

Phylogenetic position of African and Malagasy *Pimpinella* species and related genera (Apiaceae, Pimpinelleae)

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Abstract The phylogenetic position of the African and Malagasy species of *Pimpinella* is assessed using nrDNA ITS sequence data and a representative sampling of the genus, including 16 species from Africa and Madagascar and 26 species from Eurasia. The results of maximum parsimony and Bayesian analyses of these data show that the African and Malagasy species ally with their Eurasian counterparts in Pimpinelleae. The genus *Pimpinella* is rendered paraphyletic by the inclusion of African *Cryptotaenia* and the small African and Malagasy endemic genera *Frommia* and *Phellolophium*. Within a paraphyletic *Pimpinella*, three major clades are recovered, with the African species occupying two of these clades. The current sectional classification of the genus, based predominantly on fruit vestiture, is largely artificial. Chromosome base number, however, was found to be consistent with the groupings recovered in the molecular analyses. Those African and Malagasy *Pimpinella* species with a chromosome base number of $x = 11$ and largely glabrous petals and fruits, form the earliest diverging clade together with *Frommia*, which also has a base count of $n = 11$ and

glabrous petals and fruits. The remaining African species ally with several Eurasian species of *Pimpinella* and share a chromosome base number of $x = 9$ and usually hairy petals and fruits.

Keywords *Cryptotaenia* · Cytology · *Frommia* · Fruit anatomy · nrDNA ITS · *Phellolophium* · *Pimpinelleae*

Introduction

Pimpinella L., with about 150 species distributed throughout much of the Old World (Pimenov and Leonov 1993), is one of the largest genera of the family Apiaceae. However, because of its large size, wide geographical range and high levels of infraspecific variation, the generic boundary of *Pimpinella* and its infrageneric relationships remain unclear (Abebe 1992; Spalik and Downie 2007). The genus was last revised in its entirety by Wolff (1927), who subdivided it into three sections based on petal colour, fruit and petal vestiture and life history. In this system, the African and Malagasy species were accommodated within sections *Tragium* and *Tragioselinum* depending on whether the petals and fruits are papillose to hairy or glabrous respectively. More recent taxonomic studies of the African representatives of *Pimpinella* have revealed several new tropical African species (Townsend 1985, 1989; Abebe 1989, 1992) and, as a result, about 49 sub-Saharan (Van Wyk and Tilney 2004) and 5 Malagasy (Sales et al. 2004) species are generally recognised.

In a recent analysis of nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) sequence data, Spalik and Downie (2007) reported that the African species hitherto classified in *Cryptotaenia* DC. [i.e. *C. africana* (Hook. f.)

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Drude, *C. calycina* C.C.Towns., and possibly *C. polygama* C.C.Towns.] comprised a clade within the tribe Pimpinelleae, together with the Malagasy endemic species *Phellolophium madagascariense* Baker and *Pimpinella betsileensis* Sales & Hedge, and the African endemic genus *Frommia* H.Wolff. As this African clade was sister group to a clade comprising the Eurasian members of *Pimpinella*, Spalik and Downie highlighted the need for the inclusion of additional African *Pimpinella* species in future molecular phylogenetic studies to ascertain whether these species were more closely related to the Eurasian or to the Malagasy group. The present study is aimed at assessing the phylogenetic relationships of the African and Malagasy species of *Pimpinella* and related genera through comparisons of fruit anatomy, cytology and ITS sequence data, using a representative sampling of *Pimpinella* species. These results represent a contribution towards an ultimate reassessment of the generic boundary and infrageneric classification of the genus *Pimpinella*.

Materials and methods

Fruit anatomy

Mature fruits from a representative sampling of African and Malagasy species of *Cryptotaenia* (2 spp.), *Frommia* (1 sp.), *Pimpinella* (13 spp.) and *Phellolophium* (1 sp.) were studied. These fruits were first rehydrated and then placed in a mixture of formalin, acetic acid and alcohol (FAA) for a minimum of 24 h and subsequently treated according to a modification of the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA). This modification involves a final infiltration in GMA for 5 days. Transverse sections, about 3 μm thick, were cut using a Porter-Blüm ultramicrotome and stained according to the periodic acid Schiff/toluidine blue (PAS/TB) method of Feder and O'Brien (1968). Voucher specimens for the fruit anatomical study are listed in Appendix 1.

Molecular data

Total DNA was extracted from herbarium or silica dried material using a PureLink™ Plant Total DNA Purification Kit (Invitrogen, Carlsbad, CA, USA). The entire ITS region was PCR-amplified using the primer combinations of Sun et al. (1994) and the amplification methods outlined in Downie and Katz-Downie (1996). Successfully amplified PCR products were purified according to the ExoSAP protocol of Werle et al. (1994) using 5 units of Exonuclease I (New England Biolabs, Ipswich, MA, USA) and 0.5 units of Shrimp Alkaline Phosphatase (Promega, Madison, WI, USA). Sequencing reactions were carried out

using a BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) and sequenced on either an ABI (Applied Biosystems) 3130 XL or 3730 XL sequencer. Complementary strands were assembled and edited using Sequencher version 3.1.2 (Gene Codes, Ann Arbor, MI, USA) and manually aligned in PAUP* (Swofford 2002), positioning gaps so as to minimise nucleotide mismatches. Sources of material used in the study are provided in Appendix 2. All newly obtained sequences have been deposited in GenBank.

