Phylogeny of the “Ifloga clade” (Asteraceae, Gnaphalieae), a lineage occurring disjointly in the Northern and Southern Hemisphere, and inclusion of Trichogyne in synonymy with Ifloga

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Abstract Ifloga and Trichogyne constitute a small but biologically interesting lineage within the tribe Gnaphalieae (Asteraceae). Species are distributed mainly in the semi-arid parts of southern Africa, but there is a disjunction to the Saharo-Sindian region where three species occur. Due partly to an unusual capitulum structure, the phylogenetic position of the group has been little understood. In addition, the monophyly of the genera has not been assessed using phylogenetic methods. A species-level phylogenetic hypothesis is presented, based on one nuclear and two chloroplast DNA regions, analysed with parsimony and Bayesian methods. Ifloga + Trichogyne constitute the “Ifloga clade” that forms one of the early-diverging lineages within Gnaphalieae. These lineages constitute a basal grade with many poorly supported nodes, precluding robust hypotheses of relationships amongst the lineages. A sister lineage to the Ifloga clade could thus not be identified, although it diverges amongst taxa formerly united in subtribe Relhaniinae. Although this subtribe is now known to be non-monophyletic, members of the Ifloga clade share with former members of Relhaniinae a previously overlooked set of leaf characters. The genus Trichogyne is monophyletic, but Ifloga is paraphyletic with respect to Trichogyne. To retain generic monophyly, all species are here transferred to the genus Ifloga. Although two of the Northern-Hemisphere species were not included in the analysis, morphological characters suggest that the three species from this region are monophyletic, in which case the Saharo-Sindian distribution is the result of a single dispersal northwards from Southern Africa. A new combination and an updated key to the species are presented.

Keywords Gnaphalieae; Ifloga; Lasiopogon; molecular phylogeny; Southern Africa; Trichogyne

INTRODUCTION

The daisy tribe Gnaphalieae is widely distributed geographically, but most diverse in the southern continents, and is hypothesized to have arisen in southern Africa in the Eocene (Bergh & Linder, 2009). Subtribal divisions have been problematic, with the most complete treatment, by Anderberg (1991), resulting in five subtribes and a basal grade of unchanged taxa. These groups, however, have since been shown to be non-monophyletic (based on the molecular phylogenies of Bayer & al., 2000, 2002; Bergh & Linder, 2009; Ward & al., 2009). The small genera Ifloga Cass. and Trichogyne Less. are distributed largely in southern Africa and together comprise 15 to 16 species, the number depending on different concepts of the widespread Northern-Hemisphere taxon I. spicata (Forsk.) Sch. Bip. The two genera are thought to be sister-taxon (Anderberg, 1991) and were formerly united under the name Ifloga (Hilliard, 1981). Within Ifloga and Trichogyne, four species are woody shrublets mainly native to the winter-rainfall Greater Cape Floristic Region (GCFR) of the southwestern tip of Africa, while the remainder are annual and occur either in the GCFR (seven species), in the summer-rainfall regions of Southern Africa (two species), or in the Saharo-Sindian region (two to three species).

The genus Trichogyne was initially a subgenus of Ifloga (Bentham, 1873; Hilliard, 1981), formally elevated to genus status by Anderberg (1991). Ifloga was originally considered to be a close relative of Filago L. (Schultz Bipontinus, 1845; Bentham, 1873), and both genera were placed by Anderberg (1991) in his subtribe Gnaphaliinae, although cladistic analysis of morphological characters indicated that the two genera are not closely related (Anderberg, 1991). An alternative affinity for Ifloga was suggested by Leins (1973) on the basis of pollen morphology, which shows similarity to Stoebe L. and Disparago Gaertn., both members of Anderberg’s (1991) former subtribe Relhaniinae. Phylogenetic analysis based on both nuclear and chloroplast DNA sequence data has shown that members of the former Relhaniinae are not monophyletic, but form the earliest-diverging lineage as well as the basal grade of tribe Gnaphalieae (Bergh & Linder, 2009). In contrast, Filago has been shown to be nested within the “crown radiation” (Ward & al., 2009) of Gnaphalieae, and is more closely related to genera such as Helichrysum Mill., Gnaphalium L. and Anaphalis DC. (Galanby-Casals & al., 2010). The first molecular phylogenetic study to include a representative of Ifloga or Trichogyne (Bayer & al., 2000) placed Trichogyne ambiguus amongst the grade of early-diverging lineages in the tribe, although using only chloroplast DNA and with little support. A recent multi-locus analysis using two different species (Ifloga spicata and Trichogyne repens; Galanby-Casals & al., 2010) has confirmed this placement, validating the observations of Leins (1973).

