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ScienceDirect
South African Journal of Botany 78 (2012) 2–11
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Inclusion of the Cape genus *Anisothrix* in the Namibian-centred genus *Pentatrichia* (Asteraceae, Gnaphalieae) based on a molecular phylogenetic analysis

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Received 11 January 2011; received in revised form 6 April 2011; accepted 8 April 2011

Abstract

A phylogenetic analysis of the small genus *Pentatrichia*, containing three species endemic to South Africa and Namibia, was undertaken using nuclear (ITS and 3′ ETS) and chloroplast (*trnT–trnL*) DNA sequence data. Generic circumscription was examined via the inclusion of appropriate outgroup taxa (*Anisothrix* and *Athrixia*). A fully-resolved phylogenetic hypothesis found all *Pentatrichia* species and subspecies to be reciprocally monophyletic based on three sampled specimens of each taxon. A well-supported sister relationship between the radiate *P. rehmii* subsp. *avasmontana* and non-radiate *P. rehmii* subsp. *rehmii* confirmed the results of a previous morphometric study. *Pentatrichia* was found to be non-monophyletic with the exclusion of *Anisothrix kuntzei* and *A. integra*, which were placed as a subclade within *Pentatrichia*, and sister to the type species *P. petrosa.* Morphological synapomorphies supporting the inclusion of *Anisothrix* with *Pentatrichia* are discussed, as well as the evolution of capitulum structure in the group. *Anisothrix* is synonymised with *Pentatrichia* and two new combinations were made. The expanded morphological concept of the genus *Pentatrichia* is presented with a key to all five species.

Keywords: *Anisothrix*; *Athrixia*; ETS; Gnaphalieae; ITS; Molecular systematics; *Pentatrichia*; Phylogeny; Southern Africa; *trnT–trnL* spacer

1. Introduction

The small genus *Pentatrichia* comprises three species of rock-loving shrublets occurring mainly in Namibia, with one species extending into the Northern Cape province of South Africa, and an additional species endemic to the northern Drakensberg region of South Africa. The genus is a member of the daisy tribe Gnaphalieae (immortelles or everlastings), but is unusual in the tribe in its dentate leaves (Fig. 1) as most other gnaphalioid genera have entire leaves.

Understanding of relationships in the Gnaphalieae has changed considerably with the use of DNA sequence data, although a subtribal classification is still lacking (Bayer et al., 2007; Ward et al., 2009). The phylogenetic relationships at the base of the tribe have recently begun to be clarified (Bayer et al., 2000; Bayer et al., 2002; Bergh and Linder, 2009; Ward et al., 2009; Montes-Moreno et al., 2010) and the earliest-diverging lineage has been identified as the "*Relhania* clade". This clade consistently comprises two subclades in all analyses. The first of these, which for clarity we will refer to as the "*Oedera* clade" contains several genera distributed mainly in Southern Africa (*Oedera*, *Relhania* and *Macowania*, amongst others). *Pentatrichia* belongs to the second subclade, which we here refer to by the informal name "*Athrixia* clade". Circumscription of this clade has been clarified in several phylogenetic analyses (Bayer et al., 2000; Bergh and Linder, 2009; Ward et al., 2009; Montes-Moreno et al., 2010). Although Anderberg (1991) grouped the ditypic genus *Philyrophyllum* together with *Pentatrichia* and *Anisothrix* on the basis of morphological characters, Montes-Moreno et al. (2010) found that *Philyrophyllum* is only distantly related. In their analysis, the

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doi:10.1016/j.sajb.2011.04.005

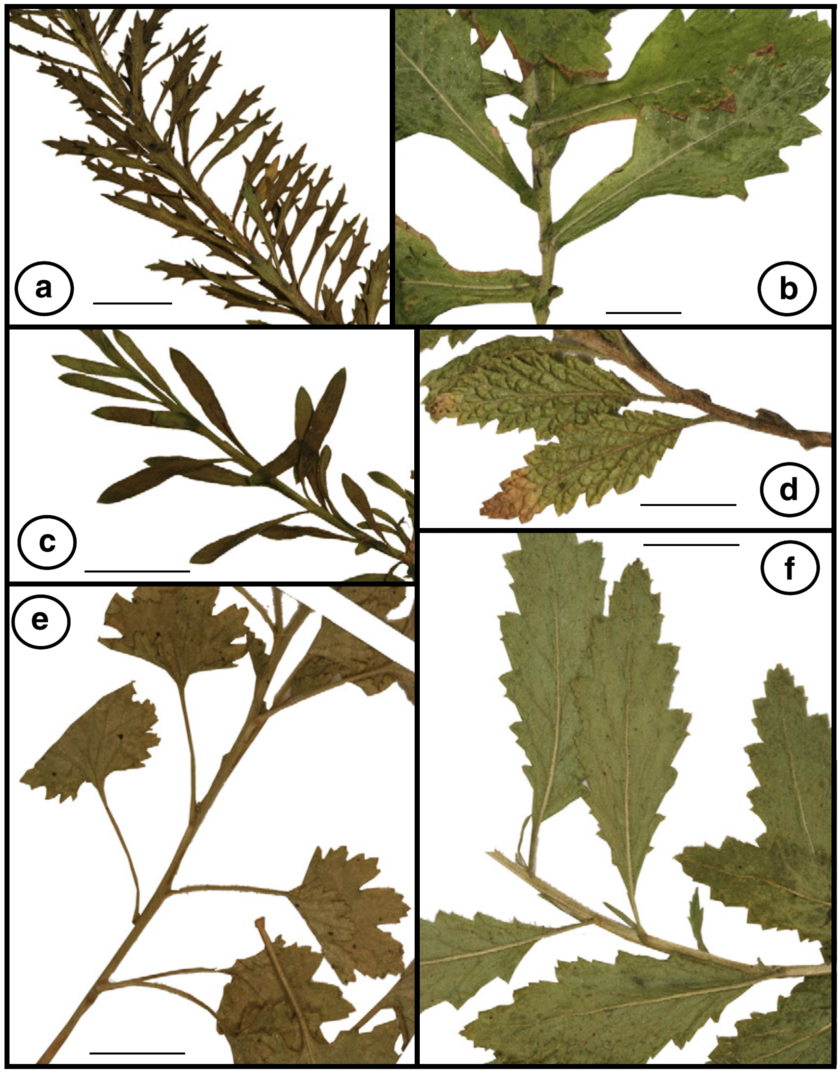

Fig. 1. Photographs of *Pentatrichia* and *Anisothrix* leaves to show different margin dentitions and shapes of the leaf bases. (a) *A. kuntzei*, Bergh 2075; (b) *P. alata*, Bergh 2209; (c) *A. integra*, Bergh 2059; (d) *P. rehmii* subsp. *rehmii*, Klaassen 1752; (e) *P. petrosa*, Williamson s.n. (NBG) and (f) *P. rehmii* subsp. *avasmontana*, Klaassen 1743. All scale bars represent 10 mm.

