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Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data; implications for the size, morphology, distribution and evolutionary history of *Coffea*

AARON P. DAVIS FLS[1]*, JAMES TOSH[2], NICOLAS RUCH[1]† and MICHAEL F. FAY FLS[1]

[1]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*
[2]*National History Museum, Cromwell Road, London SW7 5BD, UK*

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Morphological and molecular phylogenetic studies show that there is a close relationship between *Coffea* and *Psilanthus*. In this study we reassess species relationships based on improved species sampling for *Psilanthus*, including *P. melanocarpus*, a species that shares morpho-taxonomic characters of both genera. Analyses are performed using parsimony and Bayesian inference, on sequence data from four plastid regions [*trnL–F* intron, *trnL–F* IGS, *rpl16* intron and *accD–psa1* intergenic spacer (IGS)] and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (ITS 1/5.8S/ITS 2). Several major lineages with geographical coherence, as identified in previous studies based on smaller and larger data sets, are supported. Our results also confirm previous studies showing that the level of sequence divergence between *Coffea* and *Psilanthus* species is negligible, particularly given the much longer branch lengths separating other genera of tribe Coffeeae. There are strong indications that neither *Psilanthus* nor *Coffea* is monophyletic. *Psilanthus melanocarpus* is nested with the *Coffea–Psilanthus* clade, which means that there is only one critical difference between *Coffea* and *Psilanthus*; the former has a long-emergent style and the latter a short, included style. Based on these new data, in addition to other systematically informative evidence from a broad range of studies, and especially morphology, *Psilanthus* is subsumed into *Coffea*. This decision increases the number of species in *Coffea* from 104 to 124, extends the distribution to tropical Asia and Australasia and broadens the morphological characterization of the genus. The implications for understanding the evolutionary history of *Coffea* are discussed. A group of closely related species is informally named the '*Coffea liberica* alliance'.

ADDITIONAL KEYWORDS: *accD–psa1* intergenic spacer (IGS) – Coffeeae – crop wild relatives (CWR) – internal transcribed spacer (ITS) – Old World biogeography – molecular phylogenetics – morphology – *rpl16* intron – *trnL–F* intron – *trnL–F* IGS.

INTRODUCTION

Taxonomic background

Psilanthus Hook.f. is a genus of 20 species, occurring in the Old World Tropics from West Africa to northern Australia (Davis, 2003; Govaerts *et al*., 2011). Since its inception (Hooker, 1873a, b), when it contained a single species (*P. mannii* Hook.f.), *Psilanthus* has been closely associated with *Coffea* L. Hooker (1873a) stated that: 'As a genus it is evidently clearly allied both in habit and characters to *Coffea*, differing in accrescent eglandular calyx, and in the structure of the fruit, which is crustaceous and 2-celled, not drupaceous with 2 pyrenes'. However, he went on to say: 'I do not, however, place much dependence on this last point, for though fully formed, being seedless, the fruits of *Psilanthus* may be abnormally developed' (Hooker, 1873a). In the *Flora of Tropical*

*Corresponding author. E-mail: a.davis@kew.org
†Current address: Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK.

Africa Hiern (1877) provided a description for *Psilanthus*, separating it from *Coffea* mainly on the basis of its included or partly included anthers (vs. anthers exserted or partly included in *Coffea*), included style (vs. shortly exserted), and fruit crowned by subfoliaceous, accrescent calyx lobes (vs. non-accrescent). Hiern (1877) added two more species of *Psilanthus*, *P. ebracteolatus* Hiern and *P. tetramerus* Hiern (= *P. mannii*) and, although he made no direct comparison with *Coffea*, he placed the two genera together in his numbering sequence. The placement of *P. ebracteolatus* was, however, not entirely consistent with his delimitation of *Psilanthus*: this species has a short, included style and included anthers but lacks the distinct large accrescent calyx lobes of *P. mannii*. De Wildeman (1910) added a third species, *P. sapinii* De Wild, a species closely related (Maurin *et al*., 2007) and morphologically similar to *P. mannii*.

