

Generic Delimitations in Tuberos Periplocoideae (Apocynaceae) from Africa and Madagascar

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• **Background and Aims** The number of genera included in Apocynaceae subfamily Periplocoideae is a matter of debate. DNA sequences are used here as an independent dataset to clarify generic relationships and classification of the tuberos periplocoid genera and to address the question of the phylogenetic interpretation of pollinia formation in *Schlechterella*.

• **Methods** Representatives of nearly all African and Malagasy genera of Periplocoideae possessing root tubers were analysed using ITS and plastid DNA sequence characters.

• **Key Results** Sequence data from non-coding molecular markers (ITS of nrDNA and the *trnT-L* and *trnL-F* spacers as well as the *trnL* intron of plastid DNA) give support for a broad taxonomic concept of *Raphionacme* including *Pentagonanthus*. Together with *Schlechterella*, which is sister to *Raphionacme*, all *Raphionacme*-like taxa form a derived monophyletic group of somewhat diverse species. Sister to the *Schlechterella*/*Raphionacme* clade is a clade comprising *Stomatostemma* and the not truly tuberos vine *Mondia*. In the combined analysis, sister to these two clades combined is a clade formed by *Petopentia natalensis* and *Periploca*.

• **Conclusions** The recent inclusion of the monotypic South African *Petopentia* in the monotypic Malagasy endemic *Ischnolepis* is to be rejected. The Malagasy *Camptocarpus* is sister to the remainder of Periplocoideae in the ITS and combined analyses, and a Malagasy origin for the subfamily is discussed.

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Key words: Africa, Apocynaceae, cpDNA, *Ischnolepis*, Madagascar, nrDNA, *Raphionacme*, *Pentagonanthus*, Periplocoideae, *Petopentia*, root tubers, sequence analysis, *Schlechterella*, *Stomatostemma*.

INTRODUCTION

The number of genera to be accepted in Apocynaceae subfamily Periplocoideae is still a matter of debate. Venter and Verhoeven (1997) gave approx. 190 species in 44 genera; a number also relevant for Meve (2002). The number of genera decreased considerably to only 31 in the review paper published in late 2001 (Venter and Verhoeven, 2001). However, since many of the Asiatic genera are poorly known, the true number of genera is still unclear. The final number probably lies between 31 and 44 genera. Apart from lack of information, major differences in taxonomic views still contribute to these discrepancies (cf. new synonymies for *Cryptolepis* and *Raphionacme*; Venter and Verhoeven, 1997, 2001), and the tuberos Periplocoideae contribute largely to the changing genus numbers. The geophytic growth form is found in roughly one-third of the periplocoid genera. Of these, the genera *Decalepis* Wight & Arn., *Ischnolepis* Jum. & H.Perrier, *Petopentia* Bullock, *Raphionacme* Harv., *Sacleuxia* Baill., *Sarcorrhiza* Bullock, *Schlechterella* K. Schum. and *Stomatostemma* N.E.Br. only comprise tuberos species. All of them, except for the Indian *Decalepis* and the Malagasy *Ischnolepis*, are African (one *Raphionacme* species is Arabian). Three different types of true root tubers (without involvement of the hypocotyl) can be distinguished: semi-subterranean tubers formed by the main root (*Petopentia*; Fig. 1A), subterranean

(rarely partly exposed) tubers formed by few to many lateral roots (*Ischnolepis*, *Stomatostemma*; Fig. 1B and C), and subterranean tubers formed by the main root (*Raphionacme*, *Schlechterella*; Fig. 1D and E). The African *Mondia* Skeels and the Malagasy *Camptocarpus* Decne. are incompletely known with regard to this character. *Mondia*, although described as tuberos by Venter and Verhoeven (2001), is not or not always tuberos in our own observations. In Tanzanian representatives of *Mondia whitei* (Hook.f.) Skeels we observed woody rootstocks at the most. The reverse situation occurs in the Malagasy endemic *Camptocarpus*. Whereas Klackenberg (1998) and Venter and Verhoeven (2001) did not report on any thickened roots at all, plants of *Camptocarpus mauritanus* Decne. grown from seed in the glasshouse in Bayreuth possess some fleshy and swollen (lateral) roots. One non-tuberos Periplocoideae from Madagascar, *Petopentia* Decne., and one from Africa, *Periploca* L., have been added to the material investigated. Due to the lack of suitable material for sequencing, *Sacleuxia* (exact tuber shape unknown) and *Sarcorrhiza* (tubers developing from lateral roots) have not been analysed here. The bark of the tubers seen is usually smooth or nearly so, although rough and scaly in *Petopentia* (see also caption of Fig. 1).

