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Evolutionary relationships in the Asteraceae tribe Inuleae (incl. Pluceae) evidenced by DNA sequences of *ndhF*; with notes on the systematic positions of some aberrant genera

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Abstract

The phylogenetic relationships between the tribes Inuleae sensu stricto and Pluceae are investigated by analysis of sequence data from the cpDNA gene *ndhF*. The delimitation between the two tribes is elucidated, and the systematic positions of a number of genera associated with these groups, i.e. genera with either aberrant morphological characters or a debated systematic position, are clarified. Together, the Inuleae and Pluceae form a monophyletic group in which the majority of genera of Inuleae s.str. form one clade, and all the taxa from the Pluceae together with the genera *Antiphiona*, *Calostephane*, *Geigeria*, *Ondetia*, *Pechuel-loeschea*, *Pegolettia*, and *Iphionopsis* from Inuleae s.str. form another. Members of the Pluceae are nested with genera of the Inuleae s.str., and support for the Pluceae clade is weak. Consequently, the latter cannot be maintained and the two groups are treated as one tribe, Inuleae, with the two subtribes Inulinae and Pluceinae. The genera *Asteriscus*, *Chrysophthalmum*, *Inula*, *Laggera*, *Pentanema*, *Plucea*, and *Pulicaria* are demonstrated to be non-monophyletic. *Cratystylis* and *Iphionopsis* are found to belong to the same clade as the taxa of the former Pluceae. *Caesulia* is shown to be a close relative of *Duhaldea* and *Blumea* of the Inuleae-Inulinae. The genera *Callilepis* and *Zoutpansbergia* belong to the major clade of the family that includes the tribes Heliantheae sensu lato and Inuleae (incl. Pluceae), but their exact position remains unresolved. The genus *Gymnarrhena* is not part of the Inuleae, but is either part of the unresolved basal complex of the paraphyletic Cichorioideae, or sister to the entire Asteroideae.

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Introduction

The subdivision of the former tribe Inuleae into three smaller groups has gained support in a number of

papers (Bremer, 1987; Anderberg, 1989; Karis et al., 1992; Kim and Jansen, 1995; Eldenäs et al., 1999; Panero and Funk, 2002). The separation of tribe Gnaphalieae from the rest of the Inuleae complex is well supported and evidently correct, but the delimitation of the other genera of the Inuleae, referred to as tribes Inuleae s.str. and Pluceae by Anderberg (1989,

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1991a,b), have not been fully understood. Early analyses of molecular data based on *ndhF* sequence data (Kim and Jansen, 1995; Eldenäs et al., 1999) showed that the Inuleae and Plucheeae are sister groups, and that they together constitute the sister group of Heliantheae s. lat., Eupatorieae, and the African *Anisopappus*, *Athroisma* and *Blepharispermum*, three genera formerly included in the Inuleae (Merxmüller et al., 1977).

Only three genera from Inuleae s.str. and Plucheeae were included in the study by Kim and Jansen (1995), and this small sample size did not allow for any statements on whether the two represented monophyletic groups, or one was derived within the other. To clarify this issue, Eldenäs et al. (1999) investigated the relationships between the two tribes with a larger sample of taxa. They showed that the tribe Plucheeae formed one clade and that the majority of taxa from the Inuleae s.str., as circumscribed by Anderberg (1989, 1991a), formed another. However, their study failed to resolve the positions of three genera from the Inuleae s.str., i.e. *Antiphiona*, *Geigeria*, and *Pegolettia*, in relation to the Plucheeae and Inuleae clades, and thus left tribal delimitation somewhat unclear. In the same paper, Eldenäs et al. (1999) described the presence of a characteristic 3-bp insertion in *ndhF* (CCT in position 1588 from 5' end of the *Inula* sequence in GenBank Accession No. L39453) as characteristic of taxa belonging to the Inuleae s.str. clade. The same genera that were shown to have this 3-bp insertion are also characterized by a large, rhomboid oxalate crystal in each cell of the cypselas epidermis (Anderberg 1989, 1991a). The 3-bp insertion is absent from all other Asteraceae, including all the genera of the Plucheeae clade, and notably also from the three genera of unclear systematic position mentioned above (*Antiphiona*, *Geigeria*, and *Pegolettia*).

Our present investigation includes a still larger sample of taxa, aiming at a better understanding of the relationships between Inuleae and Plucheeae in order to resolve their tribal status. Apart from elucidating tribal interrelationships, we have also tried to shed light on the issue of monophyly of some of the larger genera, such as *Blumea*, *Pluchea*, *Inula*, and *Pulicaria*, by including several representatives of each in the analysis. Furthermore, we have included a number of genera whose systematic positions have been debated, or which have an aberrant morphology compared to typical members of these tribes. Such genera are *Caesulia*, *Callilepis*, *Cratystylis*, *Gymnarrhena*, *Iphionopsis*, *Rhodogeron*, *Sachsia*, *Zoutpansbergia*, and also *Dielitzia*. The latter was described as a member of Inuleae, but was included in the Gnaphalieae by Anderberg (1991c). Like *Dielitzia*, many Australian Gnaphalieae have more or less cartilaginous bracts without the papery lamina that is typical of most representatives of that tribe, and it was of interest to test if a taxon such as *Dielitzia* would

group with other taxa of Gnaphalieae, with Inuleae s.str., or with the Plucheeae. The genus *Peluchea*, which with great hesitation was accepted in the Plucheeae by Anderberg (1994), has been found to belong in the Heliantheae (Baldwin and Wessa, 2000), and was not investigated further by us.

Material and methods

Molecular methods

DNA was extracted from leaves taken from herbarium specimens or from material dried in silica gel. Voucher information for new *ndhF* sequences is presented in the Appendix A, other sequences are those used by Eldenäs et al. (1999), or more recent depositions in GenBank (*Asteriscus* spp., *Pallenis* spp., *Schizogyne*, *Vierea*, *Xerolekia*). For many extractions, leaves were ground in liquid nitrogen with mortar and pestle, and DNA subsequently extracted with the method of Saghai-Marooof et al. (1984) as modified by Doyle and Doyle (1987). For others, leaves were ground with a Mini-Bead Beater (BioSpec Products, Bartlesville, Oklahoma, USA) and subsequently treated with the DNEasy plant DNA extraction kit from Qiagen (Qiagen Inc., Valencia, California, USA), following the manufacturer's protocol. All PCR reactions were performed with 10 µmol/l primers in 25-µl reactions using "Ready-to-go" PCR beads from Pharmacia Biotech (Amersham Pharmacia Biotech, Uppsala, Sweden), following the manufacturer's standard protocol and generally with the thermal cycling profile, 95 °C for 5 min, followed by 45 or 55 cycles of 95 °C for 30 s, 45 °C 30 s, 72 °C 2 min, and finally 72 °C for 8 min. Primers used are those of Eldenäs et al. (1999).