"*Athrixia* clade" consisted of *Phagnalon*, *Aliella*, *Athrixia*, *Anisothrix* and *Pentatrichia*. Thus the "*Athrixia* clade" comprises, according to current knowledge, *Athrixia* (14 spp., distributed in Southern and East Africa), *Phagnalon* (36 spp., distributed in North-eastern Tropical Africa, Mediterranean basin and the Irano–Turanian region), *Aliella* (4 spp., endemic to Morocco) and *Anisothrix* (2 spp., endemic to the Cape Floristic Region [CFR] of South Africa). Now that the membership of this clade has been defined by molecular data, its morphological synapomorphies can be listed. Member species are perennial herbs or subshrubs with leaf margins generally sparsely or closely dentate; the heads (capitula) are discoid (with all florets tubular and hermaphrodite), disciform (containing two kinds of tubular–filiform florets) or radiate (with central tubular florets and peripheral strap-shaped ray florets). The involucral bracts are imbricate in many rows, and are acute, attenuate, and frequently recurved; ray florets (when present) are pink or white, disc florets are yellow. The stigmatic lines on the style branches are basally separated but apically confluent and the pappus consists of barbellate bristles sometimes alternating with scales. Achenes in the group are sparsely or densely villous. Relationships amongst the genera, however, have not been clearly resolved. *Phagnalon* and *Aliella* were well-sampled and recovered as monophyletic by Montes-Moreno et al. (2010), as was *Athrixia* (with four sampled species). However, these studies are either sparsely-sampled (Bayer et al., 2000) or have focussed mainly on the Northern Hemisphere taxa (Montes-Moreno et al., 2010), and only three representatives of *Pentatrichia* and *Anisothrix* (*P. petrosa*, *P. rehmii* and *A. kuntzei*) have been included in previous analyses. Relationships within *Pentatrichia*, and between these two genera, have thus not been examined in detail.

Anisothrix comprises two species, *A. integra* and *A. kuntzei* that grow in sandstone rock-crevices in the Little Karoo region of the CFR. Both, like the non-radiate species of *Pentatrichia* (Fig. 2), have discoid heads with yellow florets surrounded by numerous linear, acute involucral bracts. They are distinguished

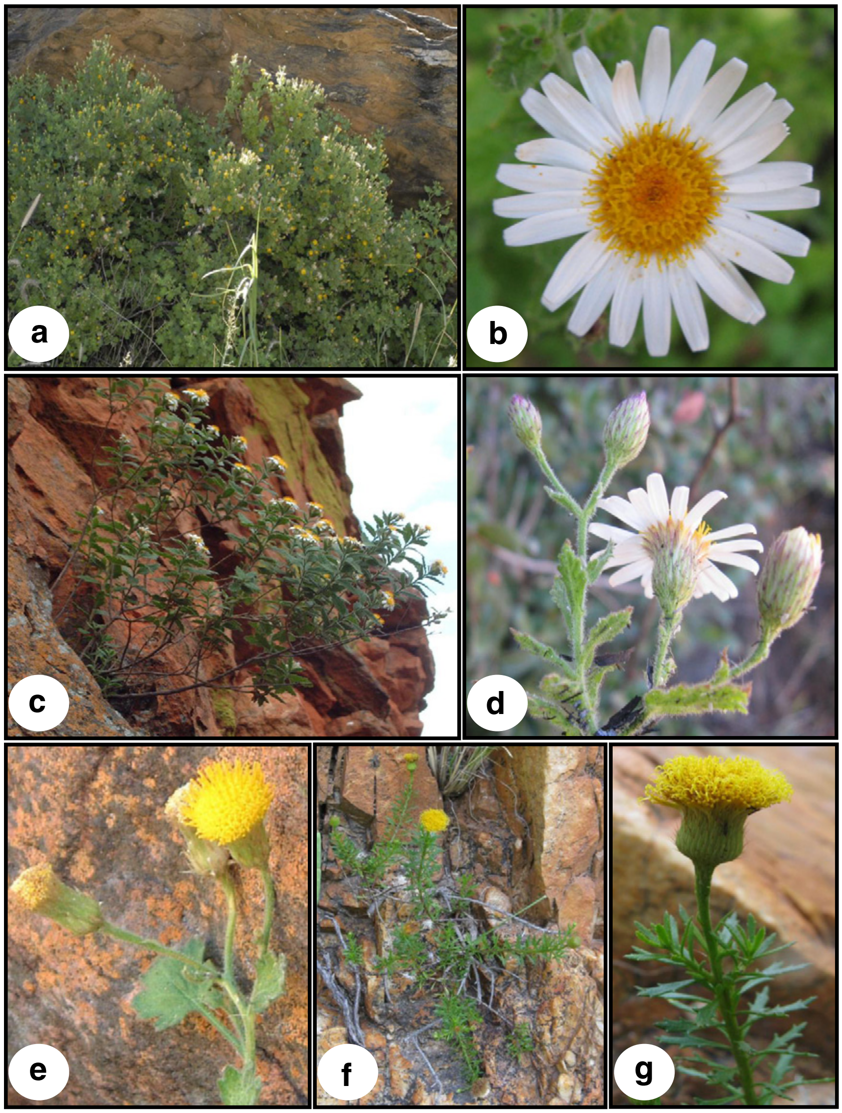

Fig. 2. Habit and inflorescence structure of *Pentatrichia* and *Anisothrix* species: (a) *P. petrosa* in flower, (b) *P. alata* flowerhead, (c) *P. rehmii* subps. *avasmontana*, (d) *P. alata* flowering shoot, (e) *P. petrosa* flowering shoot, (f) *A. kuntzei* habit, (g) *A. kuntzei* flowering shoot. Photographs by C. Mannheimer (a); N. Bergh (b, d, f, g) and E. Klaassen (c, e).

from *Pentatrichia* by their narrower, more thickened and schlerophyllous leaves (Fig. 1), which differ from the broad, flat and thin-textured leaves of *Pentatrichia* species. *Anisothrix* also differs from *Pentatrichia* in distribution, as the latter genus is absent from the CFR.