Chevalier (1942) enumerated five *Psilanthus* species: *P. mannii*, *P. ledermannii* A.Chev. ined. (= *P. mannii*), *P. minor* A.Chev. ined. (= *P. sapinii*), *P. ebracteolatus* and *P. jasminoides* Hutch. & Dalziel (= *Argocoffeopsis eketensis* (Wernham) Robbr.). This delimitation has been considered (Davis, Bridson & Rakotonasolo, 2005) illogical given the key characters used by Chevalier (1942) to separate *Psilanthus* and *Coffea*, which would exclude *P. ebracteolatus* and *P. jasminoides*: calyx limb surmounted by five accrescent lobes, which are filiform at first but rapidly become foliaceous (vs. calyx limb entire or slightly toothed in *Coffea*); fruit with five narrow wings, extending from the limb of the calyx to the base of fruit (vs. fruits without wings). In a later study, Chevalier (1947: 226) placed *P. ebracteolatus* in its own genus, *Cofeanthus* A.Chev. *Psilanthus jasminioides* was retained in *Psilanthus*, even although the name is based on the same type specimen as *Coffea jasminoides* Welw. ex Hiern, which he placed in *Coffea* section *Argocoffea* Pierre ex De Wild. (Chevalier, 1947: 131); both of these names have since been transferred to the genus *Argocoffeopsis* Lebrun and are synonyms of *A. eketensis* (Wernham) Robbr. (Robbrecht, 1986: 158).

The first person to contest the delimitation of *Psilanthus* spp. was Brenan (1953: 115–116). Upon examination of fully mature fruits of *P. ebracteolatus*, Brenan was in little doubt as to the affinities of this species. He observed, correctly, that the fruits of *P. ebracteolatus* are 'quite smooth outside, and the calyx is scarcely visible at all' (Brenan, 1953: 116), in other words, markedly different from those of *P. mannii*, fruits of which have five narrow longitudinal wings on the outside and are crowned by five large, accrescent foliaceous calyx lobes. Brenan (1953: 116) stated that: 'I am unable to see any reason against *P. ebracteolatus* being a *Coffea*, the fruits and seeds in particular showing very good agreement. The seeds of *P. ebracteolatus* when roasted smell distinctly of coffee'. In conclusion, Brenan formally placed *P. ebracteolatus* in *Coffea* (*C. ebracteolatus* (Hiern) Brenan).

Upon detailed study of African and Asian *Coffea*, Leroy (1962a, b, 1967a, b, c, 1968b) transferred nine species of *Coffea* section *Paracoffea* Miq. to the genus *Paracoffea* J.-F.Leroy (Davis, 2003), his circumscription of this genus being largely based on the works of Chevalier (1929, 1938, 1942, 1947). After further consideration of African and Madagascan *Coffea* relatives, Leroy (1980a) decided to make *Paracoffea* a subgenus of *Psilanthus*, and proposed two subgenera: subgenus *Psilanthus* for species from Africa (*P. mannii* and *P. sapinii*, i.e. those species with the distinctive accrescent calyx lobes and winged fruit) and subgenus *Paracoffea* (Miq.) J.-F.Leroy for all other species, from Africa, Asia and New Guinea, but excluding any Madagascan species (Leroy, 1980b). Following his reorganization of *Psilanthus*, Leroy (1981) placed several species of *Coffea* (*C. benghalensis* B.Heyne ex Schult., *C. cochinchinensis* Pierre ex Pit., *C. floresiana* Boerl., *C. fragrans* Wall. ex Hook.f., *C. mabesae* (Elmer) J.-F. Leroy, *C. madurensis* Teijsm. & Binn. ex Koord., *C. melanocarpa* Welw. ex Hiern, *C. wightiana* Wall. ex Wight & Arn.) in *Psilanthus*, presumably as representatives of *Psilanthus* subgenus *Paracoffea*. However, Leroy overlooked *Coffea* subgenus *Afrocoffea* Moens (Moens, 1962), which predates and competes with the use of *Paracoffea* at subgeneric rank (Bridson, 1987; Davis, 2003). The placement of *Paracoffea* spp. in *Psilanthus* subgenus *Afrocoffea* has since been upheld (e.g. Bridson, 1987, 1988a, b; Sivarajan, Biju & Mathew, 1992; Stoffelen, 1998; Davis, 2003). *Paracoffea brassii* J.-F.Leroy (Leroy, 1968a) was added to *Psilanthus* (subgenus *Afrocoffea*) by Davis (2003).