Phylogenetic analyses

The phylogenetic analyses are based on a representative sampling of the genus *Pimpinella*, which includes 16 species from Africa and Madagascar and 26 species from Eurasia. The 19 ITS sequences of African and Malagasy *Pimpinella* (of which 18 were newly obtained) were analysed with 29 accessions of Eurasian *Pimpinella* (Tabanca et al. 2005) and a sampling of several other genera from the tribe Pimpinelleae obtained from Spalik and Downie (2007). Included was a poorly known and undescribed Zambian species referred to by Cannon (1973) as *Pimpinella* sp. B. Two species from the closely related tribe Apieae and four species from the tribe Pyramidoptereae were included as outgroups, with the latter tribe used to root the trees. In total, 68 ITS accessions were included in the phylogenetic study. Data sets are available on request from the corresponding author. Phylogenetic analyses of all data sets were conducted using the maximum parsimony (MP) algorithm of PAUP* with character transformations treated as unordered and equally weighted (Fitch parsimony; Fitch 1971) and gaps treated as missing data. Tree searches were performed using a heuristic search with 1,000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, and the MULPARS option in effect. A limit of ten trees per replicate was set to reduce the time spent on swapping in each replicate. Internal support was assessed with 1,000 bootstrap replicates using TBR swapping and holding ten trees per replicate. Only bootstrap percentage (BP) values greater than 50% are reported, and the following scale was used to evaluate support percentages: 50–74%, weak; 75–84%, moderate; and 85–100%, strong. Appropriate models of nucleotide evolution were selected for each dataset using MODELTEST version 3.06 (corrected Akaike information criterion; Posada and Crandall 1998). Bayesian inference (BI) was performed for 3 million generations of Monte Carlo Markov Chains with a sampling frequency of 100 using MRBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Suboptimal trees were discarded as the “burn-in” phase (25% of all trees obtained), and from the remaining trees a majority

Table 1 Chromosome numbers of *Pimpinella* species and other taxa included in the ITS analyses

Taxa	Chromosome numbers	Sources
<i>Aphanopleura capillifolia</i>	$n = 11$	Vasil'eva et al. 1991, 1993
<i>Bunium bulbocastanum</i>	$n = 10$; $2n = 20$	Buttler 1985; Vasil'eva et al. 1985; Verlaque and Filosa 1992
<i>Cryptotaenia africana</i>	$n = 11$; $2n = 22$	Auquier and Renard 1975; Morton 1993
<i>Crithmum maritimum</i>	$n = 10$; $2n = 20$	Al-Bermani et al. 1993; Vasil'eva et al. 1993; Pimenov et al. 1998
<i>Deverra burchellii</i>	$n = 11$	Al-Eisawi 1989 (as <i>Pituranthos triradiatus</i>)
<i>Frommia ceratophylloides</i>	$n = 11$	Constance et al. 1971, 1976
<i>Nothosmyrnium japonicum</i>	$2n = 20$	Pan et al. 1995
<i>Pimpinella affinis</i>	$n = 9$; $2n = 16$	Constance et al. 1976; Yurtseva 1988
<i>P. anisum</i>	$2n = 18, 19$	Pimenov et al. 2003
<i>P. buchananii</i>	$n = 11$; $2n = 22$	Constance and Chuang 1982; Abebe 1992
<i>P. caffra</i>	$n = 9$	Constance and Chuang 1982
<i>P. corymbosa</i>	$n = 10$; $2n = 20$	Al-Eisawi 1989; Pimenov et al. 1996
<i>P. cretica</i>	$n = 10$	Al-Eisawi 1989
<i>P. diversifolia</i>	$n = 9$; $2n = 18$	Cauwet-Marc et al. 1982
<i>P. eriocarpa</i>	$n = 8$	Al-Eisawi 1989
<i>P. favifolia</i>	$n = 22$	Constance and Chuang 1982
<i>P. hirtella</i>	$2n = 18$	Abebe 1992 (as <i>P. volkensis</i>)
<i>P. huillensis</i>	$n = 9$	Constance and Chuang 1982
<i>P. ledermannii</i>	$n = 9$	Constance and Chuang 1982 (as <i>P. engleriana</i>)
<i>P. lutea</i>	$2n = 20$	Verlaque et al. 1992
<i>P. oreophila</i>	$2n = 18$	Hedberg and Hedberg 1977 (as <i>P. kilimandscharica</i>)
<i>P. peregrina</i>	$n = 9$; $2n = 16, 18, 20$	Yurtseva 1988; Abebe 1992; Pimenov et al. 1996
<i>P. puberula</i>	$2n = 18$	Yurtseva 1988
<i>P. rhodantha</i>	$n = 9, 10$; $2n = 20, 36, 40$	Constance et al. 1976; Pimenov and Vassilieva 1983; Yurtseva 1988; Daushkevich et al. 1995
<i>P. saxifraga</i>	$2n = 18, 20, 36, 40$	Gawlowska 1967
<i>P. sp. B</i>	$n = 11$	Constance and Chuang 1982
<i>P. tragium</i>	$2n = 18, 20, (22)$	Yurtseva and Tikhomirov 1998; Pimenov et al. 2003
<i>P. trifurcata</i>	$2n = 22$	Abebe 1992
<i>Psammogeton biternatum</i>	$2n = 18$	Ahmad and Koul 1980
<i>Psammogeton canescens</i>	$n = 11$	Khatoon and Ali 1993
<i>Stoibrax dichotomum</i>	$n = 10$	Silvestre 1990

Taxa for which chromosome counts were unavailable were coded as missing in the reconstruction analyses (see Fig. 3)

rule consensus was produced using the “sumt” command. Only posterior probability (PP) values greater than 0.5 are reported, and the following scale was applied: 0.50–0.84, weak; 0.85–0.94, moderate; and 0.95–1.0, strong.