Pentatrichia species are characterized by capitula that are either discoid or radiate, the latter possessing an outer whorl of white, female ray florets (Fig. 2). Four species of *Pentatrichia* were recognised by Merxmüller (1950, 1954, 1967); Anderberg (1991); Herman et al. (2000) and Herman (2003). These are *P. petrosa* from Namibia and the Northern Cape province of South Africa (discoid), *P. alata* from the Mpumalanga and Limpopo provinces of South Africa (radiate, and the only species that does not occur in Namibia), *P. rehmii* which is confined to north-eastern Namibia (discoid), and *P. avasmontana* from the Namibian central highlands and Waterberg plateau (radiate). Klaassen et al. (2009) used morphometric data to examine the circumscription of the species, and found *P. rehmii* and *P. avasmontana* to be conspecific. They are currently recognised as subspecies of *P. rehmii*, distinguished respectively by their discoid and radiate capitula.

The present study uses chloroplast and nuclear DNA sequence data to examine phylogenetic relationships within *Pentatrichia*. Three members of each taxonomic entity within the genus were sampled in order to test the previous morphometric study of Klaassen et al. (2009). Several outgroup taxa from the '*Athrixia* clade' (the two species of *Anisothrix* plus three species of *Athrixia*) were included in order to examine generic circumscription. Although we were unable to include material of the Mediterranean members of the "*Athrixia* clade", these genera (*Phagnalon* and *Aliella*) were recovered as monophyletic in relation to *Anisothrix*, *Pentatrichia* and *Athrixia* by Montes-Moreno et al. (2010).

2. Materials and methods

2.1. Material

Leaf material was obtained from silica-gel dried field-collected specimens or from herbarium material from BOL and LYD. Specimen voucher information is listed in Table 1.

2.2. DNA extraction, amplification and sequencing

Approximately 20–50 mg of dried leaf tissue was ground with sterilised sand in liquid nitrogen using a mortar and pestle, or pulverised in a shaker with metal ball bearings. Total DNA was isolated using the CTAB method of Doyle and Doyle (1987) and diluted in sterilised Millipore™ water. Herbarium specimens and problematic material was run through GFX™ PCR DNA and Gel Band Purification Kit cleanup-columns (Healthcare Bio-Sciences AB, Uppsala, Sweden).

The polymerase chain reaction (PCR) was performed for all regions in 25 µl volumes on a GeneAmp PCR System 9700 machine (Applied Biosystems, Singapore).

The 3′ portion of the external transcribed spacer (ETS) of nuclear ribosomal DNA was amplified using the primers AST-1 (Markos and Baldwin, 2001) and 18S-ETS (Baldwin and Markos, 1998). The reaction mixture consisted of polymerase buffer at recommended concentration, 1.5 mM $MgCl_2$, 0.4 mM

Table 1
Voucher details, collecting localities and Genbank accession numbers for specimens sequenced in the present study.

Taxon	Voucher specimen details	GenBank Accession Nos.		
		ITS	ETS	trnT–trnL spacer
Anisothrix integra (Compton) Anderberg	South Africa, Western Cape: Ladismith, Seweweekspoort. Bergh 2059 (NBG)	FR832499	FR823339	FR832573
Anisothrix kuntzei O.Hoffm.	South Africa, Western Cape: Montagu, Kogmanskloof. Bergh 2075 (NBG)	FR832500	FR823340	FR832574
Athrixia arachnoidea J.M.Wood & M.S.Evans ex J.M.Wood	South Africa, Kwazulu Natal: Drakensberg, Cathedral Park area, Mlambonja River Valley, path en route to Xeni Cave. Bergh 2198 (NBG)	FR832501	FR823341	FR832575
Athrixia capensis Ker Gawl.	South Africa, Western Cape: upper east slopes of the Vlakkeberg. Pillans s.n. (BOL)	FR832502	FR823342	–
Athrixia elata Sond.	South Africa, Mpumalanga: 15 km N of Dullstroom on R540 road to Lydenburg. Bergh 2203 (NBG)	FR832503	FR823343	FR832576
Pentatrichia alata S.Moore	South Africa, Mpumalanga: Pilgrim's Rest above golf course, at base of cliffs. Bergh 2209 (NBG)	FR832504	FR823344	FR832577
Pentatrichia alata S.Moore	South Africa, Mpumalanga: Pilgrim's Rest. Burn b (Lyd9015) (LYD)	FR832506	–	–
Pentatrichia alata S.Moore	South Africa, Mpumalanga: Pilgrim's Rest. Burn h (Lyd9016) (LYD)	FR832505	FR823345	–
Pentatrichia petrosa Klatt	Namibia, Otjozondjupa Region: Waterberg Plateau Park–Omuverume Plateau, mountain views, gorge above bungalows. Klaassen & Hochobes 1745 (WIND)	FR832507	FR823346	FR832578
Pentatrichia petrosa Klatt	Namibia, Otjozondjupa Region: Waterberg Plateau Park–Omuverume Plateau, Okatjikona Environmental Centre, Fig Tree Walk, base of boulder along hiking trail. Klaassen & Hochobes 1747 (WIND)	FR832508	FR823347	FR832579
Pentatrichia petrosa Klatt	Namibia, Erongo Region: Brandberg, Amis Gorge. Klaassen & Hochobes 2143 (WIND)	FR832509	FR823348	FR832580
Pentatrichia rehmii (Merxm.) Merxm. subsp. *avasmontana* (Merxm.) Klaassen & Kwembeya	Namibia, Otjozondjupa Region: Waterberg Plateau Park–Omuverume Plateau, mountains north of Huilboom campsite, crevices on cliff face. Klaassen & Hochobes 1759 (WIND)	FR832510	FR823349	FR832581
Pentatrichia rehmii (Merxm.) Merxm. subsp. *avasmontana* (Merxm.) Klaassen & Kwembeya	Namibia, Khomas Region: Aredareigas Nature Estate (Farm Regenstein 32), slopes below Telekom Tower. Klaassen & Hochobes 2146 (WIND)	FR832511	FR823350	FR832582
Pentatrichia rehmii (Merxm.) Merxm. subsp. *avasmontana* (Merxm.) Klaassen & Kwembeya	Namibia, Khomas Region: Aredareigas Nature Estate (Farm Regenstein 32), below Telekom Tower. Klaassen & Hochobes 2147 (WIND)	FR832512	FR823351	FR832583
Pentatrichia rehmii (Merxm.) Merxm. subsp. *rehmii*	Namibia, Otjozondjupa Region: Guchab Mountain, Klaassen & Hochobes 1751 (WIND)	FR832513	FR823352	FR832584
Pentatrichia rehmii (Merxm.) Merxm. subsp. *rehmii*	Namibia, Otjozondjupa Region: Guchab Mountain, Klaassen & Hochobes 1752 (WIND)	FR832514	FR823353	FR832585
Pentatrichia rehmii (Merxm.) Merxm. subsp. *rehmii*	Namibia, Otjozondjupa Region: Guchab Mountain, Klaassen & Hochobes 2137 (WIND)	FR832515	–	FR832586