The morphological description of *Paracoffea*, and hence *Psilanthus* subgenus *Paracoffea*, as given Leroy (1967b: 1044) is as follows, with characters for *Coffea* in parentheses, redundant or identical characters removed and exceptions in square brackets: bushy shrubs (vs. shrubs, well-branched shrubs, trees); growth habit mixed, monopodial and sympodial, rarely monopodial [only *Paracoffea melanocarpa*] (vs. exclusively monopodial); generally deciduous but in Africa sometimes semi-persistent or persistent (vs. leaves generally persistent); leaves thin (vs. leaves thick), often with hairs well developed, sometimes glabrous (vs. glabrous); inflorescences generally terminal, or terminal and axillary, or axillary [only *P. melanocarpa*] (vs. inflorescences exclusively axillary, sometimes subterminal); lacking calyculi [except *P. melanocarpa*] (vs. one or many calyculi); corolla tube long (vs. corolla tube relatively short); anthers included and sessile [except Madagascan species], generally supramedifixed or medifixed (vs. exserted, submedifixed); style included [except Madagascan species] (vs. style exserted); fruits didymous sometimes winged (vs. not or slightly didymous, not winged); tegument of seed with dorsal vascularization (vs. with or without dorsal vascularization); pollen tri- or tetracolporate or tetracolporate (vs. generally tricolporate), sexine clavulate or clavulate–reticulate, sometimes very thick (vs. sexine baculate). Leroy (1967a, c) used the term 'epicalice', epicalyx in English, and Chevalier (1942) used (connate) bracteoles, but the term now employed is calyculus (singular)/calyculi (plural) (Davis *et al*., 2005). A calyculus is a tubular or cup-like structure subtending the flowers and resembling a calyx. It is formed from reduced stipules and leaves (Davis *et al*., 2005).

Based on all the characters given by Leroy (1967a, b, c, 1980a, b, 1981), Bridson (1988b) summarized the key differences between *Psilanthus* and *Coffea* as: anthers included, filaments absent or very short (vs. exserted, filaments long); style short, included (vs. long, exserted); corolla tube always longer than lobes (vs. corolla tube usually about the same length as the lobes); inflorescences (and flowers) terminal on reduced shoots or some species both terminal and axillary [vs. inflorescences (flowers) axillary or less often terminal on reduced shoots]. According to Bridson (1987, 1988a), the differences between subgenus *Psilanthus* (*P. mannii* and *P. sapinii*) and subgenus *Afrocoffea* (all other *Psilanthus* spp.) were: evergreen habit, with a sympodial growth pattern (vs. mostly deciduous, mostly with a monopodial growth pattern), and large, accrescent calyx lobes (vs. small, non-accrescent calyx lobes). Bridson (1982) added two new African species of *Psilanthus*, *P. leroyi* Bridson and *P. semsei* Bridson, and made one new combination for an African *Coffea* species: *P. lebrunianus* (Germ. & Kesler) J.-F.Leroy ex Bridson (Bridson, 1987). The most recent *Psilanthus* spp. were Indian taxa described by Sivarajan *et al*. (1992), *P. bababudanii* Sivar., Biju & P.Mathew, *P. bridsoniae* Sivar., Biju & P.Mathew and *P. malabaricus* Sivar., Biju & P.Mathew, although these species were later placed into the synonymy of *P. benghalensis* (B.Heyne ex Schult.) J.-F.Leroy, *P. wightianus* (Wall. ex Wight & Arn.) J.-F.Leroy and *P. fragrans* (Wall. ex Hook.f.) J.-F.Leroy, respectively, by Deb (2002).

