910	
<i>Feltia filifolia</i> (DC.) Burt-Davy subsp. <i>schaeferi</i> (Dinter) Grau	Bayer and Puttock SAF-96166 (CANB)	South Africa: Northern Cape Province	AF318929	AF318120	AF318911	
<i>Flaveria australasica</i> Hook.	Brockway CB-167 (PERTH, CANB)	Australia: Western Australia	AF452503	AF452503	AF456788	
<i>Gaillardia aristata</i> Pursh	Bayer GH-95006 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82033	U82032	AF318912	
<i>Gazania rigens</i> (L.) Gaertn.	Bayer GH-95012 (CANB)	Commercially grown plants; wild origin unknown, but native of South Africa	U82035	U82034	AF456789	
<i>Gerbera jamesonii</i> Bolus ex Hook.	Bayer GH-95004 (CANB)	Commercially grown plants; wild origin unknown, but native of South Africa	U82037	U82036	AF456790	
<i>Helianthus annuus</i> L.	Bayer GH-95007 (CANB)	Commercially grown plants; wild origin unknown, but native of North America	U82039	U82038	AF151469	
<i>Inula helenium</i> L.	Bayer GH-95013 (ALTA)	Commercially grown plants; wild origin unknown, but native of Eurasia	U82041	U82040	AF151473	
<i>Isoetopsis graminifolia</i> Turcz.	Bayer <i>et al.</i> SA-97017 (CANB)	Australia: South Australia	AY069925	AY069925	AF456791	
<i>Lactuca sativa</i> L.	Bayer AB-95007 (CANB)	Commercially grown plants; wild origin uncertain	U82043	U82042	AF456792	
<i>Leysera gnaphalodes</i> (L.) L.	Bayer & Puttock SAF-96021 (CANB, F, MO)	South Africa: Western Cape Province	AF100473	AF098810	AF456793	

<i>Liabum solidagineum</i> (Kunth) Less.	Dillon and Sánchez 6253 (F)	Peru: Prov. Huancabamba	U82045	U82044	AF456794
<i>Matricaria matricarioides</i> (Less.) Port.	Bayer AB-95005 (CANB)	Canada: Alberta	U82047	U82046	AF151481
<i>Oedera squarrosa</i> (L.) Anderb. & K.Bremer	Bayer & Puttock SAF-96112 (CANB, F, MO)	South Africa: Western Cape Province	AF100475	AF098812	AF456795
<i>Olearia covenyi</i> Lander	Lyne, A.M. 2218 <i>et al.</i> (CANB)	Australia: New South Wales	AF452504	AF452504	AF456796
<i>Olearia tomentosa</i> (Wendl.) DC.	Makinson 1234 & Butler (CANB)	Australia: New South Wales	AF452505	AF452505	AF456797
<i>Osteospermum clandestinum</i> (Less.) Norl.	Bayer WA-94070 (CANB)	Australia: Western Australia	U82049	U82048	AF151488
<i>Pegolettia oxyodonta</i> DC.	Bayer and Puttock SAF-96161 (CANB)	South Africa: Northern Cape Province	AF452506	AF452506	AF456798
<i>Pluchea dentex</i> R.Br. ex Benth.	Short, Watanabe, Kosuge & Denda 4405 (AD, CANB, MEL, PERTH, TI)	Australia: Western Australia	AF100521	AF098858	AF151495
<i>Printzia polifolia</i> (L.) Hutch.	Bayer & Puttock SAF-96284 (CANB)	South Africa: Western Cape Province	AF098854	AF098816	AF456799
<i>Relbania fruticosa</i> (L.) K.Bremer	Bayer & Puttock SAF-96294 (CANB, F, MO)	South Africa: Western Cape Province	AF100476	AF098813	AF456800
<i>Rosenia glandulosa</i> Thunb.	Goldblatt & Manning 10528 (MO, NBG)	South Africa: Western Cape Province	AF100478	AF098815	AF456801
<i>Senecio vulgaris</i> L.	Bayer AB-95006 (CANB)	Canada: Alberta	U82053	U82052	AF151509
<i>Stokesia laevis</i> Greene	Bayer GH-95014 (ALTA)	Commercially grown plants; wild origin unknown, but native of North America	U82055	U82054	AF456802
<i>Streptoglossa cylindripes</i> (J.M.Black) Dunlop	Bayer WA-94049 (ALTA)	Australia: Western Australia	U82057	U82056	AF151513
<i>Stuartina muelleri</i> Sond.	Bayer, Breitwieser, Puttock & Ward SA-97010 (CANB)	Australia: South Australia	U82059	U82058	AF151514
<i>Tagetes patula</i> L.	Bayer <i>s.n.</i> (CANB)	Commercially grown plants; wild origin unknown, but native of Mexico	U82061	U82060	AF151515
<i>Ursinia trifida</i> (Thunb.) N.E.Br.	Bayer and Puttock SAF-96283 (CANB)	South Africa: Western Cape Province	AF452507	AF452507	AF456803