For sequencing reactions in some taxa, the Thermo Sequenase Fluorescent Sequencing Kit from Pharmacia (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used. Fragments were sequenced using fluorescently labeled (CY-5) primers. Fragments were separated on an ALF-Express (Pharmacia Biotech AB, Uppsala, Sweden). For most taxa the "Big Dye Terminator Sequencing" kit (Applied Biosystems, Warrington, Cheshire, UK) was used, and fragments were separated on an ABI377 from Applied Biosystems. Primers used for PCR and for sequencing are the same as those used by Eldenäs et al. (1999). Sequences were assembled with the Staden software (Staden et al., 1998), and aligned with the BioEdit software (Hall, 1999). Two sequences, unpublished at the time of our analysis, from *Rhodogeron coronopifolius* (GenBank Acc. No. AY226799) and from *Sachsia polycephala* (AY226800), were kindly provided by Javier Francisco-Ortega, Florida International University. All 63 new *ndhF* sequences have been

submitted to GenBank (Accession Nos. AY780811–AY780873; see Appendix A).

Phylogenetic analyses

The alignment of *ndhF* sequences resulted in a data matrix with 184 taxa and 2280 characters of which 660 were informative for phylogenetic analysis. The data was analyzed with parsimony jackknifing (Farris et al., 1996), using the computer software “Xac” (Farris, 1997) with the following settings: 1000 replications, each with branch-swapping and ten random-addition of sequences. For the analysis, *Boopis* (Calyceraceae) was used as outgroup, as this family constitutes the sister group of the Asteraceae. Gaps found in the *ndhF* sequences were treated as missing information; gaps were few, and the alignment was unproblematic. Analyses were performed with all codon positions included, following the recommendations of Källersjö et al. (2000).

Results

The results of our investigation (Fig. 1) show the same basic topology and relationship between tribes as those found earlier with a smaller taxon sample (Eldenäs et al., 1999), but give a much more detailed picture of generic relationships within the Inuleae-Pluceeae complex. The two tribes form a highly supported (100% jackknife) monophyletic group, which is composed of two main clades: Inuleae s.str. with robust support (100%), and Pluceeae together with some genera from Inuleae s.str. with low support (51%).

In Inuleae s.str. (Fig. 2), the basal bifurcation is between a group formed by *Duhaldea*, *Blumea*, and *Caesulia* (93% jackknife support) and the rest of the genera (98%). In the first clade, *Duhaldea* is sister to a group (100%) that has *Caesulia* as sister to five species of *Blumea* (78%). *Blumea balsamifera* is here sister to *B. riparia*, *B. saxatilis*, *B. integrifolia*, and *B. psammophila*, and *B. integrifolia* is sister to *B. psammophila* (100%). In the clade comprising the rest of the Inuleae s.str., there are three main monophyletic groups. The first of these (82%) comprises *Schizogyne*, *Vierea*, *Pulicaria crispera*, and two species of *Rhanterium* (99%). The second clade (61%) has *Xerolekia* as sister to *Buphthalmum* (99%) and these two genera are then sister to a large group (86%) containing *Inula oculus-christi* (GenBank Accession No. L39453, under the synonym *I. sericea*), *I. peacockiana*, *Pentanema alanyense* as sister to *Chrysophthalmum gueneri* (98%), *Carpesium* as sister to *Telekia* (98%), and a group with *Amblyocarpum*, *Chrysophthalmum montanum*, *Pentanema glanduligerum*, *Inula bifrons*, *I. britannica*, *I. conyza*, *I. germanica* and *I.*

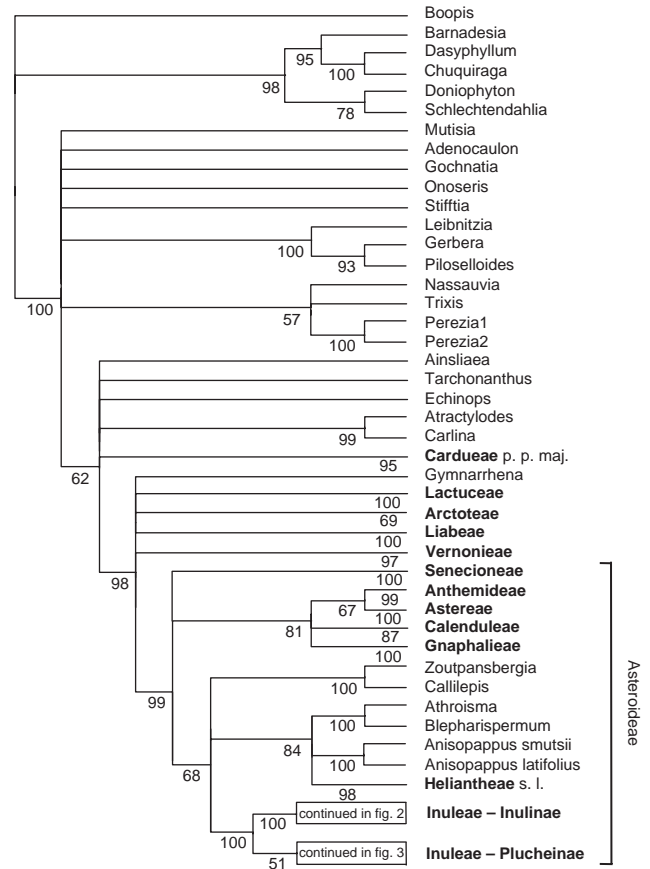


Fig. 1. Asteraceae (partly abbreviated; continued in Figs. 2 and 3). Tree based on jackknife analysis of 184 DNA sequences of *ndhF*; only nodes with jackknife support > 50% are resolved; several tribes are shown abbreviated into single lines since they are not subjects of the main discussion in the present paper.

verbascifolia (99%). The third clade (86%) has *Pulicaria canariensis*, *P. dysenterica*, *Dittrichia* and *Jasonia* in a group (80%) that is sister to one formed by *Anvillea*, *Asteriscus*, *Ighermia*, and *Pallenis* (99%). *Dittrichia* and *Jasonia* form a monophyletic group (65%) within a paraphyletic *Pulicaria*, with *P. dysenterica* as sister to *Dittrichia* + *Jasonia* and *P. canariensis* as sister to *P. dysenterica*, *Dittrichia*, and *Jasonia*. *Anvillea* is sister to a group formed by members of the genera *Asteriscus*, *Pallenis*, and *Ighermia* (64%). *Ighermia* has low support (52%) as sister to the two *Asteriscus* species, *A. imbricatus* and *A. schultzei* (99%), whereas the remaining six *Asteriscus* species form another group (83%).