Chromosome base numbers (Table 1) were reconstructed onto all maximally parsimonious trees using Mesquite version 2.5 (Maddison and Maddison 2008).

Results and discussion

Phylogenetic relationships

The ITS matrix consisted of 623 unambiguously aligned nucleotide positions of which 317 were variable and 231

parsimony informative. The MP analysis resulted in 36 equally most parsimonious trees with a tree length (TL) of 788 steps, consistency indices (CI) of 0.59 and 0.53 (with and without uninformative characters respectively) and a retention index (RI) of 0.81. MODELTEST selected the SYM + G model of evolution for use in the BI analysis. The topologies of the MP strict consensus tree and the BI majority rule consensus tree were congruent (with the differences between these trees summarised in Fig. 1) and retrieved similar groups to those shown by Spalik and Downie (2007). The tribe Pimpinelleae was recovered as monophyletic (PP 1.0, BP 98). Within this tribe, the largest genus *Pimpinella* was rendered paraphyletic by the inclusion of African *Cryptotaenia* and the small African and Malagasy endemic genera *Frommia* and *Phellolophium*.

Within the paraphyletic *Pimpinella* lineage (PP 1.0, BP 94), three major clades were recovered. The African species of *Pimpinella* were allied with both the *P. hirtella* A.Rich. group in clade A and the *P. buchananii* H.Wolff group in clade C. The majority of the Eurasian species of *Pimpinella* sampled comprised a clade with *P. saxifraga* L. (the type species) within a strongly supported (PP 1.0, BP 96) but poorly resolved clade B. Sister group to this Eurasian clade was a strongly supported clade A (PP 1.0, BP 98), comprising both African and Eurasian species of *Pimpinella*. Within this clade the African species formed a strongly supported group (PP 1.0, BP 99—the *P. hirtella* group), sister to the Eurasian species. Clade C (PP 1.0, BP 80), which was the earliest to diverge, comprised only African and Malagasy species. The African *Cryptotaenia* was paraphyletic, and a clade comprising the two Malagasy species of *Pimpinella* (PP 1.0, BP 100) was a weakly supported sister group to the Malagasy genus *Phellolophium* (PP 0.84, BP 60). The monotypic African endemic genus *Frommia* was placed within a group of tropical African *Pimpinella* species (hereafter the *P. buchananii* group) in which it was moderately to strongly supported as a sister group to *Pimpinella* sp. B. (PP 1.0, BP 79).

African and Malagasy *Pimpinella*

The infrageneric classification of *Pimpinella* proposed by Wolff (1927) does not correspond to any of the clades recovered and, as such, it is clearly artificial (Fig. 1). The main diagnostic characters of the three sections recognised by Wolff (1927) are biennial or perennial plants with yellow petals and variable fruits (sect. *Reutera*); perennial (rarely annual or biennial) plants with white petals and glabrous petals and fruits (sect. *Tragoselinum*); or annual, biennial or perennial plants with white petals and hairy to papillate petals and fruits (sect. *Tragium*). In Wolff's classification, the African and Malagasy species were, with the exception of *P. hirtella* which was placed in subsect. *Eusaxifragae*, separated from the Eurasian species into either subsect. *Afrotragium* or subsect. *Afrotragoselinum*. Although the African and Malagasy species were placed into two of the three major clades, they were found to ally within each. For the African species, their separation based on the presence or absence of hairs on the petals and fruits appears to be only slightly useful. In general, the *P. buchananii* group and allied Malagasy species have glabrous petals and fruits (although they are variable in *P. rigidistyla* C.C.Towns.), while the *P. hirtella* group has hairy petals and fruits (except for *P. kymbilaensis* H.Wolff and *P. oreophila* Hook.f., and in the *P. caffra* D.Dietr. complex, where these features are variable). Although the presence or absence of hairs is a conservative, species-specific character for the tropical African species, in the southern African members this character is

well known to be of limited value because of its infraspecific variation (Townsend 1985). However, the placement of the southern African species *P. caffra*, *P. krookii* H.Wolff and *P. transvaalensis* H.Wolff within the *P. hirtella* group suggests that the sporadic loss of hairs is likely to be a secondary adaptation. While there are some useful fruit anatomical differences among the species of *Pimpinella* (Fig. 2; Table 2), fruit anatomy appears to be more useful for distinguishing species, as previously suggested by Abebe (1992), than for the recognition of infrageneric groups. While most of the African and Malagasy species have multiple vallecular vittae between the ribs, in *P. transvaalensis* (Fig. 2d) and *P. oreophila* (Fig. 2c) only a single vallecular vitta between each rib was observed. This may prove particularly useful to distinguish between the closely related *P. caffra* and *P. transvaalensis*. However, fruits from many more populations of both of these species need to be included before any definite conclusions can be made.