Raphionacme, comprising one-fifth of the species in the subfamily, is a geophytic, erect and sometimes twining herb of tropical and subtropical Africa and Oman. For the most spectacular flowering taxa, Oliver (1887; *Chlorocyathus*

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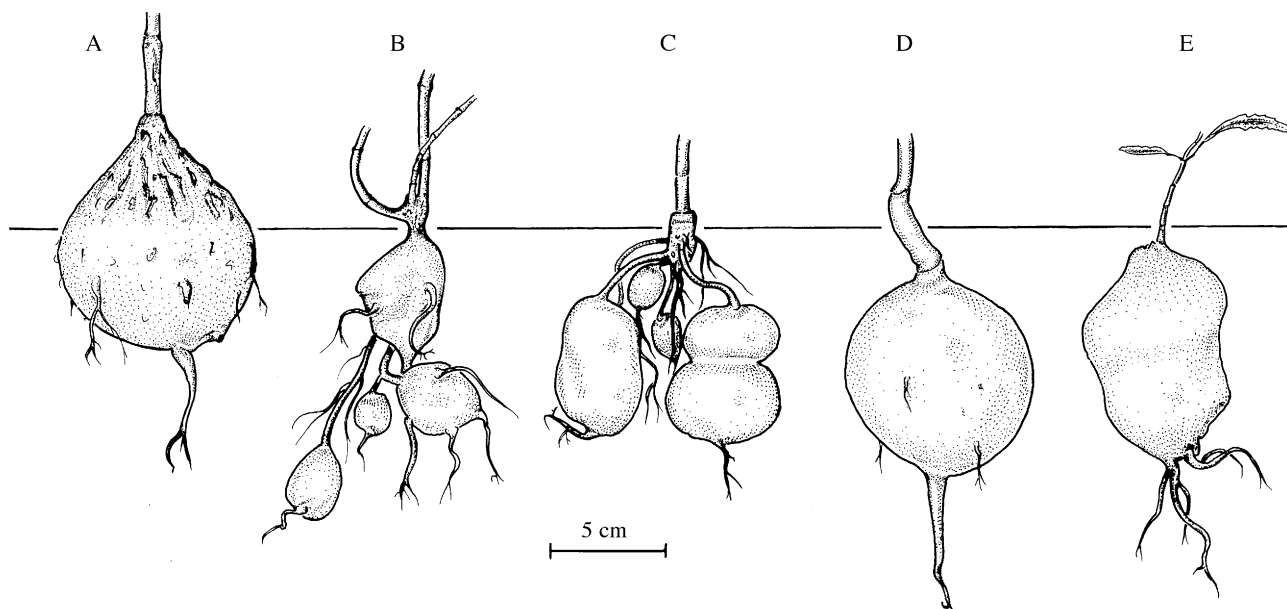


FIG. 1. Examples of different kind of root tubers in Periplocoideae. (A) *Petopentia natalensis*: semi-subterranean root tuber with upper half (third) exposed above ground, bark thick and scaly. (B) *Ischnolepis graminifolia*: multiple root tubers on primary and secondary roots, bark thin and smooth. (C) *Stomatostemma monteiroae*: multiple root tubers on primary and secondary roots, bark thin and smooth. (D) *Raphionacme vignei* E.A. Bruce: root tuber, bark stout but rather smooth. (E) *Schlechterella africana*: root tuber, bark thin and smooth (illustration slightly magnified in relation to A–D). (A) From *ex hort.* Bot. Garden Kiel; (B) from Mangelsdorff *s. n.*; (C) from Albers & Meve 524; (D) from Porembski 503, (E) from Specks 676. All drawn by U. Meve.

Oliv.) and Bullock (1962a; *Pentagonanthus* Bullock) founded new genera. However, both these segregates have been synonymized again under *Raphionacme* (cf. Venter and Verhoeven, 1997). *Schlechterella*, which superficially looks like a *Raphionacme* but possesses a corona with a basal ring-like structure and pollinia instead of loose pollen tetrads (Verhoeven and Venter, 1998), was once partially included in *Raphionacme* [e.g. Verhoeven and Venter (1994) for *Schlechterella abyssinica* (Chiov.) Venter & R.L. Verh.]. However, Venter and Verhoeven (1997, 1998, 2001) later treated both pollinia-bearing species as a separate genus *Schlechterella* (syn. *Triodoglossum* Bullock). DNA sequences are used here as an independent dataset to clarify generic relationships and classification of the tuberous periplocoid genera and to address the question of the phylogenetic interpretation of pollinia formation in *Schlechterella*. Recent molecular studies in Asclepiadeae and Ceropegieae (Liede and Kunze, 2002; Liede and Täuber, 2002; Meve and Liede, 2002) have shown that floral characters are often of limited value in generic circumscription in Asclepiadoideae, because high degrees of parallelism and homoplasy often obliterate phylogenetic relationships. Instead, vegetative characters have been found to be more reliable, including stem succulence in *Cynanchum* L. (Liede and Kunze, 2002), and stem/stipule characters in *Caralluma* R.Br. *sensu lato* (Meve and Liede, 2002). Before taxa of Periplocoideae with differently shaped root tubers are included in *Raphionacme* (which is

characterized by more or less turnip-shaped tubers, Fig. 1D) as well as herbaceous and woody plants, the relationships of these geophytes to each other and to tuberless periplocoids should be assessed.