The Pluceeae clade (Fig. 3) has a basal polytomy of five smaller monophyletic groups, three of which are formed by genera usually associated with Inuleae s.str. One group is that with *Geigeria* and *Ondetia* (100%), the second has 94% support for a group with *Calostephane* and *Mollera* (79%) as sister to the three *Pegolettia* species (100%). The third group has *Laggera decurrens*

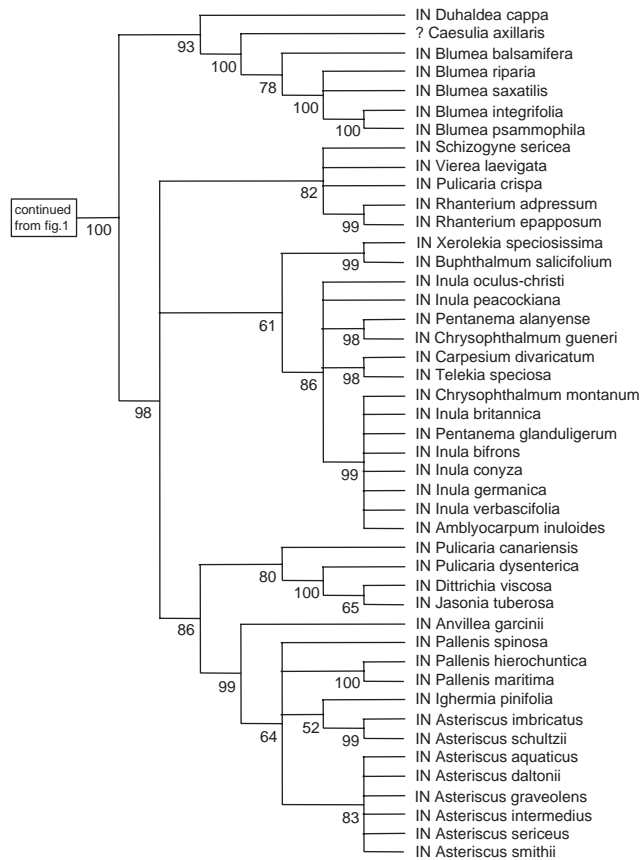


Fig. 2. Inuleae-Inulinae (continued from Fig. 1). Tree based on jackknife analysis of DNA sequences of *ndhF*; only nodes with jackknife support > 50% are resolved; IN = Inuleae s.str. (Anderberg, 1991a).

as sister to the two species of *Antiphonia* (100%). One of the two remaining clades has two samples of *Stenachaenium* (100%), and the final clade in the polytomy comprises the remaining genera (54%). In that large group, the genera *Iphionopsis*, *Cratystylis*, *Pterocaulon*, a group with *Rhodogeron* and *Sachsia* (99%), and the remaining Plucheeae genera (59%) constitute an unresolved pentatomy. The core Plucheeae group has *Pechuel-loeschea* from Inuleae s.str. as sister to the rest of the taxa (50%), a group formed by *Coleocoma*, *Cylindrocline*, *Doellia*, *Epaltes*, *Karelinia*, *Laggera* p.p., *Pluchea*, *Porphyrostemma*, *Pseudoconyza*, *Sphaeranthus*, *Streptoglossa*, and *Tessaria*. In the larger (50%) group, *Pluchea arabica*, *P. kelleri*, *P. littoralis*, *P. lucens*, and *P. sarcophylla* form a group (62%), and weak support (52%) is also obtained for a group indicating a close relationship between *Coleocoma*, *Pluchea dentex*, and *Streptoglossa*. The other relationships among the taxa of this group are unresolved.

The genus *Dielitzia* in our analysis was confirmed to belong in Gnaphalieae and was placed near *Antemaria* (100%). This means that it belongs in the core Gnaphalieae, in spite of its many specialized features

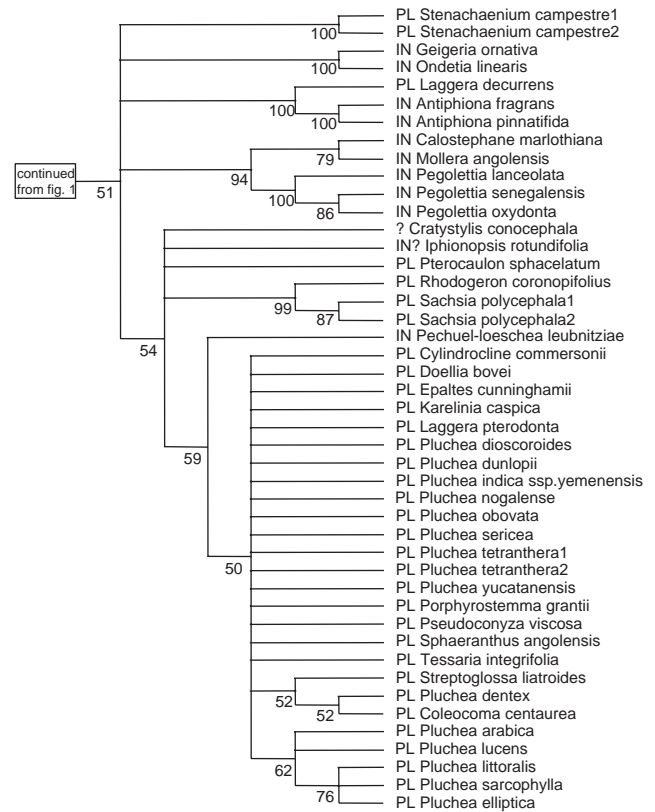


Fig. 3. Inuleae-Pluchinae (continued from Fig. 1). Tree based on jackknife analysis of DNA sequences of *ndhF*; only nodes with jackknife support > 50% are resolved; IN = Inuleae s.str. (Anderberg, 1991a), PL = Plucheeae (Anderberg, 1991b).

that differ from the typical everlasting of that tribe. The position of this genus is not shown in the tree, and will not be discussed further in the present context.

Discussion

Our present results (Fig. 1) corroborate the hypotheses of Bremer (1987) and Anderberg (1989), that two separate monophyletic groups can be identified within the Inuleae-Plucheeae complex, but extend those hypotheses to show that Inuleae and Plucheeae together form a monophyletic group. Most taxa placed in Inuleae s.str. by Anderberg (1991a) form a clade (Fig. 2), but some of its genera belong to the same clade as the taxa of Plucheeae (Anderberg, 1991b) and constitute part of an unresolved ancestral complex in that clade (Fig. 3). Therefore we propose that Plucheeae henceforth be treated as a part of Inuleae. Although support for this clade is low (51%), there are subtribal names already available for the two clades: Inuleae-Inulinae and Inuleae-Pluchinae, respectively.