Although no obvious morphological or anatomical characters could be correlated with the results of the molecular phylogenies, the reconstruction of cytological data (Table 1) onto the MP trees revealed interesting results. This is in contrast to the study of Abebe (1992) who could not find any correlation between cytological variation and the subgeneric divisions based on fruit and leaf morphology within *Pimpinella*. In the subfamily Apiioideae, the predominant chromosome base number is $x = 11$ (Moore 1971). Within *Pimpinella*, however, $x = 8, 9, 10$ and 11 have all been recorded, sometimes for the same species, with $x = 9$ being the most prevalent. For all the species for which counts are available within the early diverging clade C (Fig. 3) comprising the *P. buchananii* group, including *Cryptotaenia africana*, a count of $x = 11$ has been recorded, while those species in the *P. hirtella* group consistently have a count of $x = 9$, as found also in the Eurasian members of this clade. Optimizations of chromosome base numbers (Fig. 3) over all 36 minimal length trees differed only in the reconstruction of *P. sintenisii* H.Wolff as having either a base number of $x = 10$ or ambiguously reconstructed as $x = 9$ or $x = 10$. Reconstruction of the cytological data suggests $x = 10$ to be the plesiomorphic state for the genus. A chromosome count of $x = 11$ found in the early diverging clade C is recovered as an apomorphy for this lineage, while $x = 9$ is apomorphic for clade A, with a subsequent decrease to $x = 8$ in *P. eriocarpa* Sol. The ancestor to the entirely Eurasian clade B, including the type species *P. saxifraga*, appears to have had a basic chromosome number of $x = 10$, although subsequent decreases to $x = 9$ have occurred in many species. The possibility that $x = 11$ (the predominant number in the Apiioideae) is the plesiomorphic state within the genus, with subsequent aneuploidy resulting in decreases to $x = 10, 9$ and 8 in the more derived lineages, seems to be a plausible hypothesis. However, counts for more species are

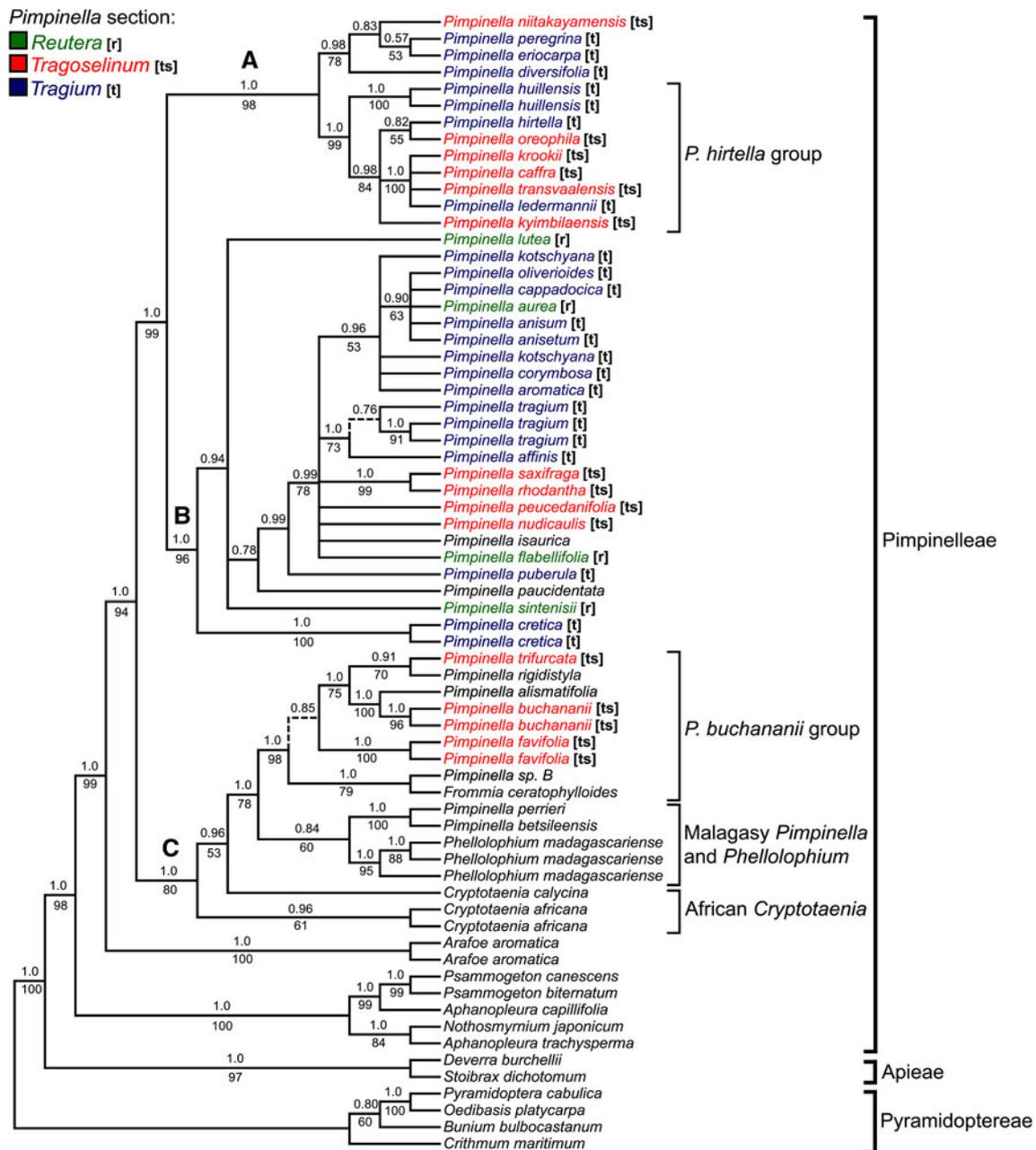


Fig. 1 Bayesian inference (BI) tree of ITS sequence data for *Pimpinella* species and some related taxa (tribe Pimpinelleae) and outgroups from tribes Apieae and Pyramidoptereae. Posterior probability (PP) values are presented above the branches. Bootstrap percentage (BP) values are presented below the branches. Branches supported only in the BI tree are indicated by dashed lines. The