Similar flowers (and similar stem texture) were used by Venter and Verhoeven (2001) to support the inclusion of the South African *Petopentia natalensis* (Schltr.) Bullock in the Malagasy genus *Ischnolepis*. *Petopentia* is a woody climber with enormous, mostly single tubers (Fig. 1A). *Ischnolepis*, established for the single species *I. graminifolia* (Costantin & Gallaud) Klack. is a shrub and not a climber. It possesses numerous underground tubers on lateral roots (Fig. 1B), which can reach a combined mass of 100 kg in mature plants. With *Petopentia* it shares the reddish, smooth bark of the stems and the large yellow, flat and star-shaped flowers. However, the tubers and leaf morphology and anatomy of *Ischnolepis* and *Petopentia* are different. Molecular data are used to test their congenerity.

MATERIALS AND METHODS

Taxa

The material used in the molecular studies is summarized in Table 1, including voucher specimens, authors of species and donors of material.

Secamone alpinii Schult. (Apocynaceae subfamily Secamonoideae) was selected as the outgroup. Tribes Echiteae and Apocyneae of Apocynaceae subfamily

TABLE 1. Voucher information and EMBL numbers for plant material used in the molecular studies

Species	Origin	Voucher		EMBL acc. no.: trnT-L spacer trnL intron trnL-F spacer	EMBL acc. ITS no.
Outgroup <i>Secamone alpinii</i> Schult. (Secamonoideae)	South Africa: E Cape, Baviaanskloof	Liede 2931	UBT	AJ428828 AJ428829 AJ428830	AJ581692
Ingroup: Periplocoideae <i>Camptocarpus mauritanicus</i> Decne.	Madagascar: Toliara	Petignat s. n.	UBT	AJ581794 AJ581795 AJ581796	AJ581677
<i>Ischnolepis graminifolia</i> (Constantin & Gallaud) Klack.	Madagascar: W Fianarantsoa	Röösli s. n.	UBT	AJ581799 AJ581798 AJ581797	AJ581678
<i>Mondia whitei</i> (Hook.f.) Skeels	Tanzania: Kilimanjaro, Msaranga Valley	Liede & Meve 3351	UBT	AJ581800 AJ581801 AJ581802	AJ581679
<i>Pentopetia grevei</i> (Baill.) Venter	Madagascar: Ambinanitelo	Mangelsdorff 516	UBT	AJ581805 AJ581804 AJ581803	AJ581681
<i>Periploca graeca</i> L.	<i>ex hort.</i> Z	Endress s.n.	Z	AJ581806 AF102468 AJ581807	AJ581682
<i>Periploca visciformis</i> K.Schum.	Socotra: Wadi Ayhaft	Mies 1444	UBT	AJ431734 AJ431735 AJ431736	AJ581683
<i>Petopentia natalensis</i> Bullock	<i>s. loc.</i>	<i>ex hort.</i> Kiel	in cult. UBT	AJ581810 AJ581809 AJ581808	AJ581684
<i>Raphionacme angolensis</i> (Baill.) N.E.Br.	Tanzania: W Songea	Specks 287	UBT	AJ431770 AJ431771 AJ431772	AJ581685
<i>Raphionacme dyeri</i> Retief & Venter	South Africa: KwaZulu-Natal	Nicholas 2862	NH	AJ581811 AJ581812 AJ581813	AJ581686
<i>Raphionacme elata</i> N.E.Br.	South Africa: Durban-Westville	Borchers 47	NH	AJ581816 AJ581815 AJ581814	AJ581687
<i>Raphionacme flanagani</i> Schltr.	South Africa: Pietermaritzburg	Albers K 1577	MSUN	AJ581817 AJ581818 AJ581819	AJ581688
<i>Raphionacme grandiflora</i> N.E.Br. [<i>Pentagonanthus grandiflorus</i> (N.E.Br.) Bullock]	Tanzania: W Songea	Specks 770	UBT	AJ581822 AJ581821 AJ581820	AJ581680
<i>Raphionacme hirsuta</i> (E.Mey.) R.A.Dyer	South Africa: KwaZulu-Natal	Nicholas 2863	NH	AJ581823 AJ581824 AJ581825	AJ581689
<i>Raphionacme madiensis</i> S.Moore	Tanzania: Dodoma	Specks 682	UBT	AJ581828 AJ581827 AJ581826	AJ581690
<i>Schlechterella abyssinica</i> (Chiov.) Venter & R.L.Verh.	Kenya: Furole	Newton 4555	UBT	AJ581829 AJ581830 AJ581831	AJ581691
<i>Stomatostemma monteiroae</i> Oliv.	<i>s. loc.</i>	IPPS 1427	UBT	AJ431779 AJ431780 AJ431781	AJ581692
<i>Stomatostemma monteiroae</i> Oliv.	Zimbabwe: NW Hwange NP, Thomson Junction	Albers & Meve 518	UBT	AJ581834 AJ581833 AJ581832	AJ581693

Apocynoideae, equally suitable as outgroups following Potgieter and Albert (2001), were not used because it was not possible to align ITS sequences. In the ingroup, 16 tuberous and non-tuberous periplocoide species were analysed. Of these, two different specimens of *Stomatostemma*

monteiroae N.E.Br. were included (Table 1). For *Periploca graeca* L., the published sequence AF102468 (Potgieter and Albert, 2001) was used for the *trnL* intron. ITS, *trnT-L* and *trnL-F* spacers of *P. graeca* were sequenced from the material also used by Potgieter and Albert (2001).