Most species in Ifloga and Trichogyne have a peculiar capitulum structure, in which the female florets are borne outside
the terminal involucres, in the axis of cymbiform bracts running down the length of the peduncle (although an alternative interpretation is that the heads possess several rows of palea surrounding the central florets, and each outer female floret is subtended by its own additional palea or bract). Bentham (1873) and Schultz Bipontinus (1845) considered the heads of Ifloga to be similar to those of the Northern-Hemisphere genus Filago, in which the female florets at the periphery of the receptacle are each enclosed by an inner and an outer bract. In Filago, however, all florets are borne on the plane of the receptacle, whereas in Ifloga the axillary florets are borne below the receptacular plane and distributed down the peduncle. Several authors have puzzled over this anomalous capitulum. Bentham (1873) and Schultz Bipontinus (1845) considered the capitulum in Ifloga and Trichogyne to consist of outer involucral bracts and inner palea, amongst which the female florets were distributed, hence the similarity with Filago. Hilliard & Burtt (1971), however, considered the Ifloga-type capitulum to be a compound structure, the isolated female flowers each representing a reduced primary capitulum. Burtt (1977) subsequently questioned this interpretation, but it was not until the discovery of Ifloga thellungiana Hilliard & B.L. Burtt (Hilliard, 1981) that Hilliard & Burtt (1981) could use the fact that this new species has both terminal, peripheral and (occasionally) axillary female florets, to support the argument that the capitulum is a simple one. Hilliard & Burtt (1981) hypothesized that the morphogenetic potential for the production of female florets moved, during the course of evolution, from the periphery of the terminal receptacle to a position axillary to the outer bracts. This argument relies on the assumption of an evolutionarily basal position in the group for the two species (I. molluginoides (DC.) Hilliard, I. thellungiana) that usually lack axillary female florets (Hilliard, 1981). Ifloga molluginoides has in the past been placed in a separate genus, Comptonanthus B. Nord. (Nordenstam, 1964). Hilliard & Burtt (1981) however, considered both I. molluginoides and I. thellungiana to be most closely related to other Ifloga species, and transferred the remaining Comptonanthus species to Lasiopo- gon Cass. Thus, any complete assessment of relationships will require inclusion of at least some other members of the former genus Comptonanthus (viz. Lasiopo- gon brachypterus O. Hoffm. ex Zahli- br. and L. debilis (Thunb.) Hilliard).

We generate a molecular phylogenetic hypothesis based on DNA sequence data from the 3′ end of the nuclear ribosomal external transcribed spacer, and the chloroplast psbA-trnH spacer and trnL-trnF′ region. The study includes all species of Trichogyne and most species of Ifloga as well as extensive outgroup sampling in an attempt to ascertain the correct phylogenetic placement of the genera within the Gnaphalieae, and to assess their monophyly.

**MATERIALS AND METHODS**

**Sampling.** — Outgroup samples include representatives of six different putative clades from across the Southern African Gnaphalieae that are potentially close relatives of Ifloga and/or Trichogyne (Nordenstam, 1964; Anderberg, 1994; Bayer & al., 2000; Bergh & Linder, 2009; Galbany-Casals & al., 2010). The ingroup sample comprised 13 of the 16 recognised species of Ifloga and Trichogyne. Not sampled, due to lack of fresh or suitable herbarium material, were three Ifloga species: *I. obovata* Bolle from the Canary Islands, *I. labillardieri* (Pamp.) Fayed & Zareh from Egypt (Fayed & Zareh, 1988), and the rare and little-collected *I. thellungiana* from the southwestern Cape of South Africa. For some taxa, DNA was extracted from PRE herbarium material. Sampled species and voucher details are given in the Appendix. We generated 99 new DNA sequences and obtained the remainder from the GenBank-deposited sequences of Bayer & al. (2000) and Bergh & Linder (2009).

**DNA isolation, amplification and sequencing.** — Fresh leaf material was collected directly into silica gel. Approximately 30 mg of dried leaf material was ground with sterilized sand and/or liquid nitrogen and total genomic DNA isolated using the CTAB method (Doyle & Doyle, 1987). DNA was suspended and diluted in Tris-EDTA buffer. PCR reactions were performed on a Hybrid PCR Sprint thermal cycler (Fisher Scientific International, Hanover Park, Illinois, U.S.A.).