current sectional classification of *Pimpinella* is indicated with both coloured text and abbreviations alongside the species names. Those species of *Pimpinella* not included by Wolff (1927) or Abebe (1992) are indicated in black text. Tribal affinities and the placement of the African species are indicated with brackets, while the circled letters indicate the three major clades of *Pimpinella* discussed in the text

required, preferably from several populations, to assess infraspecific variation, a feature already noted from several Eurasian species (Table 1).

Frommia

The genus *Frommia* is an anomalous African endemic genus with a limited distribution in Malawi. The most

striking generic character is reflected in the epithet of its only species, *F. ceratophylloides* Wolff. The leaves resemble those of *Ceratophyllum* L. due to the sessile, finely divided pinnae with linear ultimate leaf segments (Cannon 1973). Although Cannon (1978) suggested a possible affinity to the genus *Carum* L., the generic placement of *Frommia* has remained unclear. In the analysis carried out by Spalik and Downie (2007), *Frommia*

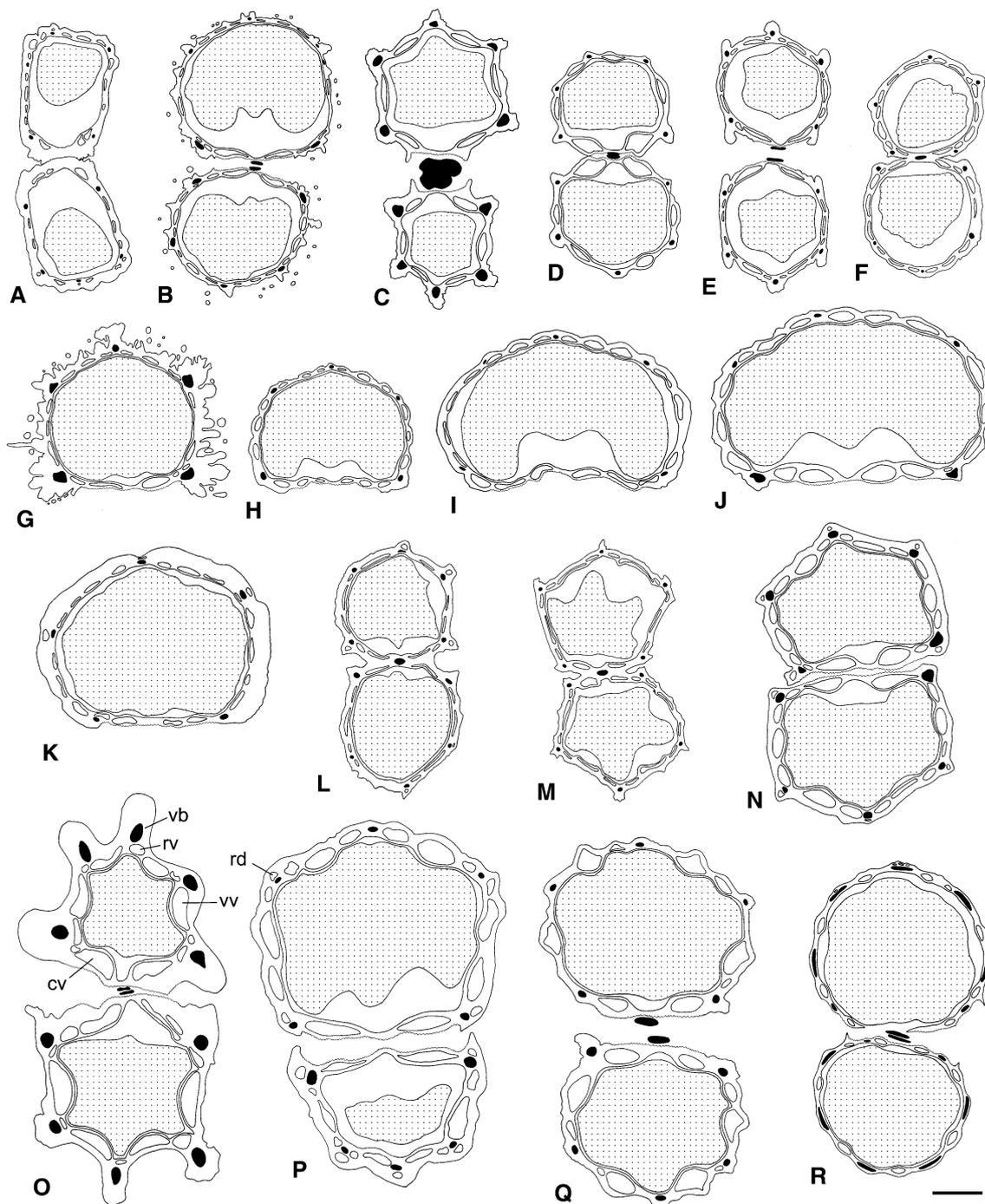


Fig. 2 Transverse sections through the fruits of African (a–j) and Malagasy (k–n) species of *Pimpinella*, *Phellolophium* (o), *Frommia* (p) and *Cryptotaenia* (q, r). a *P. acutidentata* (Fanshawe s.n., K); b *P. hirtella* (Ash 2677, MO); c *P. oreophila* (Townsend 2428, K); d *P. transvaalensis* (Miller 5910, K); e *P. caffra* (Hilliard & Burt 9777, K); f *P. caffra* subsp. *conopodioides* (Pawek 9268, K); g *P. huillensis* (Milne-Redhead 10894, K); h *P. buchananii* (Milne-Redhead 9045A, K);