DNA extraction and PCR

DNA was isolated from fresh stem tip tissue according to Doyle and Doyle (1987). PCR primers and the protocol for the plastid *trnT-L* and *trnL-F* spacers and the *trnL* intron are from Taberlet *et al.* (1991). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA was amplified using the flanking primers ITS4 and ITS5 following a slightly modified protocol from Baldwin (1992) as detailed by Meve and Liede (2001a, b). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. All sequences have been deposited at EMBL Nucleotide Sequence Database (see Table 1 for accession numbers).

Data analysis

Sequences were aligned with Perkin Elmer Sequence Navigator Version 1.0.1 and the alignment was refined manually. Indels were coded as missing characters throughout.

Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0b8a (PPC) (Swofford, 1998). Phylogenies were generated using Fitch parsimony as implemented in PAUP employing branch-and-bound search (Hendy and Penny, 1982), with addition sequence set to 'furthest'. Decay indices (Bremer, 1988; Donoghue *et al.*, 1992) and bootstrap values (Felsenstein, 1985) derived from 1000 replicates (saving a maximum of 100 trees per replicate) were calculated as measures of support for individual clades. Decay analyses were performed with AutoDecay 4.0 (Eriksson, 1998) in combination with the reverse constraint option of PAUP.

A partition homogeneity test (Bull *et al.*, 1993), as included in PAUP version 4.0b8a, showed that the plastid DNA and ITS datasets are concordant ($P = 0.27$), and they were combined.

RESULTS

The plastid DNA alignment comprises 17 taxa and a total of 1804 characters [879 sequence characters in the *trnT-L* spacer (primers a–b), 526 sequence characters in the *trnL* intron (primers c–d), and 399 sequence characters in the *trnL-F* spacer (primers e–f)]; 25 data cells are unknown, the end of the *trnL* spacer in *Periploca graeca*, for which the published sequences are shorter than ours. The ITS alignment comprises 17 taxa and 700 sequence characters, ten data cells are unknown, affecting the 18S region of *Camptocarpus mauritianus* and *Raphionacme elata*. Both alignments are available from the authors or can be viewed online at <http://www.uni-bayreuth.de/departments/planta2/> or in TreeBASE study (accession number = S956, matrix accession number = M1587; Sanderson, 1994).

Parsimony analysis of the plastid DNA sequence characters alone (29 parsimony-informative characters) resulted in one tree of 38 steps (Fig. 2), consistency index (CI) 0.84 (excluding uninformative characters) and retention index (RI) 0.90. Parsimony analysis of the ITS data alone (124 parsimony-informative characters) resulted in four trees of 274 steps (Fig. 3), CI 0.60 (excluding uninformative

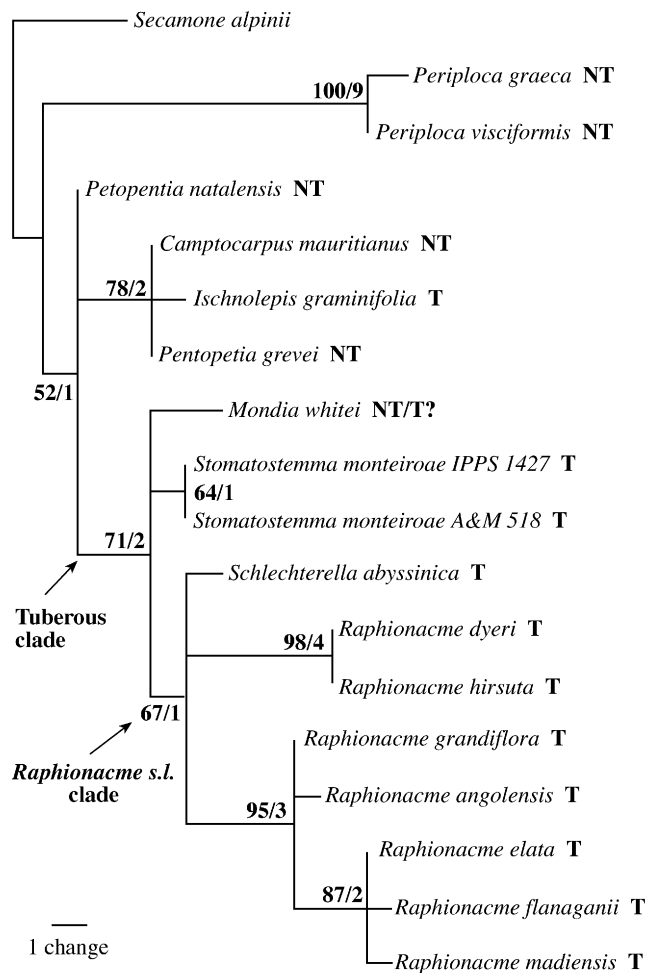


FIG. 2. Single most-parsimonious tree resulting from branch-and-bound analysis of cpDNA data (29 parsimony-informative characters, tree length = 38 steps, CI = 0.8421, RI = 0.9016, RC = 0.7593 if uninformative characters are excluded; NT = not tuberous, T = tuberous).

characters) and RI 0.68. Parsimony analysis of combined ITS and plastid DNA sequence characters (153 parsimony-informative characters) resulted in three trees of 318 steps (Fig. 4), CI 0.62 (excluding uninformative characters), RI 0.70.