Inuleae-Plucheinae

The tribes Lactuceae, Arctoteae, Liabeae, and Veroniceae, i.e. the tribes constituting the so-called LALV-complex (Kim and Jansen, 1995), are the Cichorioideae tribes that are most closely related to the subfamily Asteroideae to which Inuleae belongs. In the LALV-complex, taxa generally have style branches with acute sweeping hairs, which are also characteristic of the genera in Inuleae-Inulinae, thus being a plesiomorphic character state. In contrast, most genera belonging to the Plucheeae, as circumscribed by Anderberg (1991b), have styles with obtuse sweeping hairs reaching below the style bifurcation. However, also several taxa of the Inuleae-Plucheinae, as circumscribed here, have styles with acute sweeping hairs ending above the style bifurcation, i.e. *Geigeria*, *Laggera decurrens*, *Ondetia*, *Antiphonia*, *Pegolettia*, *Calostephane*, *Mollera*, *Sachsia*, and *Rhodogeron*. *Mollera* differs from *Calostephane* mainly in being devoid of a pappus, the former name was thus reduced to a synonym of the latter by Anderberg (1991a), and the close relationship gains support also from our present study. All the genera just mentioned, as well as *Laggera decurrens*, also have endothelial tissue with polarized wall thickenings. *Laggera decurrens* thus shares two character states with *Antiphonia* that differ from the condition in other *Laggera* species, which have obtuse sweeping hairs reaching below the style bifurcation, endothelial tissue with radial wall thickenings, and belong to the core Plucheeae clade together with *Pluchea* and other genera. All taxa of the Inuleae-Plucheinae clade with acute sweeping hairs on the style branches are positioned at lower nodes in the tree than those with obtuse sweeping hairs extending down on the style, e.g. *Pluchea*.

All taxa of the Inuleae-Plucheinae clade, as it appears here, are devoid of the large oxalate crystal in the cypsela epidermis that is characteristic of the Inuleae-Inulinae, and they also lack the 3-bp insertion (CCT) in *ndhF* that is another characteristic of the taxa belonging to that group. Anderberg (1991a) considered both *Antiphonia*, *Pechuel-loeschea*, and *Pegolettia* to be members of Inuleae s.str. because of their acute style hairs, but noted that they lacked the oxalate crystals. However, in our present study these genera are shown to be part of the Inuleae-Plucheinae. It is also interesting to note that *Pegolettia lanceolata* is sister to *P. oxyodonta* and *P. senegalensis*; except for *P. lanceolata*, the species of this genus share many distinct synapomorphies, and the only uncertainty regarding the monophyly of the genus (Anderberg, 1986) was whether or not *P. lanceolata* is part of this genus, or not. The molecular data give robust support for *P. lanceolata* as sister to the other species of the genus, thus corroborating Anderberg's results. All species of *Pegolettia* have a pappus with bristles and scales, and also *P. lanceolata* has small

scales at the base of the pappus bristles. Apart from this, *P. lanceolata* only shares the widened corolla base, a few vague pollen wall characteristics, and presence of several small crystals in the cypsela epidermis with the rest of the species of the genus.

On the next higher level of the phylogeny the relationships are still somewhat unresolved and the genera belong to one of five clades in a pentatomy. The first is formed by the mainly Caribbean genera *Rhodogeron* and *Sachsia*, which are sisters. The genus *Sachsia* has pollen with branching bacules (Leins, 1971), and in this differs from pollen of other Plucheinae. Both genera were placed in the *Sachsia* group of Inuleae-Inulinae s. ampl. by Merxmüller et al. (1977), and in spite of their styles provided with acute sweeping hairs they were treated as anomalous members of the Plucheeae by Anderberg (1991b), who also hypothesized *Rhodogeron* to be a taxon derived within *Sachsia* proper. A recent study by Hong et al. (2004) using ITS sequence data reconstructed the phylogeny of the group and reinstated *Rhodogeron* as a separate genus, as it was shown to be the sister of *Sachsia* and not a derived ingroup.

The pentatomy described above also includes the genus *Cratystylis*, another taxon for which the systematic position has been debated. It is an Australian genus of four species of dioecious shrubs having white, deeply lobed florets, styles almost devoid of sweeping hairs, and a stigmatic area covering almost the entire inner surface of the style branches. Other Inuleae are not dioecious, have short-lobed florets, distinct sweeping hairs, and a stigmatic area separated in two lateral bands that are confluent distally. Merxmüller et al. (1977) placed the genus with uncertain affinity in the so-called *Cratystylis* group of Inuleae-Inulinae s. ampl., but Zdero et al. (1988) considered the phytochemical components they found in *Cratystylis* to indicate a relationship with genera such as *Epaltes*, *Pluchea*, and *Sphaeranthus* (Plucheinae), and not with *Inula* (Inulinae), thus similar to our present results. Anderberg et al. (1992) described the pollen morphology of *Cratystylis conocephala* as being caveate with irregular baculate sexine, and with a smaller exine/pollen diameter ratio than what is typical of genera belonging to any of the Asteroideae tribes. They also found the pollen to be different from that of other Inuleae and Plucheeae, but with some resemblance to pollen from the genus *Stenachaenium*. Based on a number of features in *Cratystylis* that are normally found in the Cichorioideae, they consequently hypothesized a position for the genus in that subfamily, and hence Bremer (1994) included *Cratystylis* as a genus of uncertain position within subfamily Cichorioideae. Later, Bayer and Cross (2003) found that *Cratystylis* actually is part of the pluchoid group of genera in the subfamily Asteroideae, in spite of its somewhat differing morphological and palynological features, and our molecular data corroborate that conclusion.

The third part of the pentatomy is another genus for which the systematic position has been debated, viz. the African *Iphionopsis*. Two of the species were described as members of *Iphiona* (Inuleae-Inulinae) but subsequently transferred to a new genus of uncertain systematic position that has been associated with the Inuleae, but without a well-founded opinion of where it belongs (Anderberg, 1985, 1991a). The species of *Iphionopsis* have homogamous capitula and characteristic resin ducts in the mid-portion of the involucre bracts and in the corolla, and has an unsclerified fruit wall. The stigmatic area covers almost the entire inner surface of the style branches. In our results *Iphionopsis* is part of Inuleae-Plucheinae, and it is interesting in this context that *Pluchea serra* Franch. recently has been found to be a synonym of *I. rotundifolia* (Thulin, 2003).

The fourth clade of the pentatomy is represented by the genus *Pterocaulon*, a mainly New World genus, with a few species in Australia and adjacent areas, here represented only by the Australian *P. sphacelatum*. The diagnostic characters for *Pterocaulon* are the heterogamous capitula with filiform female florets, generally winged stems, and capitula congested into spherical or elongated secondary heads. The monophyly of the genus has not been tested, but there is some documented variation in morphology (number of disc-florets, number of row of pappus bristles, leaf trichome structure) between the Australian-Oceanic species (sometimes referred to as genus *Monenteles*) and the American ones, which may indicate that the genus is non-monophyletic. Some species have distinct spherical or ellipsoid secondary heads, whereas others have a rather loose, spike- or raceme-like arrangement of capitula that hardly can be referred to as secondary heads. *Pterocaulon* has acute sweeping hairs not reaching below the style bifurcation, as in the genera at the next deeper level of the clade (see above), but the endothecial tissue has radial wall thickenings.