DNA isolation, amplification and sequencing

Fifty-six new sequences were generated for this study (Table 1). Total DNA was isolated as outlined in Bayer *et al.* (1996). Recalcitrant DNAs were purified with Qiaquick PCR Purification Columns (Qiagen Pty Ltd, Australia). The *trnL/F* region was amplified via the polymerase chain reaction (PCR) using *Taq* DNA polymerase. The PCR reaction mixture consisted of 5 µL of 10X reaction buffer, 3 µL of 25 mM magnesium chloride solution, 4 µL of a 1.25 mM dNTP solution in equimolar ratio, 25 pmol of each primer, 10–50 ng of template DNA and 1.0 unit of polymerase in a total volume of 50 µL. The PCR samples were heated to 94°C for 3 min prior to the addition of DNA polymerase to denature unwanted proteases and nucleases. The *trnL/F* and *matK* double-stranded PCR products were produced via 30 cycles of denaturation (94°C for 1 min), primer annealing (48°C for 1 min) and extension (72°C for 2 min). A 7-min final extension cycle at 72°C followed the 30th cycle to ensure the completion of all novel strands. Primer details (sequence and reference) are given in Bayer *et al.* (2002).

The *trnL/F* region was usually amplified as a single piece with primers 'c' and 'f' to amplify across the *trnL* intron and *trnL/trnF* spacer. In some instances, recalcitrant DNA was amplified as two separate regions with primers 'c' with 'd' and 'e' with 'f'. Likewise, the *matK* region was sometimes amplified as a single c. 2.8-kb piece with primers *trnK-3914F* and *trnK-2R*, but in many cases it was amplified as two smaller separate regions by using primer 1408F with *trnK-2R* and 1541R with *trnK-3914F*. Double-stranded PCR products were cleaned by column purification with Qiaquick PCR Purification Columns prior to sequencing. Some intractable sequences were cloned before sequencing by using pGEM-T Easy Vector Systems (Promega Corporation, Madison, WI, USA).

The double-stranded PCR products were then used as templates in cycle sequencing reactions employing the *trnL/F* primers 'c' and 'f' and 'd' and 'e'. Maturase-K sequencing was conducted with primers 1110R, 1240R, 1408F, 1541R and 1694F (Bayer *et al.* 2002). The double-stranded PCR products were sequenced with the Big Dye Terminator RR Kit (Perkin-Elmer Applied Biosystems, Wellesley, MA, USA) and an ABI automated sequencer in the Division of Plant Industry, CSIRO. Sequencing reactions for the *trnL/F* region and *matK* used 57°C annealing temperatures. The cycle sequencing protocol followed manufacturer's instructions. Sequences were assembled with Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI, USA). New sequences have been submitted to GenBank (Table 1).

Alignment of sequences proceeded by hand following the principles of non-coding sequence alignment discussed in Bayer *et al.* (2000). Gaps were inserted to maintain sequence homology. Consideration was given to the mutational mechanisms that may have resulted in the observed length mutations. Indels were scored to minimise the number of inferred length mutations, except where there was clear evidence that particular length mutation events were homogenetic.

Sequence data analysis

Sequence data were analysed by PAUP 4.0.b4a (Swofford 2001). The data matrix consisted of two outgroup species, 41 ingroup taxa, *Printzia* and *Isoetopsis*. Phylogenetic reconstruction was performed on unweighted characters by heuristic searches with 1000 replicates of random addition of taxa, so as to search for islands of most parsimonious trees (Maddison 1991). The following two data sets were analysed: the first excluded all the coded indels, while the second included all indels and nucleotide characters. Forty-one 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. The robustness of clades

was assessed by using a re-sampling protocol, jackknife analysis (Felsenstein 1988) with 10 000 replicates and 33% character deletion.