dNTPs, 2% DMSO, 0.5 μM of each primer, 1 unit of Super-Therm Polymerase (Southern Cross Biotechnology, Cape Town) taq and 4 μl of template DNA.

Amplification of ITS1, 5.8S and ITS2 was performed together using the primers ITS4 and ITS5 of White et al. (1990). The reaction mixture consisted of polymerase buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 2% DMSO, 0.5 μM of each primer, 1 unit of Super-Therm Polymerase (Southern Cross Biotechnology, Cape Town) and 2 μl of template DNA.

The chloroplast *trnT–trnL* intergenic spacer was amplified using the 'a' and 'b' primers of Taberlet et al. (1991). The reaction mixture consisted of polymerase buffer at the manufacturer's recommended concentration, 6 mM $MgCl_2$, 1.2 mM dNTPs, 0–0.5% DMSO, 0.75 μM of each primer, 1 unit of Super-Therm Polymerase (Southern Cross Biotechnology, Cape Town) and 2–6 μl of template DNA.

Thermal profiles consisted of 2 min at 94 °C (ITS) or 2 min at 95 °C (ETS and *trnT–trnL*) followed by 35 (ITS) or 30 (ETS and *trnT–trnL*) cycles of (i) 94 °C for 1 min (ITS and ETS), 95 °C for 1 min (*trnT–trnL*); (ii) 1 min at 45 °C (ITS), 55 °C (ETS), or 52 °C (*trnT–trnL*); and (iii) 72 °C for 1 (ITS), 2 (ETS) or 1.5 min (*trnT–trnL*). A final extension step of 72 °C for 7 min (ETS), 10 min (ITS) or 8 min (*trnT–trnL*) was performed for all regions.

Successfully amplified target DNA was cleaned and sequenced in both directions using the original PCR primers by Macrogen Inc., 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, USA).

Chromatograms were checked and assembled with Geneious Pro.V. 4.6.4. (Drummond et al., 2009). Consensus sequences were aligned manually in BioEdit 7.0.5.3 (Hall, 1999) for PC. Insertion/deletion (indel) events were coded independently as binary characters using the simple gap coding method of Simmons and Ochoterena (2000).

2.3. Phylogenetic analyses

Tree searching was conducted using the unweighted parsimony criterion as implemented in PAUP* version 4.0 b10 (Swofford, 2002). Only parsimony-informative (PI) sites as identified by the software were included in analyses, in order to standardise tree scores. Heuristic searches were conducted using 10 000 random-addition replicates, implementing tree-bisection-reconnection (TBR) branch swapping and saving all minimal-length trees per replicate. Branch support was assessed via 10 000 nonparametric bootstrap replicates, each based on 100 random addition sequences and saving up to 500 trees per replicate, implementing TBR and saving multiple shortest trees as above.

Initial analyses examined the three gene regions (ETS, ITS and *trnT–trnL*) separately. The resulting support values were compared in order to check for incongruence especially between the nuclear and plastid partitions. There were no conflicting placements so all three regions were concatenated and analysed together. Trees were rooted on the three *Athrixia* species and the outgroup visualised as a monophyletic sister group to the ingroup (the 'outroot = monophyly' command in Paup).

3. Results

The final aligned matrix comprised 1620 characters, within the following partitions: *trnT–trnL*: 544 DNA and 2 indel characters; ETS: 398 DNA and 11 indel characters; ITS: 647 DNA and 18 indel characters. A total of 176 (10.0%) of the matrix characters are parsimony-informative (PI), comprising 6 (1.0%) of the *trnT–trnL* DNA characters and both the *trnT–trnL* indels; 75 (18.0%) of the ETS DNA characters and 4 (36.0%) of the ETS indel characters; 81 (12.0%) of the ITS DNA and 8 (44.0%) of the ITS indel characters. Analyses of each gene region separately found no incongruent groupings, and many nodes had moderate to good support in all independent analyses (Fig. 3). The analysis of the total combined dataset found 4 shortest trees of length = 233, CI = 0.91, RI = 0.94, RC = 0.86 and HI = 0.09. All multiple-sample *Pentatrichia* taxa were recovered as monophyletic (Fig. 4). The genus *Anisothrix* is monophyletic (node D), but is nested within *Pentatrichia* and sister to the type species, *P. petrosa*. Bootstrap (BS) support at every node was equal or better for this combined analysis (Fig. 4), indicating that the separate gene regions all support the same species tree. For example, no individual gene region provided BS support >65% for the monophyly of the three *P. rehmii* subsp. *avasmontana* specimens (node G), but this node received 85% BS in the combined analysis. The highest BS for the sister relationship of the two *Anisothrix* species with *P. petrosa* (node C) from any single region was 72% (*trnT–trnL*), but this node received 88% BS in the combined analysis. Similarly, the highest support from a single region for the node ancestral to all *Pentatrichia* and *Anisothrix* species, excluding *P. alata* (node B) was 73% for ETS, but this node received 93% BS in the combined analysis.