The last group in the pentatomy comprises the core plucheoid taxa, i.e. *Coleocoma*, *Cylindrocline*, *Doellia*, *Epaltes*, *Karelinia*, *Laggera* p.p., *Pechuel-loeschea*, *Pluchea*, *Porphyrostemma*, *Pseudoconyza*, *Sphaeranthus*, *Streptoglossa*, and *Tessaria*. Most taxa in this group have styles with obtuse sweeping hairs extending below the bifurcation. Capitula are generally disciform with filiform female outer florets and male or hermaphroditic central florets, but some species have discoid capitula with only hermaphroditic florets. The Australian *Streptoglossa* differs in having conspicuously radiate capitula with distinct purple ray florets, but otherwise conforms to the normal condition in the group. Anderberg (1991b) found *Pluchea* to be heterogeneous and believed the genus would prove to be paraphyletic, whereas King-Jones (2001) considered Old World and Australian species of *Pluchea* to form a homogeneous monophyletic group, a conclusion that is not supported by our present

data. We included 14 taxa of *Pluchea*, the New World species *P. sericea* and *P. yucatanensis*, the Australian *P. dentex*, *P. dunlopilii*, and *P. tetranthera* (two sequences), and the Old World *P. arabica*, *P. dioscorides*, *P. indica*, *P. kelleri*, *P. lucens*, *P. littoralis*, *P. nogalense*, *P. obovata*, and *P. sarcophylla*. These *Pluchea* species do not form a clade, and their relationships to other genera of the clade are almost unresolved. King-Jones (2001) placed *Streptoglossa* outside of *Pluchea*, and had *P. dentex* embedded between other *Pluchea* species. In our results, the Australian *P. dentex* takes a position closer to the genera *Coleocoma* and *Streptoglossa* than to any of the other *Pluchea* species. The support for this is low; thus the question whether or not *Pluchea* is monophyletic remains to be solved, and this is true also for the generic status of *Karelinia*, *Streptoglossa*, and *Tessaria* in relation to *Pluchea*. Another taxonomic problem is the phylogeny of *Laggera*, in our study represented by the two species *L. pterodonta*, and *L. decurrens*. Whereas *L. pterodonta* has a position in the *Pluchea* clade, *L. decurrens* was found to be sister to *Antiphiona* in the basal complex of Inuleae-Plucheinae. As mentioned above, *Laggera* s. str. has the same kind of obtuse sweeping hairs extending below the style bifurcation as in *Pluchea*, *Epaltes*, and other *Plucheinae*, but *L. decurrens* differs by having acute sweeping hairs not reaching below the bifurcation. Formerly, this species has been placed in *Blumea* (syn. *B. gariiepina*), but it is apparent that it can be included neither in *Blumea*, nor in *Laggera*. The genus *Pseudoconyza* (syn. *Laggera aurita*, *Blumea viscosa*) did not group with any of the *Laggera* species in our analysis. The three species *Laggera gariiepina*, *Pseudoconyza viscosa*, and *Doellia bovei* (syn. *Blumea bovei*) have at some point been included in *Blumea*, but this genus, noted to be heterogeneous by Anderberg (1991a), belongs to Inuleae-Inulinae. The phylogenetic relationships among the genera of Inuleae-Plucheinae is part of our ongoing research and will be the topic of a forthcoming paper.

Inuleae-Inulinae

The taxa of this clade, except for *Caesulia*, are characterized by the presence of one large oxalate crystal in each cypsela epidermis cell, and furthermore they all share the unique 3-bp CCT insertion in *ndhF* mentioned above. The subtribe has two well-supported main monophyletic groups, i.e. *Duhaldea* together with the *Caesulia-Blumea* group, and its sister group comprising all the taxa from the remaining genera, including the species rich *Inula* and *Pulicaria* (Fig. 2).

Anderberg (1989, 1991a) found that *Blumea* shares several characteristic features with genera of Inuleae s.str., rather than with those of the *Pluchea* group, and moved it from a position near *Pluchea* to Inuleae s.str. A

close relationship between the type of the genus (*B. balsamifera*) and *Duhaldea* was also hypothesized from Anderberg's analysis of morphological data. Among other considerations, the presence in *Blumea* of the *Inula*-type cypselas and acute stylar sweeping hairs were interpreted as indications of a position in Inuleae s.str. Anderberg also found *Blumea* heterogeneous in these character states, and therefore put forward the idea that the genus was not monophyletic. Later, Anderberg (1995) placed two *Blumea* species with styles provided with obtuse sweeping hairs extending far below the bifurcation in the genus *Doellia*, here found to belong in the *Pluchea* clade of Inuleae-Plucheinae (see above). As mentioned above, two other former *Blumea* species were found to belong in Plucheinae as well. In our present study we have included five species of *Blumea* s. str., all of which group together with the type and fall within the same monophyletic group that also includes *Duhaldea*, thus corroborating Anderberg's (1995) view.

A surprising result is that the molecular data strongly suggest that the morphologically aberrant genus *Caesulia* belongs to the clade that includes *Blumea* and *Duhaldea*, being a derived member of Inuleae-Inulinae. The relationships and tribal position of *Caesulia* have been unclear so far because of its many odd morphological character states. The cypselas of *Caesulia* do not have the large oxalate crystal in the epidermis cells that is typical of other genera of this clade, but the *ndhF* sequence from *Caesulia* has the same characteristic 3-bp insertion (CCT, discussed above) as the other genera of Inuleae-Inulinae, an insertion that is not present in *ndhF* of any sequenced Asteraceae genera outside the subtribe.

Hoffmann (1889) included *Caesulia* in Inuleae-Angianthinae (now part of tribe Gnaphalieae) because of its secondary heads consisting of one-flowered capitula surrounded by two hyaline bracts. Besold (1971) showed that the style and pollen in *Caesulia* are not structured like in that group, and only with hesitation did Leins (1971) place *Caesulia* near the genus *Sphaeranthus* (now Inuleae-Plucheinae). Merxmüller et al. (1977) followed Leins, but noted that the chromosome number ($2n = 14$) and the synflorescence structure of *Caesulia* was aberrant from that of the other members of the *Sphaeranthus* group. The style in *Caesulia* has a stigmatic area that seems to cover the entire inner surface of the style-branches, whereas the typical Inuleae style has two separate stigmatic bands that merge apically. The sweeping hairs are also of intermediate shape, neither distinctly acute nor obtuse. Like *Duhaldea* the anthers have polarized endothelial tissue and well-developed branched tails, but the apical anther appendix is not truncate as in *Duhaldea*. The fruits in *Caesulia* are strongly flattened when mature, crowned by two hyaline scales, and have no visible

epidermis crystals. The fruits are unique within Inuleae, and in appearance more closely resemble those of many Heliantheae, albeit without the black phytomelanin content. Anderberg (1989) did not accept *Caesulia* in either Inuleae or Plucheae, because of its atypical style and stigma and the lack of other characters diagnosing genera of these tribes. Later treatments by Anderberg (1991b) and Bremer (1994), included *Caesulia* as an unplaced member of subfamily Asteroideae awaiting more conclusive evidence regarding its tribal position.