Results

Phylogenetic analysis yielded one island of 126 most parsimonious trees of length 1148 steps. The strict consensus of these trees (Fig. 1) and a phylogram (Fig. 2) detailing branch length indicate that the Asteroideae are a strong monophyletic lineage [Figs 1 and 2, synapomorphies (SYN) = 8; jackknife value (JKV) = 90%]. They are sister to an evolutionary grade of tribes from the Cichorioideae with the two outgroup taxa from the Barnadesioideae at the base corroborating previous studies and summarised in Bayer and Starr (1998).

Both trees strongly support the monophyly of the following tribes: Astereae (including *Printzia*; SYN = 9; JKV = 81%), Anthemideae (SYN = 35; JKV = 99%), Calenduleae (SYN = 68; JKV = 100%), Gnaphalieae (SYN = 8; JKV = 94%), Helianthiae s.l. (SYN = 18; JKV = 100%), Inuleae + Plucheeae (SYN = 9; JKV = 85%), Lactuceae (SYN = 13; JKV = 100%) and Senecioneae (SYN = 37; JKV = 100). In general, tribal support throughout the topologies (Figs 1 and 2) is high, with most clades being supported by multiple synapomorphies.

With respect to the two enigmatic genera, *Isoetopsis* and *Printzia*, both genera are placed in Astereae (Figs 1 and 2), with *Printzia* occupying a basal position in the tribe (SYN = 9, JKV = 81%, one homogenetic indel). *Isoetopsis* is embedded within Astereae forming part of a polytomy (which was previously recognised by E. W. Cross, C. J. Quinn and S. J. Wagstaff in press) containing *Aster novae-angliae* and *Olearia tomentosa*. This clade is moderately supported by one homogenetic indel and JKV of 76% (SYN = 8).

Discussion

Throughout their long histories, neither *Printzia* nor *Isoetopsis* have been indisputably placed within a tribe of the Asteraceae. In all cases where tribal position has been contentious, this problem usually arises because the taxon in question possesses morphological traits of more than one tribe, or, as in the case of *Isoetopsis*, the taxon is so reduced morphologically that its placement is subject to misinterpretation. Below we discuss the taxonomic histories of the two genera and re-evaluate them in light of the new molecular evidence.

Taxonomic history of *Printzia*

The history of *Printzia* begins in pre-Linnean times, when two representatives of the today's genus *Printzia* were known. One species, currently *P. polifolia* (L.) Hutch., was first known by the multinomials *Aster fruticosus africanus* (Ray 1704) or *Asteropterus fruticosus coeruleus polii folius* (Vaillant 1720). The other species is currently *P. aromatica* (L.) Less., which was originally described and illustrated as