The 3′ portion of the external transcribed spacer (ETS) of nuclear ribosomal DNA was amplified using the primers AST1 (Markos & Baldwin, 2001) and 18S-ETS (Baldwin & Markos, 1998); the chloroplast psbA-trnH region was amplified using the primers psbA-F and trnH-R of Sang & al. (1997); and the chloroplast trnL intron and the trnL-trnF intergenic spacer were amplified together using the “c” and “f” primers of Taberlet & al. (1991). Reaction mixtures consisted of 5.0 mM MgCl2, dNTPs at 0.1 mM each, primers at 0.5 μM, 0.3 μM and 0.33 μM (ETS, psbA-trnH and trnL-F, respectively) and 0.75 (psbA-trnH and trnL-F) or 1.0 (ETS) unit(s) of BioTaq (Bioline, Taunton, Massachusetts, U.S.A.). The ETS mixture also contained 2% DMSO. Reaction volumes were made up to 25 μl (ETS) or 30 μl (psbA-trnH, trnL-F) with sterilized Millipore water, and included 3–4 μl of template DNA. Thermal profiles consisted of 2 min at 95°C (ETS, psbA-trnH) or 97°C (trnL-F) followed by 30 cycles of 94°C (ETS, psbA-trnH) or 97°C (trnL-F) for 1 min; 1 min at 55°C (ETS) or 52°C (trnL-F), or 45 s at 54°C (psbA-trnH); and 2 min (ETS, trnL-F) or 1 min (psbA-trnH) at 72°C. A final extension step at 72°C lasted for 7 min (ETS, trnL-F) or 8 min (psbA-trnH).

Successfully amplified target DNA was cleaned and sequenced by MacroGen, Korea (www.macrogen.com/eng/sequencing/sequence_main.jsp) under BigDye terminator cycling conditions. The products were purified using ethanol precipitation and visualised on an ABI Automated Sequencer 3730XL (Life Technologies Corporation, Carlsbad, California, U.S.A.).

Each region was sequenced in both directions using the original PCR primers; chromatograms were checked and assembled with Chromas software (v.1.45; Conor McCarthy, Griffith University, Brisbane, Queensland, Australia) and Sequencher v.4.5 (Gene Codes Corporation, 2005, Ann Arbor, Michigan, U.S.A.). Consensus sequences were aligned manually in BioEdit v.7.0.5.3 (Hall, 1999) for PC or MacClade v.4.05 (Maddison & Maddison, 1992). Several short segments of DNA that could not be unambiguously aligned were recoded.
as missing and excluded from the analyses. Insertion/deletion (indel) events were coded independently as binary characters using the simple gap coding method of Simmons & Ochoterena (2000) implemented in the software Gapcoder (Young & Healy, 2003).

**Phylogenetic analysis.** — Unweighted parsimony tree searching was conducted in PAUP* v.4.0b10 (Swofford, 2002). Parsimony uninformative characters were excluded in order to standardize parsimony statistics. An initial shallow search used 10,000 random-addition replicates, NNI branch swapping and saved only one tree per round of branch swapping. The resulting set of shortest trees was subjected to TBR branch swapping while saving multiple trees. Node support was assessed via 1000 non-parametric bootstrap replicates, each replicate saving a maximum of 500 trees based on 20 random-addition sequences and TBR branch swapping. Trees were rooted on *Rhynchosporidium* DC. and *Leysera* L., members of the “*Relhania clade*” identified by Bergh & Linder (2009) and Ward & al. (2009) as the earliest-diverging lineage of tribe Gnaphalieae.

Each of the four partitions (*psbA-trnH* spacer, *trnL* intron, *trnL-trnF* spacer, ETS) consisted of a stretch of nucleotides and the associated indel data. The plastid markers were concatenated and analysed together (because all genes in the chloroplast genome are linked and so should share the same phylogenetic history) while the nuclear data were analysed independently. Overall congruence between the two datasets was assessed using an incongruence length difference (ILD) test (Farris & al., 1995) with significance values calculated in PAUP* using 1000 matrix randomisations. Significant ILD values were found, so incongruence was further explored via comparison of independent chloroplast and nuclear bootstrap trees to determine the support at conflict nodes (De Queiroz, 1993). Combined analysis of the two gene partitions was subsequently conducted using parsimony (as described above) and Bayesian methods.