In all analyses, the tuberous African Periplocoideae formed a derived clade with moderate support (bootstrap 71 %) in the plastid DNA analysis (Fig. 2, 'tuberous clade'), and 100 % bootstrap support in the ITS and the combined analyses (Figs 3 and 4). Only *Petopentia* did not belong to this clade, despite its tuberous habit. The tuberous African clade comprised two groups: the *Mondia/Stomatostemma* clade, and the clade containing the *Raphionacme* relatives. In the latter, *Schlechterella* was the sister to 'true' *Raphionacme*, while *Raphionacme* itself was divided into two subclades. One, well supported by ITS data, placed *Raphionacme* (*Pentagonanthus*) *grandiflora* N.E.Br. together with *Raphionacme dyeri* Retief & Venter and *R. hirsuta* (E.Mey.) R.A.Dyer (Fig. 3). The other, also well-

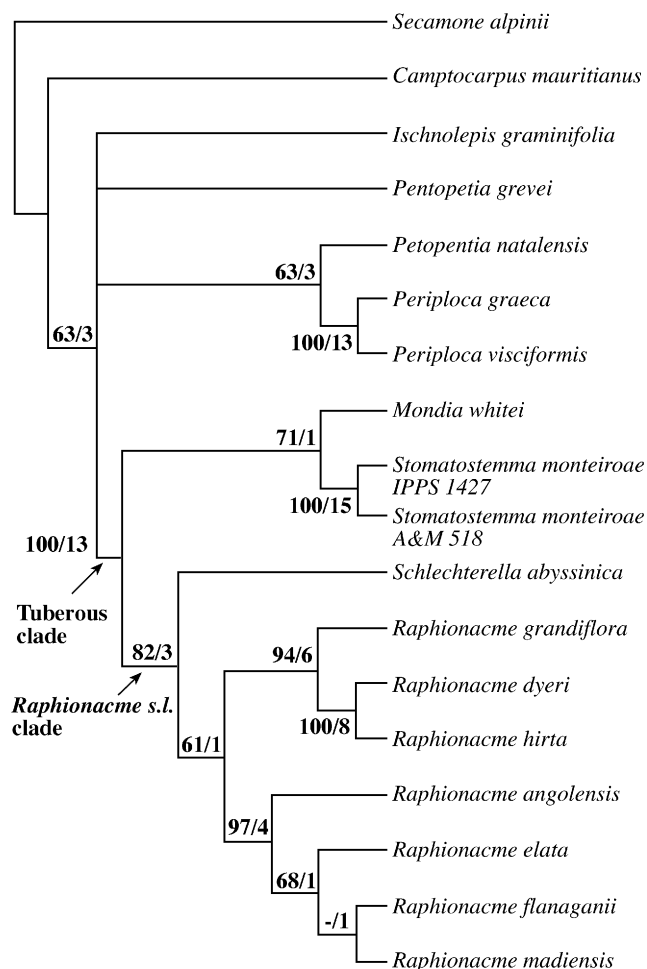


FIG. 3. One of the four most-parsimonious trees resulting from branch-and-bound analysis of ITS data (124 parsimony-informative characters, tree length = 274 steps, CI = 0.5985, RI = 0.6812, RC = 0.4077 if uninformative characters are excluded).

supported subclade, comprised four *Raphionacme* species representing fairly different growth forms.

The remaining taxa were less well resolved than the ‘tuberos clade’. Weakly supported by ITS data, *Petopentia* was sister to *Periploca*, while *Camptocarpus* was sister to all investigated periplocoid taxa in the ITS and combined analyses (Figs 3 and 4). In the plastid DNA dataset, the Malagasy *Camptocarpus*, *Ischnolepis* and *Pentopetia* formed a monophyletic group (Fig. 2).

DISCUSSION

The bitypic *Pentagonanthus* was separated from *Raphionacme* because it possesses five spur-like sacs at the corolla base, a feature to which the genus name refers (Bullock, 1962a). Verhoeven and Venter (1994), however, recognized that each pollen grain in *Raphionacme* has eight to 16 pores instead of four to six, a character unique in Periplocoideae. These authors then included *Pentagonanthus grandiflorus*, which also has eight to 16