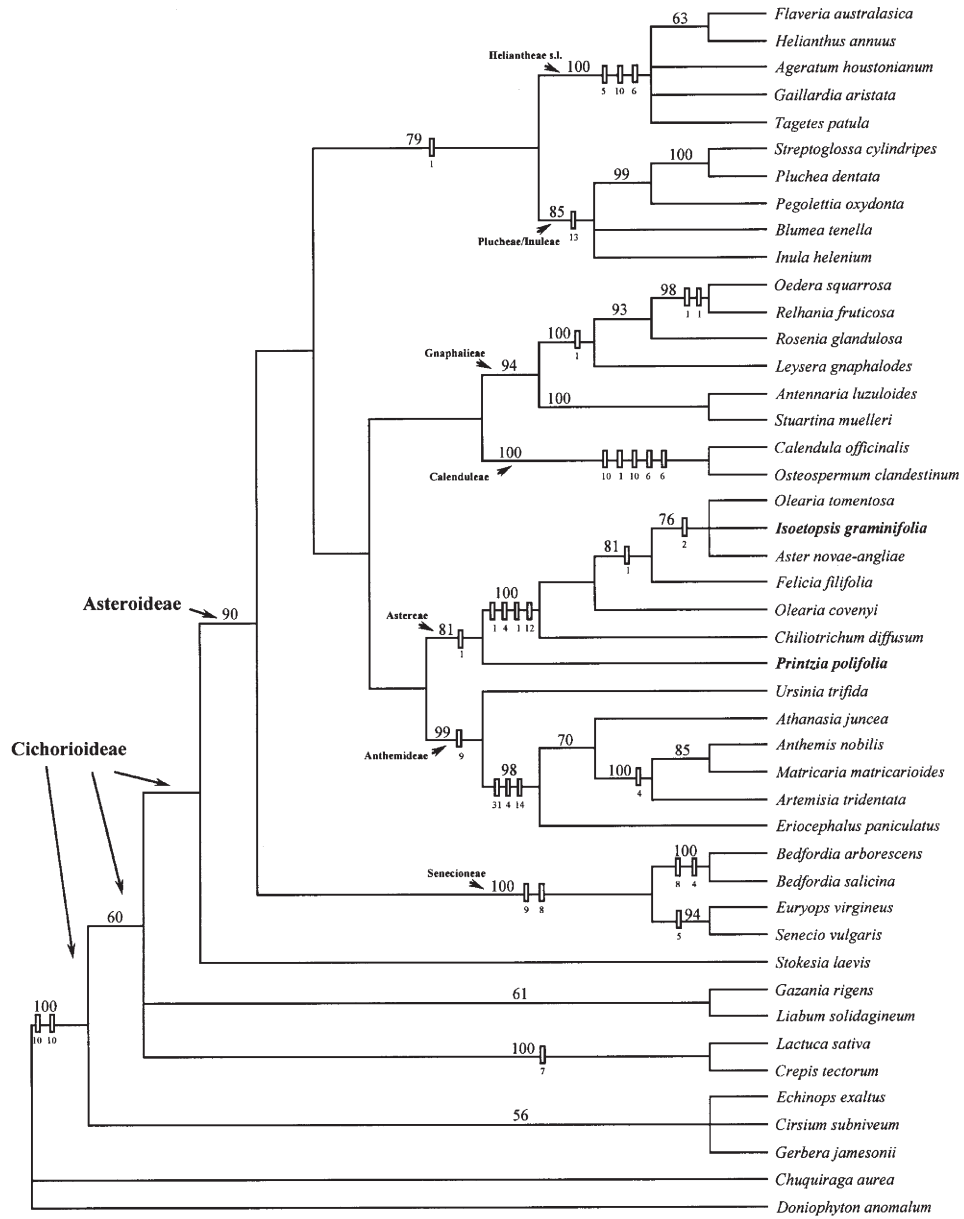


Fig. 1. Strict consensus of 126 trees of length 1148 in one island found from a heuristic search of the combined molecular data sets. CI = 0.7169, RI = 0.7596. Jackknife values >50% are shown on the branches. Informative indels are mapped on the tree as broad bars. The length of the indels is indicated below the bars.

Aster frutescens luteus Mauritanicus (Plukenet 1696). Linnaeus (1763) placed the two species into the following two different genera: the first species mentioned above as *Aster polifolius* L. and the other species as *Inula aromatica* L. In 1767, Linnaeus placed his *Aster polifolius* into *Inula* as *Inula caerulea*. Thunberg (1800) recognised the first species as *Leysera polifolia* (L.) Thunb., a genus currently classified in the Gnaphalieae, subtribe Relhaniinae (Anderberg 1991a). Cassini (1826) created the new genus *Printzia* for this taxon, calling it *P. bergii* Cass. Cassini, who was the first to erect a formal tribal system in the Asteraceae, placed

Printzia in the tribe Astereae, suggesting it was closely related to the Australasian genus *Olearia* Moench and the South American genus *Chilioitrichum* Cass. In fact, Cassini (1826) emphatically stated that *P. polifolia* [*P. Bergii* Cass., nom illeg.; = *P. polifolia*] does not belong in *Inula*, nor within the Inuleae, but instead, is well within Astereae, in which it must constitute a new genus, a very close ‘neighbour’ of *Olearia*.

Lessing (1830) moved Linnaeus’ *Inula aromatica* to *Printzia aromatica* and described *P. pyrifolia* (Lessing 1832), bringing the total species of *Printzia* to three. The