Simultaneous Bayesian inference of nucleotide substitution parameters and topology was performed in MrBayes v.3.1 (Huelsenbeck & Ronquist, 2001). Indel characters were analysed according to the restriction site (binary) model with a gamma-shaped distribution for rate variation and ascertainment (coding) bias accounted for using the ‘variable’ option (Ronquist & al., 2005). The default prior and likelihood settings were used for all remaining parameters except the nucleotide substitution model which was set to the general time-reversible model with a gamma distribution of rate variation across sites (GTR+Γ), as this was the best-fit model selected for all three model with a gamma distribution of rate variation across sites. Bayesian methods.

**RESULTS**

**Molecular characters.** — The sequence alignment (TreeBase submission ID: 11242) consists of 1756 nucleotide and indel characters. Details of the datasets and the numbers of potentially parsimony-informative (PI) characters (as calculated by PAUP*) are given for each gene region in Table 1. The ETS region contains more than twice the number of PI characters of the most variable plastid region, *psbA-trnH*. Despite having the largest number of characters, the *trnL-trnF* region is the least informative of the three.

**Phylogenetic analyses: separate gene trees.** — The ILD test indicated significant length differences between the plastid (*psbA-trnH + trnL-trnF* region) and ETS trees (*P* = 0.001). However, this test is based only on tree-length differences. A positive ILD test does not indicate which (or how many) nodes are in conflict, whether the incongruence is localized or general, or whether it involves deep or shallow nodes. It also does not indicate the degree of character support for conflicting nodes; this is better examined by comparing, for example, bootstrap support for independent gene trees (De Queiroz, 1993). Separate plastid and nuclear bootstrap trees are presented for comparison in Fig. 1, representing only those nodes supported by bootstrap (BS) values of ≥75%. The two independent loci recover similar topologies overall, although neither dataset resolves relationships amongst the major clades in the tree: the “spine” of the tree, depicting the relationships among the major clades, is a polytomy in both cases. Amongst the outgroups, only the ETS analysis recovers the *Metalasia clade* (containing *Lachnospernum fasiculatum*, *Metalasia densa* and *Phaeocoma prolifera*; BS = 91%) and a clade comprising sampled members of *Helichrysum*, *Gnaphalium*, and *Lasiopogon* (except *L. glomerulatus*; node G: BS = 92%). Both gene trees recover the *Stoebe clade* (node E) of Bergh & Linder (2009) with BS of 84% (plastid) and 83% (ETS). A clade comprising all sampled members of *Ifloga* and *Trichogyne* (node L)
Phylogenetic analyses: combined data. — Burn-in length for the MrBayes runs on the combined dataset was set to $2.3 \times 10^6$ based on convergence diagnostics, resulting in a sample of 5400 MrBayes trees (2700 from each run). The combined analysis is represented with BS ≥ 75% (above) and posterior probability (PP) values ≥ 0.95 (below) on one of the MrBayes trees with the same topology as the Bayesian analyses of combined data. Data. Although the Metalasia clade of Bergh & Linder (2009) is not recovered, both the Stoebe clade (node E; PP = 1.00) and a clade representing the “crown radiation” of the Gnaphalieae (Ward & al., 2009) are present (node D; BS < 75% but PP = 0.95). Three nodes recovered within the Stoebe clade were not present in the independent analyses; these comprise BS values ≥ 75%, PP values ≥ 0.95, or both (Fig. 2). Within the Stoebe clade, the combined analysis favours the nuclear placement for the conflict taxon Disparago anomala, although support is reduced for this relationship (BS of 62% down from 86% in the ETS dataset).

Tree topologies: the ingroup. — Ifloga and Trichogyne constitute a monophyletic group (node L; BS = 100%; PP = 1.0), hereafter called the Ifloga clade, that is sister (node C; PP = 1.00) to the “core radiation” clade. Identical internal topologies were recovered within the Ifloga clade in both parsimony and Bayesian analyses of combined data. Except for the nodes directly involved in placement of the conflict taxon T. paronychioides, combined analysis of relationships within the Ifloga clade results in increased resolution and support at all nodes, with all BS ≥ 80% and all PP ≥ 0.99. Ifloga molluginoides is sister to the remaining species, which fall into two clades: one comprising the rest of Ifloga (including I. spicata, the species of which the type of the generic name, I. cauliflora, is a taxonomic synonym, and thus termed by us the “Ifloga s.str.” clade; node U), and a second corresponding to the genus Trichogyne (node N). Within Trichogyne there is a well-supported split into a mostly-annual subclade (node O) and a strictly perennial subclade (node P) containing the type of the generic name, T. ambigua (L.) Druce. The conflict taxon T. paronychioides takes the placement given by the plastid data in independent analysis, sister to T. decumbens (node S).