pores, in *Raphionacme*. Our molecular data support the conclusion of Verhoeven and Venter (1994) that *Pentagonanthus* is a member of the *Raphionacme* group. The close relationship of *P. grandiflora* to *R. dyeri* and *R. hirsuta* (Figs 3 and 4) cannot be explained morphologically. *Raphionacme hirsuta* and *R. dyeri* differ from *R. grandiflora* with respect to tuber shape and size, foliage, corolla and corona structure. This subclade is relatively strongly supported in the ITS and combined analyses (Figs 3 and 4). However, in the analysis of plastid DNA alone, *R. grandiflora* switches over to the subclade of the genus comprising *R. angolensis*, *R. elata*, *R. flanaganii* and *R. madiensis* (Fig. 2). *Schlechterella* is sister to the remaining species of *Raphionacme* according to the nrDNA dataset (Figures 3 and 4), but forms a trichotomy with the *R. dyeri/R. hirsuta* subclade and the *R. grandiflora/R. angolensis/R. elata/R. flanaganii/R. madiensis* subclade in the plastid analysis (Fig. 2). Since the *Raphionacme s.l.* clade (including *Schlechterella*) is monophyletic in both analyses (Figs 2–4), a close relationship of *Raphionacme s.s.* (with loose pollen tetrads) to *Schlechterella* (with pollen tetrads agglutinated into pollinia) is likely. Nevertheless, the generic status of *Schlechterella* should be maintained, because it is well defined morphologically and genetically. Although the character ‘pollen tetrads forming pollinia’ is clearly a derived state in Periplocoideae (cf. also Nilsson *et al.*, 1993), *Schlechterella* is most probably an early segregate of the *Raphionacme* group because it shows some characters regarded as ancestral in *Raphionacme*: the small, edible tubers, the membranous leaves with thin, long petioles, and the membranous, short-lived flowers (lasting just 1 d at the most, not fleshy and long-lived as in *Raphionacme*). Finally, biogeographic factors support this conclusion. Both species of *Schlechterella* are East African endemics reaching as far north as Ethiopia, where *Raphionacme* is nearly absent (Venter and Verhoeven, 1988). (North-) East Africa has been recognized as centre of origin for many asclepiad groups such as Fockeeae (Kunze *et al.*, 1994) or Ceropegieae (Meve and Liede, 2002). There are three current centres of distribution in *Raphionacme*: west Africa (up to four species per 5° square), east Africa (up to ten species per 5° square) and south-east Africa (up to seven species per 5° square) (Venter and Verhoeven, 1988). Apart from east Africa, which cannot be separated distinctly from north-east Africa, the west and the south-east African centres must be regarded as secondary.

Stomatostemma forms several globoid root tubers per plant. Usually, these tubers are produced on secondary roots. They reach the size and fleshiness of large potatoes (Fig. 1C). This kind of tuber formation is similar to that found in *Ischnolepis* (Fig. 1B). We cultivated several plants of two different clones of *Stomatostemma*, which were both investigated (Table 1 and Figs 2–4). All these plants developed the characteristic tubers which, when growing in cultivation, easily break the pot. Venter and Verhoeven (2001) coded *Stomatostemma* as non-tuberos, whereas they coded *Mondia* as tuberos, which could not be confirmed here (see Introduction). Nevertheless, the molecular data point to a close relationship between these two genera (Figs 2–4). Again, the differences in floral

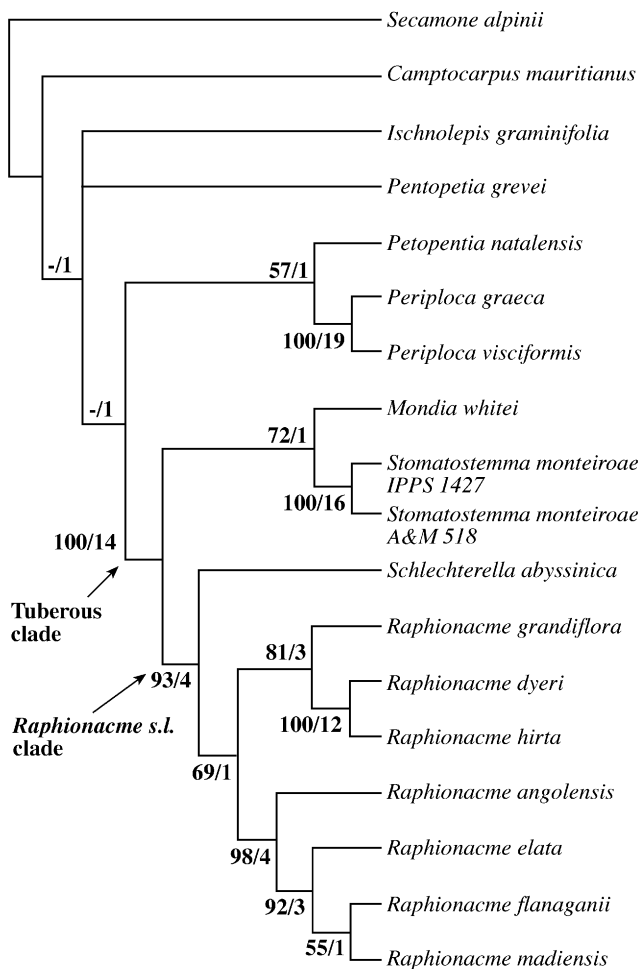


FIG. 4. One of the three most-parsimonious trees resulting from branch-and-bound analysis of combined cpDNA and ITS data (153 parsimony-informative characters, tree length = 318 steps, CI = 0.6164, RI = 0.9695, RC = 0.4311 if uninformative characters are excluded).

morphology between *Mondia* and *Stomatostemma* are considerable, so that Venter and Verhoeven (1997) placed them in two different tribes. In this case, even vegetative morphology fails to indicate a relationship, because in addition to the different root systems, *Mondia* possesses large, slightly coriaceous leaves on smooth, stout stems, whereas *Stomatostemma* has small, membranous leaves on wiry, rough stems. Only in their pollen morphology are the two genera similar (Verhoeven and Venter, 1993, fig. 1). However, rhomboidal-to-tetrahedral pollen tetrads as seen in these genera seem to represent a plesiomorphic rather than an apomorphic state, because this condition is fairly widespread in the other Periplocoideae (Verhoeven and Venter, 1993). The close and well-supported relationship between *Mondia* and *Stomatostemma* was also found by Potgieter and Albert (2001) (*Stomatostemma monteiri* instead of *S. monteiroae*), who analysed a different set of periplocoid genera.