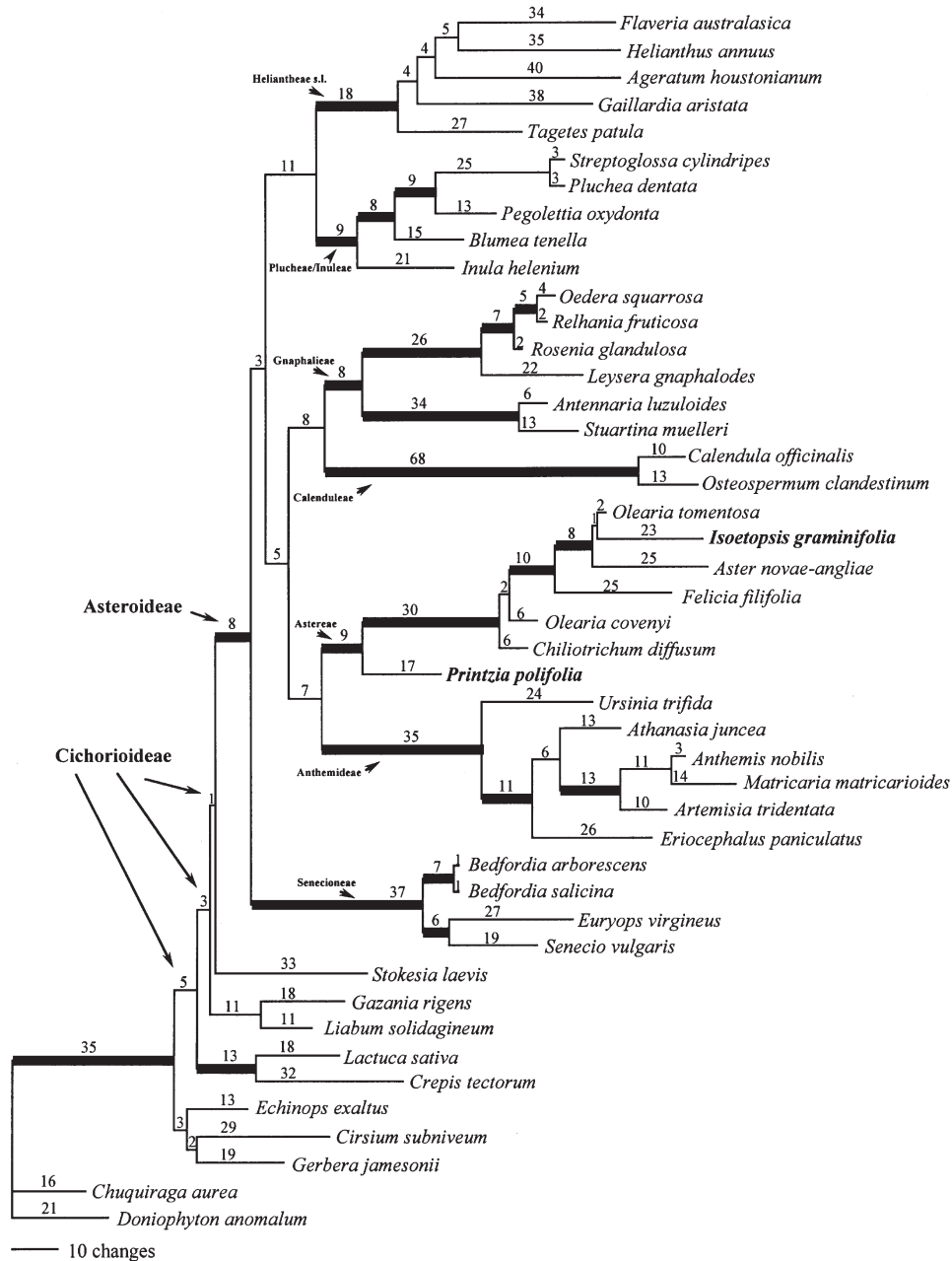


Fig. 2. A phylogram derived from one of the 126 most parsimonious trees. Branch lengths and the numbers above the branches, are the number of changes. Bold branches receive >80% jackknife support as in Fig. 1.

species, however, were placed in his tribe Mutisieae (Lessing 1832), which contained genera from the modern Barnadesioideae and parts of the Inuleae *s.str.* and Gnaphalieae. De Candolle (1838), following Lessing's lead, placed the three species of *Printzia* in his Mutisieae. The same tribal affiliation for *Printzia* was echoed by Lindley (1847). Harvey (1865), in *Flora capensis*, discussed two additional species of *Printzia*, *P. huttoni* Harv. and *P. auriculata* Harv., which he added to the three original species of the genus. He placed them in the tribe Cynareae,

subtribe Mutisieae, again close to members of the modern Barnadesioideae. Bentham (1873) suggested that *Printzia* lacked the essential characteristics of the Mutisieae (Mutiseae) and suggested a placement in the Inuleae near to the genus *Iphiona* Cass. Hoffmann (1890–1894), in his treatment of the Asteraceae in *Die Natürlichen Pflanzenfamilien*, adhered to Bentham's scheme and placed *Printzia* in the Inuleae subtribe Inulinae.