DISCUSSION

Incongruence between plastid and ETS gene trees. — Well-supported incongruence indicates that the two gene trees are representing different phylogenetic histories. This may be
caused by paralogy (for example in tandem repeats of the ETS), incomplete lineage sorting of ancestral polymorphisms, or lateral gene transfer between species. Given that the two instances of conflict involve taxa near the tips, and that there is little independent evidence of hybridization in these groups, we feel that both are most likely the result of incomplete lineage sorting (retention of unsorted ancestral polymorphisms) in one or the other locus. If so, the underlying relationships of the species follow a bifurcating pattern that can be accurately represented as a phylogenetic tree. We have followed the approach advocated by Wiens (1998), comparing topologies and support values in separate analyses, followed by data combination. The advantage of this approach, clearly demonstrated in our combined analysis, is that if conflict is localized, the unaffected parts of the tree may benefit from increased resolution and support when data are combined (Wiens, 1998). The improved bootstrap values at all unaffected nodes within the Ifloga clade indicate the value of data combination for these datasets. Given the complexity of a phylogenetic problem involving this number of taxa, and the fact that we have data from only two independently segregating loci, we have opted to present the topology indicated by the concatenated analysis of both gene regions. Incongruence within the Stoebe clade does not affect inferences of relationship in the Ifloga clade. In the combined analysis, the parts of the tree that were strongly supported as incongruent by the separately analysed data sets are considered less certain and...
Fig. 2. One of the MrBayes phylograms, with the same topology as the Bayes 75% majority-rule consensus tree. As this is a single tree, all nodes are shown resolved, including those that are not statistically supported. Numbers at nodes represent parsimony bootstrap percentages (above line) and Bayesian posterior probabilities (below line). Only bootstrap values greater than 70% and posterior probabilities above 0.90 are indicated. Letters indicate the same nodes as in Fig. 1 and additional nodes that are referred to in the text. Vertical bars delimit clades named to the right of the bar (the “Relhania clade” and “Stoebe clade” of Bergh & Linder, 2009 and the “Crown radiation” clade of Ward & al., 2009).
Outgroup relationships. — Relationships amongst outgroup taxa are generally consistent with those found in previous molecular phylogenies. Low backbone resolution in the Gnaphalieae tree has been found repeatedly, in analyses based on trnL-trnF sequences (Bayer & al., 2000), ITS and ETS sequences (Galbany-Casals & al., 2009), trnL-trnF and matK sequences (Ward & al., 2009) and a combination of ETS, trnL-trnF and psbA-trnH (Bergh & Linder, 2009). This lack of resolution was also evident in each of the independent gene trees and may indicate the inability of the markers to resolve relationships, overly sparse taxon sampling, or rapid divergence events.

The “crown radiation” clade is likely to contain most of the ca. 1000 unsampled species included in Gnaphalieae, although it is represented in our analyses only by southern African members of Anderberg’s (1991) former subtribe Gnaphaliinae. Within this “crown radiation” the five sampled Helichrysum species fall into a well-supported clade (node K; BS = 100%; PP = 1.00) which also includes Galeomma oculus-cati (L. f.) Rauschert. Non-monophyly of the large genus Helichrysum is well-established (Bayer & al., 2000; Galbany-Casals & al., 2004; Ward & al., 2009) but the inclusion of Galeomma Rauschert in a “Helichrysum clade” is a novel result. Galeomma is a genus of just two species, considered by Anderberg (1991) to be non-monophyletic. The unsampled species Galeomma stenolepis (S. Moore) Hilliard was transferred by Hilliard (1983) from Gnaphalium; inclusion of this species in future analyses together with additional accessions of Helichrysum and other related taxa will be required to determine monophyly in these genera.