The monophyly of the three accessions of each *Pentatrichia* species or subspecies is supported in every case by BS ≥ 85%, and some have well-supported internal relationships. The radiate *P. rehmii* subsp. *avasmontana* is confirmed as sister (node E; BS = 100%) to the non-radiate *P. rehmii* subsp. *rehmii.* The monophyly of *Anisothrix* is supported by a BS = 99% (node D), and its sister relationship to *P. petrosa* receives a BS of 88% (node C). This relationship was also supported by individual analysis of the ETS and *trnT–trnL* regions, although with lower support, and ITS could not resolve the branching order between *P. rehmii, P. petrosa* and the two *Anisothrix* species. The combined analysis does resolve these, placing the two subspecies of *P. rehmii* (*P. rehmii* subsp. *avasmontana* and *P. rehmii* subsp. *rehmii*) sister to *P. petrosa* and *Anisothrix* (node B; BS = 93%). Sister to all these is *P. alata* which forms the earliest-diverging lineage of the ingroup.

Anisothrix is recovered as monophyletic, but the genus *Pentatrichia* is not monophyletic unless *Anisothrix* is included. *Pentatrichia* and *Anisothrix* form a monophyletic lineage relative to the sampled *Athrixia* species (Fig. 4).

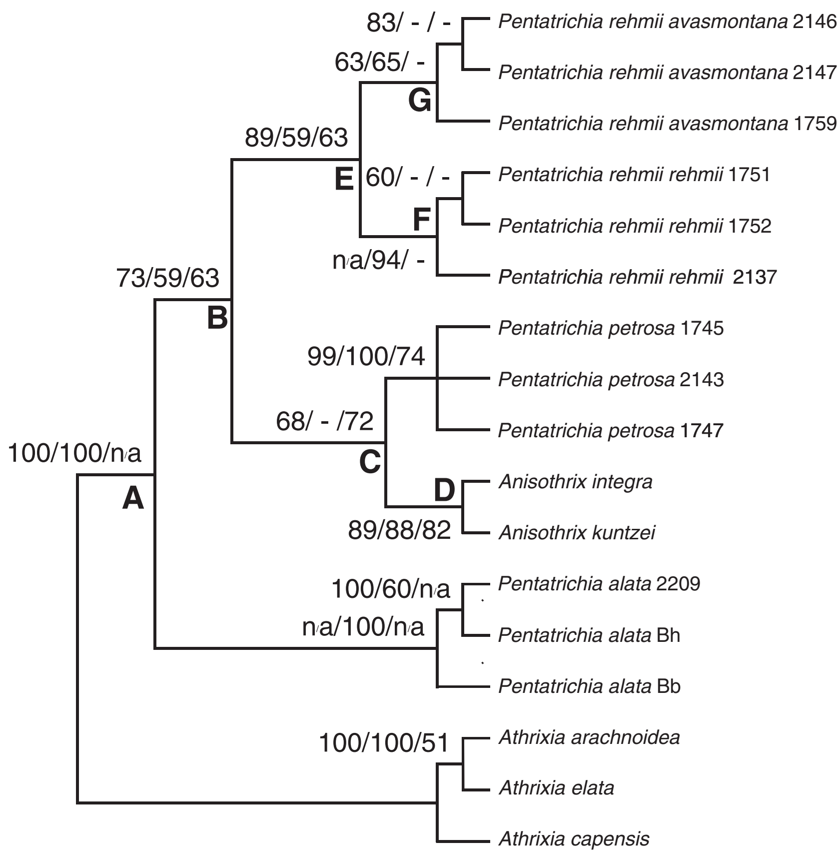

Fig. 3. Consensus tree indicating the results of separate bootstrap analyses on each of the three datasets. There were no conflicting placements in separate analyses of each dataset and so independent analyses can be represented by the same consensus topology. Numbers above branches represent bootstrap support from each of the DNA regions (ETS/ITS/*trnT–trnL*). Some groupings were not represented in individual data partitions due to different taxon sampling in each data partition (indicated by n/a; see Table 1).

4. Discussion

We recover a well-supported and resolved parsimony hypothesis of relationships amongst Southern African members of the "*Athrixia* clade". Our analysis, with complete species sampling and three accessions of all species and subspecies of *Pentatrichia*, demonstrates that *Pentatrichia* is not monophyletic as currently circumscribed. The high bootstrap support (88%) for the grouping of *Anisothrix kuntzei* and *A. integra* sister to the type species *Pentatrichia petrosa* indicates that sinking *Anisothrix* into *Pentatrichia* (the oldest name) would provide a reasonable solution to the non-monophyly. An alternative approach would be to synonymise *Anisothrix* with *P. petrosa* and provide additional generic names for the remaining lineages of *Pentatrichia*. However, this would involve erection of two monotypic genera: one to accommodate *P. alata* and one to accommodate *P. rehmii*. Apart from a greater number of name changes, this scheme would split a very small lineage into three separate genera. Given that there are several morphological synapomorphies defining the *Pentatrichia* plus *Anisothrix* lineage within the "*Athrixia* clade" (see below), we feel that it is more appropriate to treat all the species within a single genus. The generic name *Anisothrix* (Hoffmann, 1898) is thus synonymised with *Pentatrichia* (Klatt, 1895).