In the 1890s, *Printzia laxa* N.E.Br. and *P. densifolia* J.M. Wood & Evans were described, but these species are now

conspecific with *P. auriculata* (Kroner 1980). Hutchinson (1946) uncovered the connection to Linnaeus' first description, overlooked by Cassini and others, and made the necessary new combination for the type species, *P. polifolia* (L.) Hutch. Finally, Leins (1971a) moved the monotypic *Bojeria nutans* Bolus into *Printzia*, making the new combination, *P. nutans* (Bolus) Leins. Currently, *Printzia* contains six species (Kroner 1980).

Recent history of *Printzia*

Since the time of Bentham (1873), all modern researchers in the Inuleae have considered *Printzia* to be circumscribed within the Inuleae *s.l.* (including Gnaphalieae). It was placed in Inuleae, subtribe Inulinae, by Leins (1971b) on the basis of pollen morphology. Leins (1971b) found that there are two distinct pollen grain types within the subtribe Inulinae. *Printzia* was placed in the second group along with *Pentatrachia*, *Anisochaeta* and the Relhaniinae–Athrixiinae. While the Relhaniinae–Athrixiinae also possess this pollen grain type, they differ in the structure of their styles (Leins 1971b). Further, Leins (1971b) noted that the similarity in pollen morphology between the two groups may be convergence. Merxmüller *et al.* (1977) considered it in Inuleae (Athrixiinae) and Kroner (1980) endorsed their view. Hilliard (1977) placed *Printzia* in the Inuleae in her treatment for Natal. Anderberg (1991a; in Bremer 1994), moved *Printzia* into his Gnaphalieae, after demonstrating, through a series of papers, that the traditional Inuleae (*sensu* Bentham and Merxmüller) was an unnatural assemblage that was to be best treated as three distinct tribes, Inuleae *s. str.*, Plucheeae and Gnaphalieae (Anderberg 1989, 1991a, 1991b, 1991c).

The now seemingly traditional placement of *Printzia* in the Inuleae *s.l.* was called into question in our previous analysis of South African Gnaphalieae (Bayer *et al.* 2000), where *Printzia* and *Aster* formed a monophyletic group supported by six synapomorphies in 97% of the most parsimonious trees. In addition, Bohlman and Zdero (1978) found compounds (prinziatic acid methylester; isoprinziatic acid methylester; 15, 16H-16-oxohardwickiic acid methylester; 2-oxo-ent-manoyl oxide; 2-oxo-19-hydroxyl ent-manoyl oxide) in *Printzia* that were identical to those found in members of the Astereae, specifically in species of *Solidago* L. Given the evidence at that time, Bayer *et al.* (2000) concurred with Cassini's (1826) opinion that the affinities of *Printzia* lie with the Astereae, not the Inuleae–Gnaphalieae. The analysis that we present here strongly supports (SYN = 9; JKV = 81%) the placement of *Printzia* at the base of the tribe Astereae.

Printzia as a member of the Astereae

Cassini (1816), when he erected the first comprehensive tribal classification system for the Asteraceae, placed *Printzia* in the Astereae. Later, Lessing (1832), with no

explicit reason, moved it into the Barnadesioideae (his Mutisiaceae). This tribal position was followed until Bentham (1873) moved it to the Inuleae, saying simply that *Printzia* did not have characters of the Mutisiaceae, but instead to his 'eye' had similarity to the Inuloid genus *Iphiona*. Until recently, Bentham's placement of *Printzia* in the Inuleae went unquestioned.

Printzia is a genus of shrubs or perennial herbs. The leaves are alternate, flat or revolute with entire or dentate-serrate margins and are tomentose at least on the abaxial surface. The capitula are solitary and the involucre bracts are imbricate in about four rows, cartilaginous in texture and brown with a green midrib. The receptacle is flat and epaleate. Outer florets are radiate, purple to blue or white to yellow, whereas the central florets are perfect, white or yellow. Anthers are tailed with flat appendages (see illustration in Hilliard 1977). The pollen, referred to as the '*Printzia*-type' by Anderberg (1991a), has walls that are two-layered with a baculate outer sexine and irregularly interlaced inner sexine and the spines on the grains have cavities (Leins 1971b). The styles are bifid, branches are obtuse, dorsally with short papillose, obtuse sweeping-hairs not reaching the bifurcation. The stigmatic surface is basally separated in two lines that converge apically. The cypselae are ellipsoid and covered with elongated twin hairs and glandular hairs. Finally, the pappus bristles are capillary, barbellate to subplumose, tawny brown and free.