Monophyly of Lastiopogon is not supported by our analysis. The type of the generic name, L. muscoides (Desf.) DC., is placed sister to L. micropoides DC. with good support (BS = 100%; PP = 1.00); L. glomerulatus (Harv.) Hilliard is sister to the non-Stoebe clade members of the “crown radiation” (PP = 1.00) while L. debilis is placed sister to Gnaphalium declinatum L. f. (BS < 75%; PP = 1.00) within the “crown radiation”. Inclusion of the remaining Lastiopogon species (L. ponticulus Hilliard, L. volkii (B. Nord.) Hilliard, L. brachypyterus O. Hoffm. ex Zahlbr., L. minatus (B. Nord.) Hilliard & B.L. Burtt) as well as a broader range of outgroup taxa will allow full assessment of the status of the genus.

Phylogenetic placement of the Ifloga clade in Gnaphalieae. — The analysis of Bayer & al. (2000) placed Trichogyne ambiguca sister to their clade “E” (corresponding to the Metalasia clade of Bergh & Linder, 2009) plus a clade corresponding to the “crown radiation” clade of Ward & al. (2009). Galbany-Casals & al. (2010) recover a moderately supported (BS = 74%; PP = 0.99) sister relationship between the Metalasia clade and the Ifloga clade (each represented in their analysis by two species) with these in turn being sister to what is probably the “crown radiation” (although they did not include any representatives of the “Stoebe clade” of Bergh & Linder, 2009). Our results instead place the Ifloga clade sister to the “crown radiation” clade, although with low BS support (≤75%) at the relevant nodes. Lack of resolution and sparse sampling of outgroup taxa preclude the identification of a sister lineage to the Ifloga clade, although it is possible that they form part of a grade at the base of Gnaphalieae. Further sampling of early-diverging groups is required, as well as the addition of more, and more informative, independently segregating genetic markers.

The present study and the analyses of Bayer & al. (2000), Bergh & Linder (2009) and Galbany-Casals & al. (2010) identify the early-diverging lineages of Gnaphalieae as the “Relhania”, “Metalasia”, “Ifloga” and “Stoebe” clades. Except for the Ifloga clade, most members of these lineages were formerly united in subtribe Relhaniinae Less. by Anderberg (1991, 1994). Although the members of this tribe are now known to be non-monophyletic, they possess a synapomorphy in that their leaves are involute with a compact white tomentum on the adaxial surface (Anderberg, 1991, 1994). Members of the Ifloga clade also possess involute leaves and adaxially tomentose leaf surfaces, a feature noted by Hilliard (1981) but not previously recognized as an indicator of evolutionary affinity.

Generic subdivision of the Ifloga clade. — In the past, some authors subdivided the Ifloga clade into two genera on the basis of the annual or perennial life-history (Candolle, 1838; Bentham, 1873). We found that Ifloga s.str. and Trichogyne form reciprocally monophyletic, well-supported sister clades, supporting the conclusion of Hilliard (1981) that two natural groups within the lineage are defined by capitular structure and floral characters rather than by annual versus perennial life-history. Ifloga molluginoides is, however, sister to these two clades. Hilliard & Burtt (1981) cite several characters which unite this species with other annual members of the Ifloga clade, including sharply mucronate leaves with involute margins; a filiform corolla in female florets and tubular corolla in hermaphrodite florets; scattered thickenings on all walls of the endothecial tissue, possession of globose achenial hairs and pappus setae with patent, intermingling cilia basally and shortly, regularly plumose apices. Apart from these characters, this species is anomalous within the Ifloga clade because (1) its female florets are borne at the periphery of the receptacle within the terminal whorl of bracts (rather than in the axils of outer bracts); and (2) its habit is prostrate or decumbent rather than erect, and the leaves are weakly involute without a marked difference in the tomentum between the ad- and abaxial surfaces. These characters are shared with one other species, I. thellungiana, which we were unable to include in our analysis. Initially, I. molluginoides was described as Lasiopogon molluginoides DC. (Candolle, 1838) and subsequently transferred by Nordenstam (1964) to a new genus, Comptonanthus B. Nord., mainly on the basis of pappus characters (at this time I. thellungiana had not yet been described). Nordenstam (1964) also included two species of Lasiopogon (L. brachypyterus, L. debilis) in Comptonanthus. Our study supports Hilliard & Burtt’s (1981) dissolution of Comptonanthus and return of C. molluginoides (DC.) B. Nord. to the Ifloga clade.