Pandey et al. (2000) described the fruit anatomy of *Caesulia axillaris* and found this to be more complex than had been assumed before. What appears to be the fruit was shown to be a structure formed by two strongly and laterally flattened bracts, which at maturity completely conceal the pericarp. With increasing age the tissue of this 'involucral cup' dominates as it becomes hard and provided with resiniferous ducts, whereas the true pericarp only consists of a single cell layer, a sclerified epidermis. Pandey et al. (2000) referred to the complex fruit structure as a pseudopericarp. A pappus is missing in *Caesulia*, but instead the pseudopericarp is crowned by two scale-like outgrowths, which are likely to be the tips of the involucral bracts. The very reduced pericarp lacks large oxalate crystals, and in this respect *Caesulia* differs from all other members of the Inuleae clade. The most parsimonious interpretation, given the present phylogeny, is that the fruit of the ancestor of *Caesulia* was provided with cypselas like in the other taxa of Inuleae s.str., but that these were lost as a result of the increasing reduction of the pericarp.

The sister group of the *Duhaldea-Caesulia-Blumea* clade is the large group formed by the remaining genera, including the *Inula* and *Pulicaria* complexes. These complexes were hypothesized by Anderberg (1991a) to be paraphyletic residue groups devoid of the synapomorphies diagnosing a number of smaller descendant genera, and separated by technical characters, the presence (*Pulicaria*) or absence of small scales in a row outside the barbellate pappus bristles (*Inula*). Anderberg anticipated that a number of the smaller genera would prove to have their closest relatives in either of these two genera, and in this respect our results corroborate his conclusion.

The large clade including the *Inula* and *Pulicaria* complexes has an unresolved trichotomy at the base. One clade in that trichotomy includes the genera *Rhanterium*, *Vierea*, and *Schizogyne*, but also one species of *Pulicaria*, i.e. *P. crispa*, which is often placed in the genus *Francoeuria*. The two other species of *Pulicaria* belong to another monophyletic group, and this supports the previous supposition that *Pulicaria* is not monophyletic. *Vierea* has an *Inula*-like appearance, but unlike *Inula* it has a pappus of bristles of unequal length arranged in several rows. In *Rhanterium* the pappus bristles are broad and flattened with an aspect

that is clearly different from the ordinary kind of capillary pappus bristles found in other genera. The second clade within the trichotomy includes *Amblyocarpum*, *Carpesium*, *Chrysophthalmum*, *Inula*, *Pentanema*, and *Telekia*. This clade has *Buphthalmum-Xerolekia* as sister group, but only with low support. The third clade in the trichotomy includes the genera *Anvillea*, *Asteriscus*, *Ighermia*, and *Pallenis*, where *Anvillea* is sister to the others. These genera all have paleate receptacles, and except for *Anvillea* have a pappus of large scales only. Two species of *Pulicaria*, i.e. *P. canariensis* and *P. dysenterica*, group together with *Jasonia* and *Dittrichia*, with *P. dysenterica* sister to *Jasonia*+*Dittrichia*. All except for *Dittrichia* have a double pappus consisting of both bristles and scales. The pappus in *Dittrichia* consists of barbellate bristles united at the base in a cupule-like structure that is unique in the tribe. Its ellipsoid cypselas are, however, similar in shape to those of most taxa of *Pulicaria*, and the fruits also have a collar of glandular hairs distally.

Paraphyly of *Inula* and *Pulicaria*

Anderberg (1991a) put forward that *Inula* and *Pulicaria* were likely to be paraphyletic and morphologically heterogeneous assemblages, and that more detailed analyses would lead to a new circumscription of these genera. Earlier, *Dittrichia* had been separated from *Inula*, and Anderberg demonstrated *Duhaldea* to be only distantly related to *Inula*, and more closely related to *Blumea*. The simple, bristly pappus in combination with the absence of more specific characteristics of other genera has been the only feature diagnosing *Inula*, and Anderberg proposed that several smaller genera could have their closest relatives within *Inula*. Likewise, the double pappus with bristles and scales is the only characteristic diagnosing *Pulicaria*, if the smaller and more derived genera with the same kind of pappus structure are kept separate. In the present study we found that all the species of *Inula* belong to the same clade, but that other genera are also part of this clade, i.e. *Amblyocarpum*, *Carpesium*, *Chrysophthalmum*, *Pentanema* p.p., and *Telekia*. All taxa belonging to this clade have a pappus of bristles only, or are completely devoid of pappus bristles. They may be with or without paleae, but most taxa are epaleate. At the base of the *Inula* clade is a pentatomy including *Inula* (*Codonocephalum*) *peacockiana*, *I. oculus-christi*, a well-supported (98% jackknife) group with *Chrysophthalmum gueneri* and *Pentanema alanyense*, another robust (98%) group with *Carpesium* and *Telekia*, and a 99%-jackknife clade including *Amblyocarpum*, *Chrysophthalmum montanum*, *Pentanema glanduligerum*, and five species of *Inula*. Francisco-Ortega et al. (2001) found a close relationship between *Telekia* and *Inula helenium*, and this is hardly

surprising considering their similarity in general appearance. Among the tall, large-headed, large-leaved species of Inuleae-Inulinae, *Telekia* is readily recognized by its paleate receptacles and lack of a pappus. In our analysis *Telekia* is closest to the morphologically derived genus *Carpesium*; both are epappose but otherwise very different. *Carpesium* differs from all other Inuleae in its capitulum morphology and the cypselas shape, and a position near *Inula* or *Telekia* is not self-evident. However, cypselas of *Carpesium* have one large, characteristic, elongated crystal in each epidermis cell, just like the other Inuleae s.str., and its *ndhF* sequence also has their characteristic CCT insertion in the same position. The sister relationship with *Telekia* is surprising, but there are a few morphological character states in support of this, e.g. long rod-shaped cypselas and the lack of a pappus. Anderberg (1991a) had hypothesized that the long cypselas and lack of a pappus were synapomorphies uniting *Carpesium* and *Amblyocarpum*, but this postulated relationship does not receive any support here, although both genera are part of the same larger clade, and *Amblyocarpum* seems to be a derived epappose relative of some *Inula* species.