Morphological assessments

A morphological reassessment of *Coffea* and *Psilanthus* by Davis *et al*. (2005) largely supported the findings of Bridson (1988a, b), in that *Psilanthus* can be separated from *Coffea* on the basis of five morphological characters (see above for characters in *Coffea*): (1) absent or very short (< 0.5 mm long) filaments [except *P. melanocarpus*; anthers 0.5–1.0 mm long]; (2) supramedifixed anthers [except *P. melanocarpus*; submedifixed]; (3) included or just emergent anthers; (4) short styles; (5) and mean number of pollen apertures (after Stoffelen, Robbrecht & Smets, 1997). Davis *et al*. (2005) argued that all *Psilanthus* and *Coffea* spp. possess calyculi, one of the key characters used in the characterization of *Psilanthus* by Leroy (1967b, 1972a, b). Davis *et al*. (2005) made the observation that, although calyculi can be highly modified, especially in *Coffea* subgenus *Baracoffea* (J.-F.Leroy) J.-F.Leroy (currently known as the '*Baracoffea* alliance'; Davis & Rakotonasolo, 2008), the basic structure is remarkably consistent across the two genera. In particular, the foliar leaves subtending the flowers in the *Baracoffea* alliance and in many *Psilanthus* are in fact modified (enlarged) foliar lobes of the calyculus. Davis *et al*. (2007) went even further, proposing that calyculi are present in all 11 genera of Coffeeae DC., and that the presence of theses structures is one of the key characters of the tribe. Davis *et al*. (2005) also showed that two other key characters used for the characterization of *Psilanthus* by Leroy (1967b, 1972a, b) were also found in *Coffea*. Firstly, the sympodial growth pattern and terminal inflorescence position (the latter influences the former) of *Psilanthus* is also found in the *Baracoffea* alliance and *C. rhamnifolia* (Chiov.) Bridson. Secondly, although the corolla tube of *Psilanthus* is usually distinctly long–tubular (always much longer than the corolla lobes), and in most *Coffea* it is short–tubular (shorter to slightly longer than the corolla lobes), in the *Baracoffea* alliance the corolla tubes are of a similar length to those in *Psilanthus*. Davis *et al*. (2005) discussed the possibility that anther appendages could represent a morphological difference between *Coffea* and *Psilanthus*, as some *Psilanthus* spp. possess sterile appendages at the apex of the filaments (Bridson, 1982: fig. 13e) and this character is lacking in *Coffea*. These appendages are usually quite short (e.g. *c*. 1 mm long or less), and either pointed or obtuse at the apex. However, Davis *et al*. (2005) found that sterile anther appendages were absent in at least three *Psilanthus* spp.: *P. leroyi*, *P. melanocarpus* and *P. travancorensis* (Wight & Arn.) J.-F.Leroy.

Davis *et al*. (2005) reiterated the anomalous position of *P. melanocarpus* within *Psilanthus*: it has submedifixed anthers (like *Coffea*) and anther filaments 0.5–1.0 mm long (vs. 0.5 mm in *Psilanthus*; 2 mm or longer in *Coffea*). Furthermore, the evergreen habit and axillary infloresences of *P. melanocarpus* resemble *Psilanthus* subgenus *Psilanthus* (i.e. *P. mannii* and *P. sapinii*), even though it clearly lacks the accrescent calyx lobes and ribbed fruits of this subgenus. Davis *et al*. (2005) concluded that: 'Our data infer that *P. melanocarpus* should be removed from *Psilanthus* subgenus *Afrocoffea*, but we cannot say where this taxon should be placed within the "core Coffeeae" '. Earlier, Andreasen & Bremer (1996) stated that: '*P. melanocarpus* should be placed either in a genus of its own or in *Coffea*, rather than be included in *Psilanthus*. Our morphological data, however, do not support the placement of *P. melanocarpus* within *Coffea*'.