Ischnolepis graminifolia (= *I. tuberosa* Jum. & H.Perrier) is an erect shrub with linear, hypostomatic leaves arranged

in whorls of three (cf. Klackenberg, 1999). In contrast, *Petopentia* possesses large, ovate, opposite, amphistomatic leaves (Venter *et al.*, 1990). Red, waxy, smooth stems are stressed by Venter and Verhoeven (2001) in support of their merging of the two taxa. However, *Ischnolepis* has characteristically swollen nodes which are lacking in *Petopentia*. Finally, reddish stems also occur in other distantly related taxa (e.g. *Raphionacme lobulata* Venter & R.L.Verh., cf. Venter and Verhoeven, 1988; *Sarcorrhiza*, Bullock, 1962b). Thus, red stems are of multiple origin in the subfamily, and cannot be used to support a close relationship between *Ischnolepis* and *Petopentia*. *Ischnolepis graminifolia* bears numerous tubers on lateral roots. Again, this is different from the large, usually single tubers of *Petopentia natalensis*, which are formed by the main root and are partly exposed above ground (Fig. 1). In the flowers, corolla, corona and anther structure are similar, but the shape of the pollen tetrads in the two genera is different. In *Petopentia* the pollen tetrads are typically elongated, mainly linear ($55\text{--}90 \times 25\text{--}40 \mu\text{m}$, cf. Nilsson *et al.*, 1993; Verhoeven and Venter, 1994), whereas in *Ischnolepis* they are rhomboidal (approx. $52 \times 40 \mu\text{m}$; U. Meve, unpubl. res., from Rösli s.n., UBT). Although such differences do not necessarily exclude a close relationship, they do not constitute a supporting character, as do the number of pores in the *Raphionacme* complex (Verhoeven and Venter, 2001). Our sequence data show that *Ischnolepis* is a rather isolated genus, weakly associated with the Malagasy genera *Camptocarpus* and *Pentopentia* according to the plastid DNA data (Fig. 2), while *Petopentia* is equally weakly associated with *Periploca*, in this case supported by the ITS data (Figs 3 and 4). The relationship of *Petopentia* to *Periploca* is unexpected, since the shrubby-to-twining, more or less woody *Periploca* species do not show too many similarities with *Petopentia*. The mainly northern hemisphere *Periploca* (14 species) has the widest distribution of all periplocoid genera, being found from the Canary Islands to China (Venter, 1997). In contrast, the single species of *Petopentia* is restricted to South Africa (Venter *et al.*, 1990), and thus the two distribution areas do not overlap. However, Verhoeven and Venter (1994) reported the peculiar linear to T-shaped pollen tetrads, which are shared by only *Periploca* and *Petopentia*. This observation hints at one putative morphological synapomorphy of a *Petopentia/Periploca* clade. From this discussion it becomes clear that *Ischnolepis* and *Petopentia* cannot be considered congeneric, and *Petopentia* is reinstated here (see Taxonomy).

In their tribal classification of Periplocoideae, based on floral characters, Venter and Verhoeven (1997) scattered the representatives of our 'tuberous clade' throughout the three tribes they recognized. However, as highlighted in the case of *Petopentia* and *Ischnolepis*, floral characters are of limited taxonomic value in Periplocoideae, except for pollen and pollinium characters, which are at least of some value. A careful assessment of all available data, especially the vegetative characters, is necessary for an appropriate classification of Periplocoideae. This is not surprising with regard to information presented in several recently published papers in Apocynaceae subfamily

Asclepiadoideae (Liede and Kunze, 2002; Liede and Täuber, 2002; Meve and Liede, 2002), and also in, for example, two tribes of Orchidaceae: Pleurothallidinae (Pridgeon *et al.*, 2001) and Oncidiinae (Williams *et al.*, 2001a, b). Morphological similarities, in particular those of floral structures, are often a poor guide to phylogenetic relationships because of the high degree of parallelism and homoplasy brought about by selection for pollinators. For example, the genus *Caralluma s.l.* in Asclepiadoideae tribe Ceropegieae has been divided into seven genera, although their flowers are so similar in many cases that they cannot be keyed-out morphologically. However, the classification based on molecular data is supported by stem and leaf characters, and so the taxa can be keyed-out using these characters (Meve and Liede, 2002).