Except for *Inula peacockiana* and *I. oculus-christi*, the species of *Inula* belong to the same (99%-jackknife) clade, but this clade also includes *Chrysophthalmum montanum*, *Pentanema glanduligerum*, and *Amblyocarpum inuloides*. Within the larger (86%) *Inula* clade, the genera *Pentanema* and *Chrysophthalmum* also seem to be paraphyletic. *Pentanema* is heterogeneous and is merely diagnosed by having few pappus bristles, which seems to be a technical character. The dwarf shrub *P. glanduligerum* and the perennial herb *P. alanyense* do not group with each other, the latter instead forms a clade with *Chrysophthalmum gueneri*. Duman and Anderberg (1999) had doubts about the generic assignment of *P. alanyense* when the species was described. Due to its few pappus bristles and lanate resting buds the choice of genus was *Pentanema*, a decision also influenced by the assumption that *Inula*, the alternative possibility, was heterogeneous and paraphyletic. *Pentanema alanyense* and *Chrysophthalmum gueneri* show some similarities in vegetative morphology, but are distinct in capitula morphology and floral structure. The former has epaleate capitula and a pappus of capillary bristles, the latter is paleate without a pappus. The possibility of DNA contamination is ruled out because the *Pentanema alanyense* DNA was extracted and sequenced long before the work on *Chrysophthalmum gueneri* was initiated. An interesting parallel is that the two species of *Chrysophthalmum* included in the analysis do not form a monophyletic group, as the second species (*C. montanum*) is closer to a group of *Inula* species than to *C. gueneri*. The small genus *Chrysophthalmum* is diagnosed by the lack of a pappus and by paleate capitula, but both these character states are

homoplasious, as evidenced by our results. Considering also that the epappose genera *Amblyocarpum*, *Carpesium*, and *Telekia* are part of the *Inula* clade, where members are normally characterized by a pappus of capillary bristles, parallel losses or gains of pappus bristles seem to have occurred. The absence or presence of paleae is evidently a highly homoplasious character in Inuleae s.str.

The second large genus of Inulinae, *Pulicaria*, also is paraphyletic as presently circumscribed, with *P. crispa* more closely related to *Rhanterium*, *Vierea*, and *Schizogyne* than to *P. dysenterica* and *P. canariensis*. With a different sample of taxa, [Francisco-Ortega et al. \(2001\)](#) also presented a tree topology where *Pulicaria* species were found to belong in two different clades, but the bootstrap support for these clades was less than 50%, and the nodes were said to collapse in the strict consensus tree. *Pulicaria crispa* sometimes is placed in the genus *Franceouria* together with a few similar species. Our present results indicate that recognition of *Franceouria* for *P. crispa* may be warranted. Except for not having a pappus of bristles and scales, *Schizogyne* resembles some species of *Pulicaria* subg. *Platychaete*, thus it will be interesting to learn the relationships of that yet unsampled subgenus when more data become available.

Like the *Inula* clade, the taxa of the *Pulicaria*-*Asteriscus* clade may be with or without paleae. [Eldenäs et al. \(1998\)](#) found multiple losses of paleae to be less parsimonious than multiple independent gains. This interpretation is supported by the variable shape of the paleae, which ranges from robust structures subtending the florets to thin, linear, bristle-like structures between the florets.

Anvillea, which is epappose but with a paleate receptacle, is sister to *Asteriscus*, *Ighermia*, and *Pallenis*, all of them genera with paleate receptacles and a pappus of scales only. The only other two taxa of Inuleae-Inulinae with the same combination of character states are *Xerolekia* and *Bupthalmum*. The former has been associated with *Telekia*, but *Telekia* differs in having linear, subulate paleae, a less developed scaly pappus that is often missing, and long, branched anther tails. The opinion that *Xerolekia* is related to *Telekia* is rejected. Both *Xerolekia* and *Bupthalmum* have folded paleae and almost ecaudate anthers. In contrast to results of an earlier analysis of morphological data ([Anderberg, 1991a](#)), now there is good support that *Bupthalmum speciosissimum* (*Xerolekia*) is sister to *Bupthalmum*, and therefore the two taxa should be treated as congeners again. These genera are, however, not closely related to *Asteriscus* and its allies, but together form a small monophyletic group that is sister to the clade comprising *Inula* and its closest relatives.

The relationships among the taxa of Inuleae-Inulinae still are not fully understood, but the small sample of

species we have included shows that the circumscription of many of its genera may have to be revised to obtain monophyletic genera. A more detailed study of the phylogeny of Inuleae-Inulinae is another ongoing part of our present research on the Asteraceae ([Englund and Anderberg in prep.](#)).

Excluded genera

Callilepis DC. and *Zoutpansbergia* Hutch

These two South African genera, nowadays generally treated as congeners, have been associated with Inuleae ([Merxmüller et al., 1977](#)). Our present results show that *Callilepis* and *Zoutpansbergia* are sisters ([Fig. 1](#)), and the notion that the latter may be a derived *Callilepis* has thus gained further support. Their styles are inuloid, but their strange cypselas and paleae are not matched by any taxon in Inuleae, and [Leins \(1971\)](#) showed that these taxa have a type of pollen wall different from that found in, e.g., *Inula* and *Pluchea*. The pollen wall is more similar to that in Gnaphalieae; *Callilepis* (incl. *Zoutpansbergia*) was included in an informal basal complex of the Gnaphalieae by [Anderberg \(1991c\)](#). At present there is nothing that would indicate that *Callilepis* and *Zoutpansbergia* are closer to the Inuleae clade than to the Heliantheae s. ampl. (incl. Athroismeae and Eupatorieae), or that instead they are sister to both these clades. The position of *Callilepis*-*Zoutpansbergia* is not clear, and future research based on a combination of sequences from several genes may shed more light on this issue. For the time being we therefore treat *Callilepis* and *Zoutpansbergia* as unclassified to tribe, pending more conclusive results on their relationship.

Gymnarrhena Desf.

This genus, formerly associated with Inuleae, was included in Cichorioideae but not assigned to tribe by [Bremer \(1994\)](#). It is a morphologically derived, stemless taxon provided with glomerules of heterogamous capitula bearing filiform female florets and functionally male central florets. The male florets have an undivided style and no visible stigmatic surface, vestigial cypselas, and a pappus of ciliate scales. The capitula structure of *Gymnarrhena* is complicated, and its morphology has made the genus difficult to place, except for the indication that *Gymnarrhena* was misplaced in the vicinity of Inuleae, with pollen suggesting a position near Cardueae ([Skvarla et al., 1977](#)). Apart from pollen, the calcarate anthers are almost the only feature that indicates a relationship with the Cichorioideae tribes. In our analysis, *Gymnarrhena* was found to be one branch of a polytomy also including Lactuceae, Arctoteae,

Liabeae, Vernoniae, and the subfamily Asteroideae. In other words, the position of *Gymnarrhena* in relation to the four tribes and Asteroideae was unresolved. It is either part of the unresolved basal complex of the paraphyletic Cichorioideae, or sister to the entire Asteroideae. Our results are implicitly similar to those of Panero and Funk (2002), who found *Gymnarrhena* to be even more basal – sister to both the Asteroideae and the LALV-complex – and hence proposed it as the sole member of subfamily Gymnarrhenoideae.