i *P. trifurcata* (Jean Pawek 13923, MO); j *P. lindblomii* (Gilbert 6531, K); k *P. tenuicaulis* (Baron 3241, MO); l *P. betsileensis* (Humbert 28087, P); m *P. perrieri* (Bossert 8622, P); n *P. ebracteata* (Baron 929, K); o *Ph. madagascariensis* (Pettersen & Nilsson 359, K); p *F. ceratophylloides* (Hooper et al 1844, K); q *C. africana* (Verdcourt 2481, PRE); r *C. canadensis* (PE0866713). cv commissural vita; rd rib oil duct; rv additional rib vita; vb vascular bundle; vv vallicular vita. Scale 0.4 mm

was placed sister group to a clade comprising the Malagasy endemics species *Phellolophium madagascariense* and *Pimpinella betsileensis*. In the current analysis, with a

broader sampling of African *Pimpinella*, the genus was moderately to strongly placed as a sister group to the undescribed *Pimpinella* sp. B., the latter known only from a

Table 2 Summary of selected anatomical characters for the mericarps of those species of African *Pimpinella* and related taxa included in the fruit anatomical study

	Vestiture	Ribs	Number of vascular bundles	Rib oil ducts in mature fruits	Number of vittae per vallecule	Additional rib vittae	Commissural seed surface
<i>C. africana</i>	Glabrous	±Prominent	5	Inconspicuous	2	—	Flat
<i>C. canadensis</i>	Glabrous	Inconspicuous	7	Inconspicuous	1	—	Flat
<i>F. ceratophylloides</i>	Glabrous	Inconspicuous	5	±Prominent	3	—	Concave
<i>P. acutidentata</i>	Glabrous	Inconspicuous	5	?	2–4	—	?
<i>P. betsileensis</i>	Glabrous	±Prominent	5	±Prominent	2 or 3	—	?
<i>P. buchananii</i>	Glabrous	Inconspicuous	5	Inconspicuous	3	—	Concave
<i>P. caffra</i>	Glabrous to pilose	±Prominent	5	Inconspicuous	3 or 4	—	Flat
<i>P. ebracteata</i>	Glabrous	±Prominent	5	±Prominent	3	—	Concave
<i>P. hirtella</i>	Pilose	Inconspicuous	5	Inconspicuous	3 or 4	—	Concave
<i>P. huillensis</i>	Pilose	±Prominent	5	Inconspicuous	3	—	Slightly concave
<i>P. lindblomii</i>	Glabrous	Inconspicuous	5	Inconspicuous	3	—	Concave
<i>P. oreophila</i>	Glabrous	±Prominent	5	Inconspicuous	1	—	Flat
<i>P. perrieri</i>	Glabrous	±Prominent	5	Inconspicuous	3	—	Flat
<i>P. tenuicaulis</i>	Glabrous	Inconspicuous	5	±Prominent	3 or 4	—	Slightly concave
<i>P. transvaalensis</i>	Glabrous	±Prominent	5	Inconspicuous	1	—	Flat
<i>P. trifurcata</i>	Glabrous	Inconspicuous	5	Inconspicuous	3 or 4	—	Concave
<i>Ph. madagascariense</i>	Glabrous	Very prominent	5	Inconspicuous	1	+	Flat

Question marks represent character states that were not visible in the material studied

few collections in Zambia (Cannon 1973), within the *P. buchananii* group of clade C (Fig. 1). The latter species also has finely divided, linear ultimate leaf segments like *Frommia*, although without sessile pinnae. *Pimpinella lineariloba* Cannon from Angola may form part of this group as it also has linear ultimate leaf segments and, according to Cannon (1973), a possible affinity to *Pimpinella* sp. B. In transverse section the fruits of *Frommia* (Fig. 2p) appear similar to those of the *P. buchananii* group (Fig. 2h–j), with multiple vallecular (three in each furrow) and commissural (four, sometimes appearing confluent in mature fruits) vittae, as well as a prominently concave commissural seed surface (Table 2). Furthermore, a chromosome base number of $x = 11$, as found in *Pimpinella* sp. B and the other species from the *P. buchananii* group (Fig. 3h–j), has also been recorded for *Frommia ceratophylloides* (Constance et al. 1971, 1976).