With respect to all the morphological features of *Printzia*, described above, most of these conditions are well known in the Southern Hemisphere Astereae, such as *Olearia*. Obtuse style branch apices and *Printzia* type pollen are exceptions. The similarity of the unusual *Printzia* pollen type to that in genera of subtribe Relhaniinae of the Gnaphalieae may be an example of convergence (Leins 1971b).

Given the position of *Printzia* in the molecular phylogeny and its morphological similarities to other members of the Astereae, we support the reinstatement of *Printzia* in Astereae recognising it as the basal taxon. This tribal placement was first proposed by Henri Cassini (1826) nearly 200 years ago.

Taxonomic history of *Isoetopsis*

The history of the monotypic genus *Isoetopsis* begins with its description by Nicolai Turczaninow in 1851. Turczaninow (1851) suggested that *Isoetopsis* was perhaps closely related to *Heterotheca* Cass. (Astereae) or *Heteropappus* Less. (Astereae), although, because of its unusual paleaceous pappus, he also suggested it resembled some species of *Bellis* (Astereae). It was also pointed out by Turczaninow (1851) that its monoecious heads, i.e. pistillate outer florets and functionally staminate central florets, were inconsistent with those of other members of the 'subtribe'. Later, Bentham, in *Flora Australiensis* (1867), placed it in the Anthemideae remarking that it had affinities with *Cotula* L.

and *Myriogyne* Less. [= *Centipeda* Lour.]. In 1873, Bentham reaffirmed this placement of *Isoetopsis* in the Anthemideae, but suggested that it differed from typical Anthemideae, particularly in the structure of their style and other morphological features. *Isoetopsis* was placed in the Anthemideae subtribe Chysantheminae by Hoffmann (1890–1894) following Bentham's (1867–1873) assignment. Since the publication of *Flora Australiensis* (Bentham 1867), virtually all Australian floras have traditionally placed *Isoetopsis* in the Anthemideae, except the very recent *Flora of Victoria* (Walsh 1999), which placed it in the Gnaphalieae.

Tribal affinity of Isoetopsis revisited

For nearly a century, Bentham's placement of *Isoetopsis* in the Anthemideae went unquestioned. This, however, all began to change with the publication of the chromosome number for the taxon, as $n = 17$, by Turner (1970). He suggested that, on the basis of unpublished pollen data, *Isoetopsis* might be better placed in the Inuleae. Robinson and Brettell (1973) revisited the tribal position of *Isoetopsis*. They suggested that with respect to morphology of the anthers, pollen ultrastructure, style branches and corolla, *Isoetopsis* more closely resembles members of the Astereae. The pappus, for example, which consists of about eight broad squamae, is unlike anything in Anthemideae or Senecionieae, but instead is much closer to some members of the Astereae. They state categorically that 'There is no reason to doubt that *Isoetopsis* is a member of the Astereae'. Grau (1977), although retaining *Isoetopsis* in the Astereae, commented that it did not fit as well in the Astereae as Robinson and Brettell (1973) thought. He commented that the fruit anatomy and pappus morphology of *Isoetopsis* were especially aberrant (Grau 1977). Heywood and Humphries (1977), accepting that *Isoetopsis* has an unusual base chromosome number and fruit anatomy, excluded it from Anthemideae and suggested it is better situated in the Astereae. Skvarla *et al.* (1977), in studying pollen morphology, were uncommitted as to whether the Astereae or Gnaphalieae were the closest tribal affinity of *Isoetopsis*, as was Sørensen (1977) in studying the distribution of polyacetylenes in the Asteraceae. Sørensen (1977) did, however, verify that *Isoetopsis* lacked the polyacetylenes that are characteristic of the Anthemideae.

Following an examination of the ultrastructure of pollen grains of *Isoetopsis*, Gadek *et al.* (1989) confirmed them as being of the Helianthoid type. This pollen type is much more prevalent in Astereae than in Anthemideae (Skvarla *et al.* 1977). Anderberg (1989) rejected the placement of *Isoetopsis* in the Astereae, arguing that the presence of ectomycorrhizal fungi in association with the roots of *Isoetopsis* indicated an affinity with the Gnaphalieae (in the 'Waitzia group') rather than Astereae. He drew from the work of Warcup and McGee (1983) on the mycorrhizal associations of some Australian Asteraceae, despite their