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Appendix A

Taxa investigated, vouchers sequenced, and new GenBank accession numbers [AY780811–AY780873]

Amblyocarpum inuloides: Azerbejdjan, Bobrov & Tzvelev 194 (LE); [AY780849].

Antiphiona pinnatisecta: Namibia, Nordenstam & Lundgren 678 (S); [AY780811].

Anvillea garcinii ssp. *radiata*: Algeria, Anderberg 576 (S); [AY780812].

Blumea balsamifera: Taiwan, Peng & Chen 10780 (HAST); [AY780814].

Blumea integrifolia: Australia, Western Australia, Telford 6307 (CANB); [AY780813].

Blumea psammophila: Australia, Northern Territory, Wightman & Dunlop 1287 (CANB); [AY780816].

Blumea riparia: Taiwan, Peng et al. 15102 (HAST); [AY780815].

Blumea saxatilis: Australia, Northern Territory, Cowie 6734 (CANB); [AY780817].

Caesulia axillaris: India, Pandey 3021 (S); [AY780818].

Callilepis salicifolia: South Africa, Bayer SAF-01009 (S); [AY780851].

Calostephane marlothiana: Namibia, Merxmüller & Giess 28120 (S); [AY780819].

Carpesium divaricatum: Taiwan, Chung & Anderberg 1422 (S); [AY780820].

Chrysophthalmum gueneri: Turkey, Duman 7072 (S); [AY780848].

Coleocoma centaurea: Australia, Albrecht 10563 (S); [AY780852].

Cratystylis conocephala: Australia, Nordenstam & Anderberg 604 (S); [AY780821].

Dielitzia tysonii: Australia, Nordenstam & Anderberg 394 (S); [AY780822].

Dittrichia viscosa: Greece, Trift et al. 22 (S); [AY780823].

Doellia bovei: Yemen, Kilian & Hein NK 4724 (S); [AY780858].

Duhaldea cappa: China, Luo 0253 (HAST); [AY780827].

Epaltes cunninghamii: Australia, Nordenstam & Anderberg 972 (S); [AY780824].

Gymnarrhena micrantha: Libya, Anderberg 1326 (S); [AY780825].

Inula bifrons: France, Barbezat s. n. (S); [AY780840].

Inula britannica: Sweden, Svensson AS02100 (S); [AY780826].

Inula conyza: Bulgaria, Anderberg & Anderberg B01-24 (S); [AY780841].

Inula germanica: Macedonia, Segelberg 30661/22 (S); [AY780842].

Inula peacockiana: Iran, Rechinger & Rechinger 4956 (S); [AY780839].

Inula verbascifolia ssp. *methanea*: Greece, Rechinger 20377 (S); [AY780843].

Iphionopsis rotundifolia: Somalia, Thulin & Warfa 5914 (UPS); [AY780853].

Jasonia tuberosa: Spain, Dittrich 3247 (S); [AY780844].

Karelinia caspia: Turkmenistan, Nikulina s.n. (LE); [AY780854].

Laggera decurrens: Yemen, Kilian et al. NK 4562 (S); [AY780861].

Laggera pterodonta: Yemen, Kilian & Hein NK 4705 (S); [AY780862].

Mollera angolensis: Zambia, Robinson 6557 (B); [AY780863].

Ondetia linearis: Namibia, Emanuelsson 1021 (S); [AY780828].

Pechuel-loeschea leubnitziae: Namibia, Bremer 455 (S); [AY780829].

Pegolettia lanceolata: Swaziland, McCallum & Balkwill 186 (B); [AY780860].

Pegolettia oxyodonta: Namibia, Emanuelsson 1032 (S); [AY780830].

Pentanema alanyense: Turkey, Duman 5504 (S); [AY780831].

Pentanema glanduligerum: Afghanistan, Rechinger 18595 (S); [AY780832].

Pluchea arabica: Yemen, Thulin et al. 9555 (UPS); [AY780864].

Pluchea dentex: Australia, New South Wales, Crawford 1076 (CANB); [AY780833].

Pluchea dioscorides: Eritrea, Ryding & Ermias 1279 (UPS); [AY780855].

Pluchea dunlopii: Australia, Western Australia, Hunger & Kilian 3948 (B); [AY780867].

Pluchea indica ssp. *yemensensis*: Yemen, Kilian et al. NK 4601 (S); [AY780871].

Pluchea kelleri: Somalia, Thulin & Mohamed 6953 (UPS); [AY780872].

Pluchea littoralis: Somalia, Thulin et al. 10634 (UPS); [AY780856].

Pluchea lucens: Somalia, Thulin 10775 (UPS); [AY780865].

Pluchea nogalensis: Somalia, Thulin & Warfa 6150 (UPS); [AY780868].

Pluchea obovata: Yemen, Socotra, Thulin & Gifri 8831 (UPS); [AY780857].

Pluchea sarcophylla: Somalia, Thulin et al. 10040 (UPS); [AY780866].

Pluchea tetranthera: Australia, New South Wales, Lyne 1797 (CANB); [AY780834].

Pluchea tetranthera: Australia, Western Australia, Hunger & Kilian 3797 (B); [AY780873].

Pluchea yucatanensis: USA, Mississippi, Jones & Jones 12656 (TEX); [AY780870].

Pseudoconyza viscosa: Yemen, Kilian et al. NK 4607 (S); [AY780859].

Pulicaria crispa: Iran, Karis 734 (S); [AY780845].

Pulicaria dysenterica: Bulgaria, Anderberg & Anderberg B01-23 (S); [AY780846].

Rhanterium epapposum: Kuwait, Nilsson et al. 16436 (S); [AY780847].

Sachsia polycephala: Cuba, Gutiérrez & Nilsson 6 (S); [AY780835].

Stenachaenium campestre: Argentina, Myndel Pedersen 3014 (S); [AY780836].

Stenachaenium campestre: US National Plant Germplasm System (NPGS), Accession No. PI 312840; origin Uruguay (S); [AY780869].

Telekia speciosa: Sweden, Thorán 4100 (S); [AY780838].

Tessaria integrifolia: Bolivia, Daly et al. 6392 (S); [AY780837].

Zoutpansbergia caerulea: South Africa, Koekemoer 2259 (PRE); [AY780850].

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