Phellolophium

The Malagasy endemic genus *Phellolophium* was described by Baker (1884) who proposed an affinity to *Seseli* L. and *Foeniculum* Mill. Recently, another undescribed species (Sales et al. 2004) was discovered, with an affinity to *Pimpinella* speculated (Sales and Hedge 2010). In Spalik and Downie (2007), *Phellolophium* was shown to be closely related to the African members of the tribe Pimpinelleae,

together with *Pimpinella betsileensis*, the only Malagasy species they sampled. In the current analysis, the sister group relationship between *Phellolophium* and Malagasy *Pimpinella* was confirmed, with this group successively sister to the *P. buchananii* group. While Wolff (1927) placed the Malagasy species of *Pimpinella* together with the African species in his subsect. *Afrotragoselinum*, they were separated into the series *Madagassicae*. *Phellolophium* shares the broad, pinnately divided leaves with some of the Malagasy species of *Pimpinella* but differs in the presence of prominent involucre and involucre bracts, fruits with thick corky ribs and four vallecular vittae, and five slightly smaller vittae usually situated more or less below the ribs (Fig. 2o; Table 2).

Cryptotaenia

The geographically disjunct genus *Cryptotaenia* was shown by Spalik and Downie (2007) to be polyphyletic, with its species occurring in tribes Oenantheae (*Cryptotaenia* s.s.), Scandiceae (*C. elegans* Webb ex Bolle) and Pimpinelleae (*C. africana*, *C. calycina* and possibly *C. polygama*). As in the analyses done by Spalik and Downie (2007), both the MP and BI analyses presented herein place the African species of *Cryptotaenia* (*C. africana*, *C. calycina*) successively sister to the lineage comprising *Phellolophium*, the Malagasy species of *Pimpinella*, and the *P. buchananii* group. The fruits of *C. africana*



Fig. 3 Parsimony-based reconstructions of chromosome base numbers (Table 1) in *Pimpinella* and related taxa when optimized over one of the 36 minimal length trees inferred from MP analysis of ITS

data. The placement of the African species are indicated with brackets, while the circled letters indicate the three major clades of *Pimpinella* discussed in the text

(Fig. 2q) appear only superficially similar to those of *Cryptotaenia* s.s. (Table 2). In *C. canadensis* (L.) DC. (Fig. 2r) the fruits are diagnostically different with only one vallecular vitta between the ribs and two additional vascular bundles located near the commissure. The fruits of *C. africana*, in contrast, have multiple vittae between the ribs and only five vascular bundles (Fig. 2q), as found also in most species of *Pimpinella* (Fig. 2a–n).

Biogeography

Spalik and Downie (2007) suggested that the African and Malagasy members of tribe Pimpinelleae occupied an

isolated position separate from the Eurasian species. These authors, through biogeographical analyses, also suggested that the African taxa were derived from a common ancestor of Middle Eastern origin which extended into Africa through a Middle East–East African tract and subsequently into Madagascar. With the inclusion of additional African and Malagasy species of *Pimpinella* in our study, it is now apparent that these African and Malagasy taxa are not as isolated as previously suggested. Although it is likely that the species from Madagascar are derived from a single ancestor of African origin, it would appear that more than one dispersal event into Africa from Eurasia must have taken place.

Taxonomic implications

While it is tempting to simply include the anomalous African genera *Cryptotaenia*, *Frommia* and *Phellolophium* into an expanded and already rather poorly defined genus *Pimpinella*, there remains a need for a broader sampling of the East Asian species which, according to Zhou et al. (2008, 2009), are placed within the “East Asia clade”—a group that is distantly related to Pimpinelleae. Formal taxonomic and nomenclatural changes should only be implemented once the full range of diversity within the genus has been studied. Chemotaxonomic studies may also prove to be valuable in defining the genus, as these studies indicate that *Pimpinella* is unique in the presence of norsesquiterpenes and phenylpropanoids (Kubeczka 1997; Tabanca et al. 2005). Although the morphological characters traditionally used to define infrageneric groups within *Pimpinella* appear to be of limited use, the results of this study suggest that cytological data should be explored in more detail as an important phylogenetic character.

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Appendix 1: Voucher information for fruit material studied in transverse section

Cryptotaenia DC.: *C. africana* (Hook. f.) Drude, *Verdecourt* 2481 (PRE); *C. canadensis* (L.) DC., PRE0866713 (PRE), *Howler* 11047 (PRE). *Frommia* H.Wolff: *F. ceratophylloides* H.Wolff, *Hooper et al.* 1844 (K). *Pimpinella* L.: *P. acutidentata* Norman, *Fanshawe s.n.* (K); *P. betsileensis* Sales & Hedge, *Humbert* 28087 (P); *P. buchananii* H.Wolff, *Milne-Redhead* 9045A (K); *P. caffra* D.Dietr. subsp. *caffra*, *Hilliard & Burt* 9777 (K); *P. caffra* subsp. *conopodioides* C.C.Towns., *Pawek* 9268 (K); *P. ebracteata* Baker, *Baron* 929 (K); *P. hirtella* A.Rich., *Ash* 2677 (MO); *P. huillensis* Welw. ex. Engl., *Milne-Redhead* 10894 (K); *P. lindblomii* H.Wolff, *Gilbert* 6531 (K); *P. oreophila* Hook.f., *Townsend* 2428 (K); *P. perrieri* Sales & Hedge, *Boissier* 8622 (P); *P. tenuicaulis* Baker, *Baron* 3241 (MO); *P. transvaalensis* H.Wolff, *Miller* 5910 (K); *P. trifurcata* H.Wolff, *Jean Pawek* 13923 (MO). *Phellolophium* Baker: *P. madagascariense* Baker, *Pettersson & Nilsson* 359 (K).