No tuberous species is known in Apocynaceae subfamily Secamonoideae, whereas in subfamily Asclepiadoideae root tubers as storage organs are widespread. In the Fockeeae, sister group of the remaining Asclepiadoideae, large, more or less woody tubers are the rule, sometimes including the hypocotyl and basal internodes in addition to the main root (cf. Kunze *et al.*, 1994). In tribe Marsdenieae, slightly woody root tubers, sometimes with tuberous secondary roots as well, are restricted to *Marsdenia* R.Br. (Forster, 1995; Albers and Meve, 2002). In the closely related and more derived Ceropegieae, fleshy, non-woody hypocotyl tubers, root tubers and/or tuberous secondary roots are restricted to *Brachystelma* R.Br. and parts of the species-rich genus *Ceropegia* L. (Albers and Meve, 2002). In the fourth tribe Asclepiadeae, semi-subterranean root tubers are found in one species of subtribe Gonolobinae, *Matelea cyclophylla* (Standl.) Woodson. Subtribe Metastelminae is also poor in tuberous species (e.g. leafy *Cynanchum* L. from Madagascar with root tubers or tuberous secondary roots; see photographs in Rauh, 1995), whereas in subtribe Asclepiadinae most species have globose, fusiform or napiform root or stem tubers (sometimes with additional secondary tuberous roots; Goyder, 1998, 2001; Albers and Meve, 2002).

Root succulence is undoubtedly an advanced character typically confined to taxa of arid environments or those with at least a seasonal shortage of water. Within each tribe, tubers never occur in the first-branching groups (except for the generally root-succulent Fockeeae). This is the case in *Cryptolepis* R.Br. (Periplocoideae; Potgieter and Albert, 1991; this paper), *Gymnema* R.Br. and *Cionura* Griseb. (Marsdenieae), *Heterostemma* Wight & Arn. and *Leptadenia* R.Br. (Ceropegieae; Meve and Liede, 2004), and *Calotropis* R.Br. (Asclepiadeae; Potgieter and Albert, 2001). Clear delimitations between genera on the basis of tuber morphology as in Alstroemeriaceae (Sanso and Xifreda, 2001), for example, are the exception. Only in a few less-species-rich genera such as *Raphionacme* and *Stathmostelma* does the possession of tubers serve as a reliable generic marker, whereas it is possible to distinguish between at least two main different types of root succulence in *Trachycalymma*, *Brachystelma* and *Ceropegia* (Goyder, 2001; Albers and Meve, 2002). Biogeographically, root tubers are mostly restricted to Africa, which might be highly influenced by the many different arid to semi-arid habitats

and the occurrence of relevant groups such as Periplocoideae, Fockeeae and Ceropegieae.

Chromosomes are often useful in systematics and taxonomy, but are of restricted taxonomic value in Periplocoideae. Apart from some (auto-)tetraploids with $2n = 44$ chromosomes in *Periploca* and *Raphionacme* (Albers and Meve, 2001), all investigated periplocoid taxa show the basal chromosome number of $x = 11$ ($2n = 22$). However, all 'Raphionacme-like taxa' investigated, eight *Raphionacme* species, one *Schlechterella* species (Albers and Meve, 2001), and *Raphionacme* (*Pentagonanthus*) *grandiflora* (U. Meve, unpubl. res.), possess fairly large chromosomes of approx. 1.5 μm length on average, in contrast to *Periploca*, *Petopentia* and *Stomatostemma*, where the chromosomes are only approx. 1 μm long (Albers and Meve, 2001). *Ischnolepis graminifolia* and *Camptocarpus mauritanus* also have small chromosomes, approx. 1–10 μm in length (U. Meve, unpubl. res.).

The Malagasy Periplocoideae occupy the first three branches in the subfamily in our combined analysis. This might lead to the idea that Periplocoideae in general could be of Malagasy origin. Of the 32 genera in the subfamily [31 genera *sensu* Venter and Verhoeven (2001), plus *Petopentia*, this paper]; however, only five genera are from Madagascar, whereas 15 are found exclusively in mainland Africa. Except for *Ischnolepis*, no true tubers are found in any Malagasy taxon and other derived characters are also lacking, including epiphytic growth (Africa: *Sarcorrhiza*; Bullock, 1962b), (sub)succulence (Africa: *Epistemma* H. Huber, *Raphionacme* p.p.), pollen tetrads in pollinia (present in seven genera: two in Africa, four in Asia, one in Australia), clear latex (Africa: *Raphionacme namibiana* Venter & R.L. Verh.) and linear pollen tetrads (Africa: *Petopentia*; Asia: *Periploca*). It can be hypothesized, therefore, that Madagascar houses a stock of relatively early diverged taxa. However, by far the largest number of genera (many monotypic) occur in Africa, so the area of origin of Periplocoideae cannot yet be identified.

TAXONOMY

Petopentia reinstated

Petopentia Bullock, *Kew Bulletin* 10: 362 (1954).

Type species: *Petopentia natalensis* (Schltr.) Bullock. Basionym: *Pentopentia natalensis* Schltr., *Journal of Botany and Foreign* 32: 257 (1894) = *Tacazzea natalensis* (Schltr.) N.E.Br. in Dyer, *Flora Capensis* 4(1): 541 (1907).

Type: Republic of South Africa, KwaZulu-Natal, Isipingo, Wood s.n. (K, holo).

Description and illustration: Venter and Verhoeven (1993).

Number of species: 1 species.

Distribution: Republic of South Africa (KwaZulu-Natal, Mpumalanga, Eastern Cape).

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