Biogeography. — The Ifloga clade is embedded within a predominantly Southern African grade. In addition, the
earliest divergence event produced a Southern African taxon (I. molluginoides), and most extant taxa are restricted to the arid parts of Southern Africa. Taken together, these facts strongly suggest that the group originated in Southern Africa. We could only obtain sequence data from one of the three Northern-Hemisphere species, I. spicata, which was placed in our analysis sister to I. glomerata (Harv.) Schlr. Ifloga spicata is widespread throughout the Sahara, the Mediterranean region (including the Canary Islands) and into Southwest Asia, while I. glomerata occurs in Namibia and South Africa. Such a large disjunction suggests a long-distance dispersal event or the historical opening of habitat corridors. The distribution ranges of the two unsampled Northern-Hemisphere species are nested within that of I. spicata, and phylogenetically they may be close relatives (Hilliard, 1981; Fayed & Zareh, 1988). Monophyly of these three species, as suggested by morphology, would suggest a single migration event northwards up the African continent, and subsequent diversification in the Mediterranean-Saharan region. Whether northwards dispersal occurred once or multiple times, the opening of arid corridors connecting Southern Africa to the Horn of Africa (Boe, 2006) might have provided favourable arid habitat during parts of the Miocene, a dispersal route suggested by Coleman & al. (2003) for Senecio L.

### TAXONOMIC IMPLICATIONS

Convincingly monophyletic subtribes within Gnaphalieae are yet to be circumscribed (Bayer & al., 2007; Ward & al., 2009) so we do not propose any formal subtribal assignment for the genera treated here. Anderberg’s (1991) justification for separating Trichogyne from Ifloga was that they were reciprocally monophyletic. This is contradicted by the current work, necessitating taxonomic change, which could be implemented in two ways. Firstly, the current circumscription of taxa could be retained and a new genus erected to house I. molluginoides; or secondly, Trichogyne could be sunk into Ifloga (the earliest name). Despite the larger number of name changes required we support the second option because a single generic name would seem more appropriate for such a small lineage. In addition, all but one of the combinations are already available (the exception is T. lerrouxiae, described by Beyers, 1995). Also, because we were unable to include I. thellungiana in our analysis, the placement of this species is currently uncertain. Ifloga thellungiana was considered by Hilliard (1981) to be a “linking species” between I. molluginoides and the rest of Ifloga. Morphology indicates a possible sister relationship between I. thellungiana and I. molluginoides, because they are both prostrate and both have their female florets borne within the terminal capitulum. The exact placement of this species, however, awaits its rediscovery in the wild. Although I. thellungiana is almost certain to be a member of the Ifloga clade, its placement within the clade is uncertain, which uncertainty is best anticipated by a single generic name for all members of the clade.

We therefore revert to the taxonomy of Hilliard (1981) and transfer all species of the genus Trichogyne to Ifloga.
14. Heads terminating dwarf lateral shoots, the many leaves of which are webbed together and to the outer involucral bracts; heads normally solitary at the tip of each shoot ............................................. \textit{I. ambigua}

15. Heads up to 3.25 mm long; main branches creeping .

............................................. \textit{I. repens}

15. Heads ≈4–5 mm long; erect, well-branched shrub .

............................................. \textit{I. pilulifera}

### Taxonomic treatment


\textit{Trichogyne} Less. in Linnaea 6: 231. 1831 – Type: \textit{T. laricifolia} (Lam.) Less.


\textbf{Ifloga obovata} Bolle in Bonplandia 7: 297. 1859 – Type: Canary Islands, Fuerteventura, Punta de Handia, \textit{Bolle s.n.} (?B, destroyed?).


† Note: Several species, specimens were cited as holotypes by Hilliard & Burtt (1971, 1981) and Hilliard (1981). Since these publications pre-date the 2001 requirement of the statement “designated here” for lectotypification, under Art. 9 of the ICBN we here correct these statements to lectotypifications.
collibus, Schlechter 1618 (Z, n.v., holotype; BM, G, K, n.v., isotypes; BOL!, P [photo], S [photo], isotypes).


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■ LITERATURE CITED

Appendix. Accession and voucher information of specimens included in this study. Voucher details (collector name and number; herbarium acronym); collection locality (localities listed without mention of a country are from South Africa; CFR, Cape Floristic Region; NZ, New Zealand); GenBank/EMBL accession numbers for the psbA-trnH spacer, trnL-trnF region and 3′ ETS region. GenBank numbers in bold were published previously (Bayer & al., 2000; Bergh & Linder, 2009; those generated for this study are indicated by the prefix “FR”). Sequences derived from herbarium specimens are indicated by “F”. Missing data are indicated by an n-dash (–).