Appendix 2: Voucher information of ITS sequences with corresponding voucher specimens (for the new accessions) and GenBank accession numbers

New accessions of *Pimpinella* L. *P. alismatifolia* C.C.Towns., FM986448; *Bidgood et al.* 3564 (K).

P. buchananii H.Wolff, FM986455; *Winter* 4179 (PRE). *P. buchananii* H.Wolff, FM986456; *Winter* 4188 (PRE). *P. caffra* D.Dietr., FM986447; *Van Wyk et al.* 4233 (JRAU). *P. favifolia* C.Norman, FM986458; *Winter* 3992 (PRE). *P. favifolia* C.Norman, FM986453; *Phillips* 1612 (MO). *P. huillensis* Welw. ex. Engl., FM986443; *Biegel* 3461 (PRE). *P. huillensis* Welw. ex. Engl., FM986454; *Fanshawe* 1011 (P). *P. hirtella* A.Rich., FM986444; *De Wide* 6578 (PRE). *P. krookii* H.Wolff, FM986445; *Hilliard & Burt* 14439 (K). *P. kyimbilaensis* H.Wolff, FM986452; *Gereau et al.* 3150 (MO). *P. ledermannii* H.Wolff subsp. *engleriana* (H.Wolff) C.C.Towns., FM986457; *de Witte* 6205 (PRE). *P. oreophila* Hook.f., FM986450; *Abebe* 1124 (MO). *P. perrieri* Sales & Hedge, FM986460; *Boissier* 16.027 (P). *P. rigidistyla* C.C.Towns., FM986459; *Gereau & Kayombo* 3987 (MO). *P. transvaalensis* H.Wolff, FM986449; *Winter s.n.* (PRE). *P. trifurcata* H.Wolff, FM986446; *LaCroix* 4284 (PRE). *P. sp. B*, FM986451; *Brummitt et al.* 16990 (MO). **Published GenBank accessions.** *Aphanopleura capillifolia* (Regel & Schmalh.) Lipsky, DQ516368^h. *Aphanopleura trachysperma* Lipsky, AF008629^e, AF009108^e. *Arafoe aromatica* Pimenov & Lavrova, AF077874^c. *Arafoe aromatica* Pimenov & Lavrova, U78383^b, U78443^b. *Bunium bulbocastanum* L., DQ443722^j, DQ443724^j. *Crithmum maritimum* L., AH003474^a. *Cryptotaenia africana* (Hook. f.) Drude, DQ516370^h. *Cryptotaenia africana* (Hook. f.) Drude, DQ516371^h. *Cryptotaenia calycina* C.C.Towns., DQ516372^h. *Deverra burchellii* (DC.) Eckl. & Zeyh., AM408887ⁱ. *Frommia ceratophylloides* Wolff, DQ647630^h. *Nothosmyrnum japonicum* Miq., DQ516367^h. *Oedibasis platycarpa* Koso-Pol., AF008632^d, AF009111^d. *Phellolophium madagascariense* Baker, DQ647627^h. *Phellolophium madagascariense* Baker, DQ647628^h. *Phellolophium madagascariense* Baker, DQ647629^h. *P. affinis* Ledeb., AY581780^g. *P. anisetum* Boiss. & Balansa, AY581781^g. *P. anisum* L., AY581782^g. *P. aromatica* M.Bieb., AY581784^g. *P. aurea* DC., AY581785^g. *P. betsileensis* Sales & Hedge, DQ647626^h. *P. cappadocica* Boiss. & Balansa, AY581786^g. *P. corymbosa* Boiss., AY581787^g. *P. cretica* Poir., AY581789^g. *P. cretica* Poir., AY581788^g. *P. diversifolia* DC., DQ516369^h. *P. eriocarpa* Sol., AY581790^g. *P. flabellifolia* (Boiss.) Benth. & Hook. f., AY581791^g. *P. isaurica* Matthews, AY581792^g. *P. kotschyana* Boiss., AY581793^g. *P. kotschyana* Boiss., DQ516373^h. *P. lutea* Desf., DQ516374^h. *P. nitakayamensis* Hayata, DQ516375^h. *P. nudicaulis* Trautv., AY581794^g. *P. oliverioides* Boiss. & Hausskn., AY581795^g. *P. paucidentata* Matthews, AY581796^g. *P. peregrina* L., AY581797^g. *P. peucedanifolia* Fischer ex Ledeb., AY581798^g. *P. puberula* (DC.) Boiss., AY581799^g. *P. rhodantha* Boiss., AY581800^c. *P. saxifraga* L., AY581801^g. *P. sintenisii* H.Wolff, AY581802^g.

P. tragium Vill., AY581803^g. *P. tragium* Vill., AY581-804^g. *P. tragium* Vill., AY581805^g. *Psammogeton biternatum* Edgew., AF164839^f, AF164864^f. *Psammogeton canescens* (D.C.) Vatke, AF008630^e, AF009109^e. *Pyramidoptera cabulica* Boiss., AF008631^d. *Stoibrax dichotomum* (L.) Raf., DQ516366^h.

Downie and Katz-Downie (1996)^a; Downie et al. (1998)^b; Valiejo-Roman et al. (1998)^c; Katz-Downie et al. (1999)^d; Lee and Downie (1999)^e; Downie et al. (2000)^f; Tabanca et al. (2005)^g; Spalik and Downie (2007)^h; Winter et al. (2008)ⁱ; Degtjareva et al. (2009)^j.

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