Although Anderberg (1988) recognised the close relationship between the two genera, he considered *Anisothrix* to be defined against *Pentatrichia* on the basis of growth habit (the compact woody stems of *Anisothrix* grow concealed in rock fissures, with slender, rather weak protruding branches) by its many-flowered non-radiate capitula, and by leaf characters. However, the two genera are not distinct in habit, as both are sparsely-branched woody subshrubs and several species of *Pentatrichia* also root in rock crevices (Fig. 2). The number of florets per capitulum is also not a good distinguishing character because this does not separate *A. kuntzei* from the discoid species of *Pentatrichia* (*A. kuntzei* capitula contain ±150 individual florets, whereas the discoid species of *Pentatrichia* have ±150–160 florets. Radiate *Pentatrichia* species have ±80 disc and 20 ray florets while *A. integra* has ±260 florets). The most striking difference between the two genera is the leaf morphology (Fig. 1); *Anisothrix* leaves are sessile, narrow, dark green, somewhat thickened and schlerophyllous with obscure veins, while *Pentatrichia* leaves are frequently (pseudo-) petiolate, broad, generally pale green, and very thin-textured with prominent veins. In addition, *Pentatrichia* leaves possess stalked glands, while *Anisothrix kuntzei* has glabrous leaves and those of *A. integra* have sessile glands. *Anisothrix integra* also completely lacks leaf teeth (at least in the adult leaves; Anderberg, 1988) that are present in all other

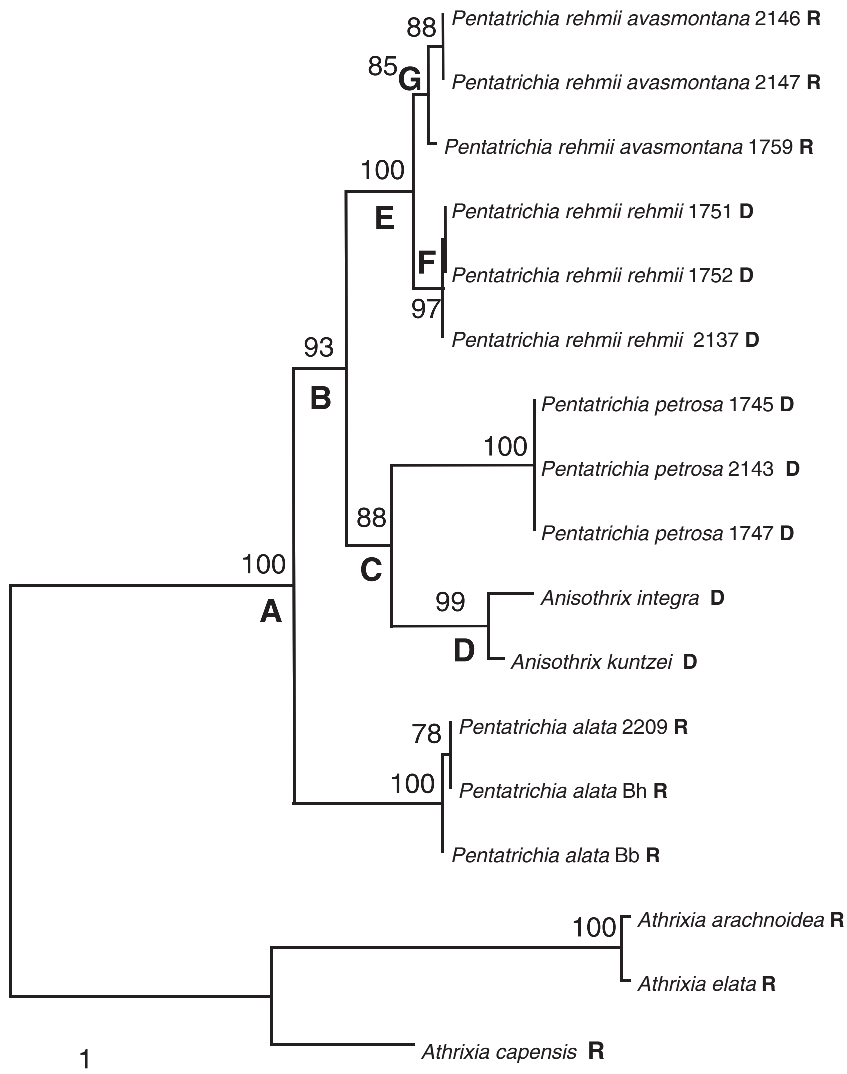

Fig. 4. One of the shortest trees from analysis of all data combined (ITS, ETS, *trnT–trnL* and indels) and including only 176 phylogenetically-informative characters. Numbers above nodes represent bootstrap percentages; labels in uppercase letters represent nodes that are referred to in the text. The capitulum type of each accession is represented by a letter after the taxon name: D (discoid); R (radiate).Tree length = 233 steps; consistency index = 0.91; retention index = 0.94; rescaled consistency index = 0.86; homoplasy index = 0.09.

members of *Anisothrix* and *Pentatrichia*. Other characters that differ between the two genera are a slightly greater number of pappus bristles (5–8) in *Anisothrix* as compared to most *Pentatrichia* species (3–5 in all except *P. petrosa* which has 5–8) and the fact that the scales between the pappus bristles are much longer in *Anisothrix* than in *Pentatrichia* (Anderberg, 1988; Klaassen et al., 2009).

Anisothrix is monophyletic and nested within *Pentatrichia*, and thus the morphological synapomorphies described above reflect the shared, independant ancestry of the two *Anisothrix* species since their divergence from a common ancestor with *P. petrosa*. *Pentatrichia* and *Anisothrix* species, however, form their own distinct lineage within the "*Athrixia* clade", and together share several synapomorphies which support their synonymy. Both genera are small woody subshrubs and are confined to rocky substrates; there is little information on the habitat of *Phagnalon* and *Aliella*, but the remaining members of the "*Athrixia* clade" are not restricted to rocks. *Anisothrix* and *Pentatrichia* are the only members of the "*Athrixia* clade" to contain species with discoid heads, in contrast to *Phagnalon* and *Aliella* where the capitula are strictly disciform, and *Athrixia* where they are strictly radiate. Although some *Pentatrichia* species are radiate, the heads are distinct from those of *Athrixia* in having white ray florets (several species of *Athrixia* have pink or lilac rays). *Athrixia* also differs from both *Anisothrix* and *Pentatrichia* in that its involucral bracts are densely white-woolly, not glabrous or glandular, and in having revolute leaves with white-tomentose lower surfaces. The non-radiate *Pentatrichia* species have heads which are most similar in structure to those of *Anisothrix*, agreeing in the involucral bracts which are pale and scarious and, although they might be glandular, are not hairy; and lacking any female florets. Both *Pentatrichia* and *Anisothrix* have a pappus of less than ten free bristles in a single row, alternating with small, acute scales.