extremely limited sampling of Astereae (e.g. 12 species of Astereae across five genera). Warcup and McGee (1983) also emphasised that one of the main limitations of their study were clear differences between field studies and laboratory-based results. Bruhl and Quinn (1990) undertook detailed analysis of many cypsela features including the extent of sclerenchyma within the seed, the nature of the trichomes and the embryo orientation. They concluded that despite *Isoetopsis* lacking a proposed Astereae synapomorphy (Grau 1977), the presence of a testa epidermis with thickening on three sides (U-shaped cells), the other morphological characters were Astereae-like. On the basis of further detailed morphological analysis, this time on the micromorphology of the inflorescence, as well as stem anatomy, Bruhl and Quinn (1991) accepted the argument of Anderberg (1991a) and supported his placement of *Isoetopsis* in Gnaphalieae. Bremer and Humphries (1993) and Nesom (1994) excluded *Isoetopsis* from the Anthemideae and Astereae, respectively, both sets of authors referring it to the Gnaphalieae. Anderberg in Bremer (1994) also maintained this placement. Finally, Watanabe *et al.* (1996), while accepting *Isoetopsis* as part of the Astereae, suggested that it might also be a member of the Gnaphalieae. Therefore, in the past 30 years there has been a great deal of uncertainty about the tribal placement of *Isoetopsis*.

Isoetopsis as a member of the Astereae

Isoetopsis is an annual herb. Its leaves are alternate, filiform with entire margins and glabrous. The capitula, few to many, are crowded together amongst the basal leaves. Involucral bracts are biseriolate, chartaceous and brownish basally, papery and green apically, with the stereome divided. The receptacles are flat and epaleate. The numerous outer florets are weakly bilabiate and white, outnumbering the functionally male, white, central florets. The anthers are short and linear with apical appendages and the exothecial cells are 'Astereae-like' (Robinson and Brettell 1973). Style branches are linear, glabrous or with a few apical hairs at the obtuse ends and the cypselae are turbinate, with elongated twin hairs. The pappus is of approximately eight hyaline, paleaceous, scales. The pollen type has been confirmed as being of the 'Helianthoid' type (Gadek *et al.* 1989). The meiotic chromosome number has been determined as $x = 17$ (Turner 1970).

With respect to all the morphological features of *Isoetopsis*, described above, most of these conditions are well known in the Astereae. A couple of features are uncommon in Astereae, including bilabiate outer florets and fibers in the phloem. However, as pointed out by Bruhl and Quinn (1991), the bilabiate corolla can evolve quite easily from reduction of the lamina of ray florets. Drury and Watson (1966) discovered that members of a portion of the 'old' Inuleae corresponding to many, but not all, genera of the modern Gnaphalieae, contain numerous bunches of fibers in the phloem. Bruhl and Quinn (1991) reported occasional fibers in the phloem of

Isoetopsis, but it is not known whether the distribution and frequency of occurrence match those that are frequent in some Gnaphalieae. The chromosome number of $x = 17$ is not known in Australian Astereae, but $x = 18$ is quite common (Turner 1970). The lower base chromosome number in *Isoetopsis* could have come about through aneuploid reduction from $x = 18$, which is quite a common evolutionary phenomenon in annuals (Stebbins 1971). The final anomalous feature of *Isoetopsis* is the presence of ectomycorrhizal fungi on the roots, a feature that is common among the members of the low percentage of Australian Gnaphalieae that have been investigated (Warcup and McGee 1983). Warcup (1980) has also shown that ectomycorrhizae are associated with plants that occur across a wide taxonomic range in Australia from Casuarinaceae to Fabaceae, Euphorbiaceae, Rubiaceae and Apiaceae, to the sister families of the Asteraceae, Goodeniaceae and Stylidiaceae. Given the rather limited sampling of the Asteraceae, particularly the tribe Astereae, carried out (Warcup and McGee 1983), it is quite likely that ectomycorrhizae are present in other tribes of the Asteraceae. Consequently, ectomycorrhizae may not be unique to the Gnaphalieae.

Given the morphological similarities of *Isoetopsis* to members of the Astereae, together with its location in the molecular phylogeny within a monophyletic Astereae, we see many reasons why *Isoetopsis* should be permanently placed in the tribe Astereae, as first implied by Nicolai Turczaninow (1851). It would seem that *Isoetopsis* would be best placed in subtribe Asterinae (*sensu* Bremer 1994), along with other herbaceous Australian genera, such as *Minuria* DC. and *Vittadinia* A. Rich. (see Bruhl and Quinn 1990, 1991).

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