Athrixia species also have scale-like structures but have a greater number of pappus bristles in several rows, while in *Phagnalon* and *Aliella* there are no scales and the pappus bristles, although in a single row, are fused basally. Morphology and DNA sequence data thus agree in placing *Anisothrix* and *Pentatrichia* in a clearly-defined group within the "*Athrixia* clade". Although both genera have corollas with strongly retrofract lobes (Anderberg, 1988; 1991), this is also found in *Athrixia* and *Aliella*, with *Phagnalon* being the only member with erect corolla lobes. In addition, the anthers are tailed in *Anisothrix*, *Pentatrichia* and *Athrixia*, shortly tailed in *Aliella* but lack tails in *Phagnalon*. The cypselas have five vascular bundles in *Anisothrix*, *Pentatrichia* and *Athrixia*, three in *Aliella* and two or three in *Phagnalon*. These characters suggest that *Phagnalon* may be more distantly related to *Pentatrichia* and *Anisothrix* than are *Aliella* and *Athrixia*. Full elucidation of relationships between this group and the remaining members of the clade will require an analysis that includes, apart from all *Pentatrichia* and *Anisothrix* species, members of *Phagnalon* and *Aliella,* more *Athrixia* species and the addition of outgroup taxa from the "*Relhania* clade".

4.1. The evolution of rays in Anisothrix and Pentatrichia

The radiate capitulum with marginal ray florets is thought to have evolved relatively early in the history of the Asteraceae (Gillies et al., 2002) but it is fairly common for different capitulum types to occur in closely-related species or even within species. The change between discoid and radiate capitula, for example, results from the replacement of the outer whorl of ray florets by disc florets, and is thought to be under the control of a relatively simple genetic system (Gillies et al., 2002). Bremer and Humphries (1993) considered discoid capitula to be derived from radiate ones in tribe Anthemidae, and this may also be the case in the present study. In the '*Athrixia* clade' of the Gnaphalieae, *Phagnalon* and *Aliella* species are all disciform, but all *Athrixia* species are radiate. In the clade comprising *Pentatrichia* and *Anisothrix*, the earliest-diverging position of the radiate *P. alata* suggests that the ancestor might have been radiate (Fig. 4), a conclusion which would be strengthened if *Athrixia* is indeed the closest sister group. A radiate ancestor for *Pentatrichia* and *Anisothrix* requires two shifts in capitulum type, involving either one loss and one gain of rays (consistent with an ACCTRAN parsimony reconstruction and comprising a shift to the discoid type at node B, and subsequent regaining of rays at node G) or two losses of rays (consistent with DELTRAN parsimony reconstruction involving nodes C and F). If the ancestor at node A was discoid, two gains of the radiate capitulum would have occurred, once in *P. alata* and once in *P. rehmii* subsp. *avasmontana*.

Shifts between radiate and discoid capitula are unusually frequent in this group compared with other members of the "*Athrixia* clade": *Athrixia* species are all radiate while *Phagnalon* and *Aliella* species are all disciform. Other members of the "*Relhania* clade" of the Gnaphalieae (sister to the "*Athrixia* clade"; Bergh & Linder, 2009 — i.e. *Arrowsmithia, Macowania, Relhania, Oedera* and relatives) are all strictly radiate.

Klaassen et al. (2009) used multivariate analysis of morphological characters to examine relationships within *Pentatrichia*. Their analysis grouped *P. alata* in a cluster with *P. rehmii* and placed *P. petrosa* accessions in a separate cluster. This separation was determined largely by leaf shape (lanceolate–ovate versus cordate, respectively). The present analysis contradicts this result, as *P. alata* is placed sister to the remaining *Pentatrichia* species as well as *Anisothrix*.

The analysis of Klaassen et al. (2009) was also used as a basis for synonymising two species (*P. rehmii* and *P. avasmontana*). The new species, *P. rehmii*, consists of one discoid (*P. rehmii rehmii*) and one radiate (*P. rehmii avasmontana*) subspecies. Our analysis agrees with the multivariate phenetic analysis in the former study in placing these subspecies as reciprocally-monophyletic sister taxa.

5. Taxonomy

Anisothrix is here synonymised with *Pentatrichia* and the necessary two new combinations are provided. The concept of *Pentatrichia* (Klaassen et al., 2009) is thus expanded to include species with entire leaves (*P. integra*) and also those with glabrous, narrowly lanceolate, thickened and obscurely veined leaves that occur in the Cape Floristic Region (CFR) of South Africa (*P. integra* and *P. kuntzei*). The distribution of the expanded genus *Pentatrichia* is shown in Fig. 5.

Previous publications have made use of the term "petiolate" to describe the leaves in *Pentatrichia* (Anderberg 1988, 1991; Klaassen et al., 2009). However, the distinction between petiolate leaves and those with a narrowed leaf-base is not always clear (Fig. 1). Here we describe the leaves as sessile or with the lamina narrowing into a petiole-like base. This pseudopetiole may be nude (like a true petiole) or possess a prominent or obscure 'wing' or extension of the leaf lamina (Fig. 1).

5.1. Key to the species of Pentatrichia

1a Leaves orbicular-cordate, membranous; capitula homogamous, discoid; growing mainly between boulders ………………………………………………………………………*P. petrosa*

1b Leaves lanceolate to lanceolate–ovate to obovate, becoming leathery at maturity; capitula heterogamous, radiate or homogamous, discoid; growing in rock crevices in cliffs ………………………………………………………………………2

2a Leaf margin entire; leaves glabrous; capitula solitary ………………………………………………………………………*P. integra*

2b Leaf margin incised; leaves glandular pilose to glabrescent; capitula solitary or subcorymbose ……………………………………3

3a Capitula solitary; ray florets absent; endemic to the CFR…………………………………………………………………*P. kuntzei*

3b Capitula subcorymbose; ray florets absent or present; distributed outside of the CFR………………………………………4

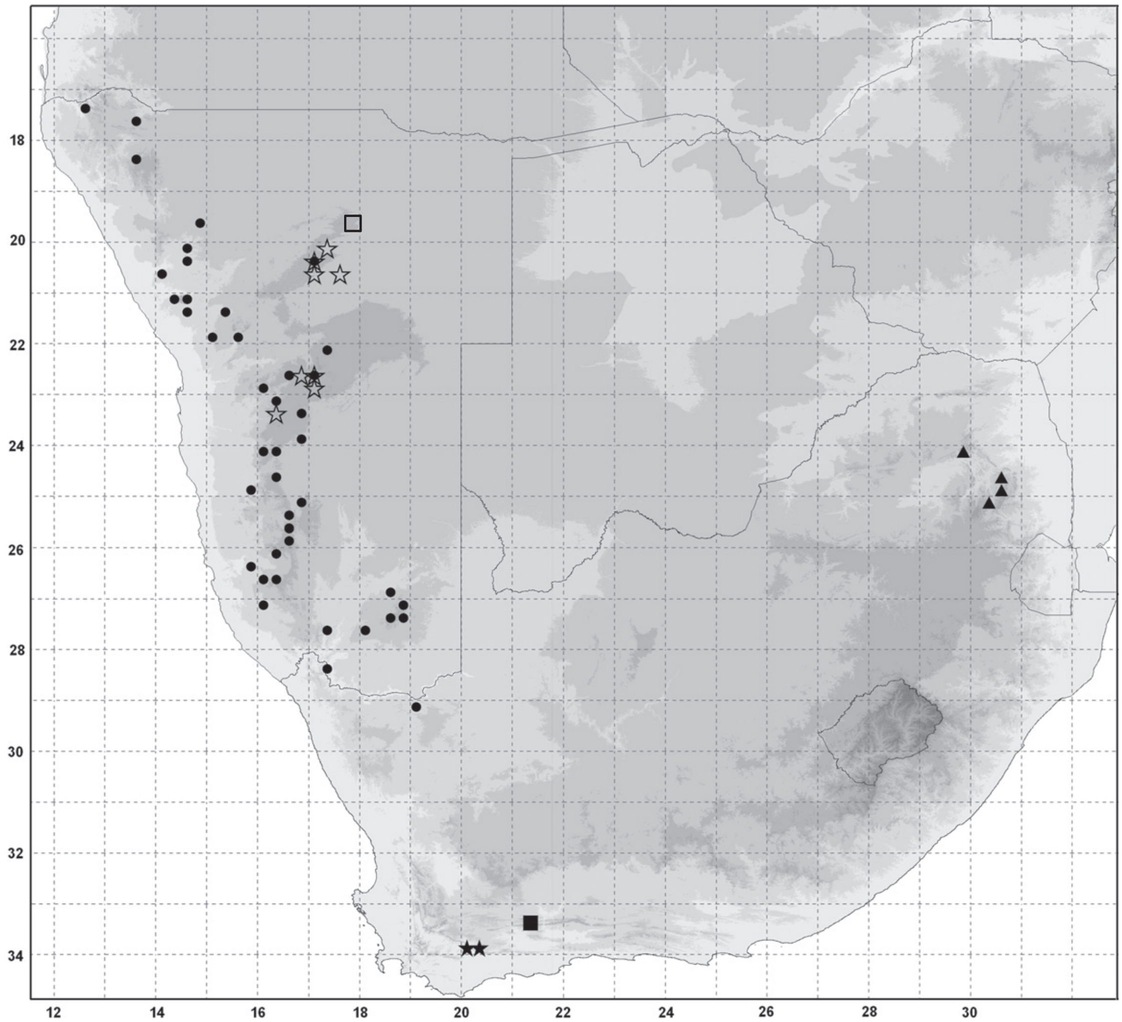

Fig. 5. Distribution of *Pentatrichia* species in Namibia and South Africa based on herbarium records from BOL, LYD, NBG, PRE and WIND. ▲ — *P. alata*; ■ — *P. integra*; ★ — *P. kuntzei*; ● — *P. petrosa*; ☆ — *P. rehmii* subsp. *avasmontana*; □ — *P. rehmii* subsp. *rehmii*.

4a Pseudopetiole prominently winged, more than 15 mm long and up to 2 mm wide; ray florets present; plants endemic to South Africa……………………………………………………*P. alata*

4b Pseudopetiole obscurely winged, less than 6 mm long and less than 0.2 mm wide; ray florets absent or present; plants endemic to Namibia…………………………………………………5

5a Ray florets present……………*P. rehmii* subsp. *avasmontana*

5b Ray florets absent……………………*P. rehmii* subsp. *rehmii*

Pentatrichia Klatt in Bulletin de L'Herbier Boissier 3:436–437 (1895); Merxmüller in Mitt. Bot. München 10:438–439 (1954); Merxmüller in FSWA 139:138–139 (1967); Anderberg in Op. Bot. 104:43 (1991); Anderberg in Asteraceae Cladistics and Classification 325 (1994); Herman et al. in Strelitzia 10:154 (2000). Type: *P. petrosa* Klatt

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Pegolettia dentata Bolus in Transactions of the South African Philosophical Society 16:385–386 (1906). *Iphiona dentata* (Bolus) Bolus in Transactions of the South African Philosophical Society 18(3):395 (1907). Type: South Africa, Western Cape, prope termas in collibus – Montagu in rimis saxorum, 1000 ft, 12/1892, *Bolus 7882* (BOL, lecto.!, designated by Anderberg: 367 (1988); K., isolecto.-photo!).

Acknowledgements

The authors thank the curators of the Bolus (BOL) and Mpumalanga (LYD) herbaria for permission to extract DNA from specimens, and the Compton Herbarium (NBG), Pretoria National Herbarium (PRE) and National Herbarium of Namibia (WIND) for specimens and specimen information. The authors are grateful to J. Bentley who generated DNA sequences for *Pentatrichia alata* and the *Athrixia* species; M. Smith who produced the distribution map, and N. Montes-Moreno and J. Manning for constructive comments on the manuscript. This study was jointly funded via a South African National Research Foundation SABI grant awarded to N. Bergh, and travel funding to E. Klaassen from the Ministry of Agriculture, Water and Forestry of Namibia as well as the German Federal Ministry of Education and Research under the BIOLOG programme, the promotion number 01 LC 0024A, as part of the BIOTA southern Africa project.

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Edited by JC Manning