Maurin *et al*. (2007) elaborated on the morphological discussion of Davis *et al*. (2005), and in particular with reference to their molecular analysis, which showed that *Coffea* subgenus *Baracoffea* (i.e. the *Baracoffea* alliance; Davis & Rakotonasolo, 2008) and *C. rhamnifolia* are nested within *Coffea* subgenus *Coffea*. The *Baracoffea* alliance is a group of nine species from the western, seasonally dry forests of Madagascar, and *C. rhamnifolia* is from the dry shrublands of north-east Kenya and south-east Somalia (Davis *et al*., 2006; Davis & Rakotonasolo, 2008). These taxa share many of the characters of *Psilanthus* subgenus *Afrocoffea* (Leroy, 1961; Davis *et al*., 2005), particularly deciduousness, axillary and terminal inflorescences, the presence of an indumentum (leaves and corolla) and long corolla tubes (only the *Baracoffea* alliance). These results effectively reduced the morphological differences between *Coffea* and *Psilanthus*. Maurin *et al*. (2007) did not sample *P. melanocarpus* but argued that if it were placed with either *Coffea* or *Psilanthus*, the differences between the genera would be minimal. As a species of *Psilanthus*, only two characters would separate *Psilanthus* and *Coffea*: (1) short (fully within corolla tube) vs. long (emergent) style; (2) mostly or fully included anthers vs. partially emergent or fully emergent anthers. If *P. melanocarpus* were nested within *Coffea*, then only one character would separate *Psilanthus* and *Coffea*: absent or very short (0.5 mm long) filaments vs. longer (0.5–2.0 mm, or longer) filaments. Clearly, these differences are not substantial.

The number of pollen apertures (Leroy, 1967b; Lobreau-Callen & Leroy, 1980; Chinnappa & Warner, 1981; Stoffelen *et al*., 1997) has been used as additional evidence to separate *Coffea* and *Psilanthus*. However, considerable polymorphism is evident and there is overlap in the number of apertures and sexine ornamentation between the two genera and between their subgenera (Stoffelen *et al*., 1997; Davis *et al*., 2005).

MOLECULAR PHYLOGENETIC DATA

Lashermes *et al*. (1997) used the internal transcribed spacer (ITS) region (ITS2) to examine relationships between 37 accessions of *Coffea* and three accessions of *Psilanthus*. Their study indicated limited sequence variation between the two genera. In some of their analyses, *P. mannii* and *P. ebracteolatus* were placed sister to a clade of east African *Coffea* spp.; the Indian species, *P. travancorensis*, was nested within a clade of Madagascan species. Cros *et al*. (1998) examined 23 *Coffea* taxa and two *Psilanthus* spp. in their study, using plastid sequences from the *trnL–trnF* intergenic spacer (IGS). They also detected low levels of sequence variation between *Coffea* and *Psilanthus* and stated that *P. mannii* and *P. ebracteolatus* do not appear to be closely related. Lashermes *et al*. (1997) and Cros *et al*. (1998) concluded that the division of *Coffea* and *Psilanthus* into two genera was unsupportable. Maurin *et al*. (2007) sampled 84 species (86 accessions) of *Coffea* and seven species of *Psilanthus* (82% and 35% of the total species diversity, respectively) using sequence data from four plastid regions (*trnL–F* intron, *trnL–F* IGS, *rpl16* intron and *accD–psa1* IGS) and the ITS region (ITS1/5.8S/ITS2). Their combined plastid analysis shows that African *Psilanthus* (*P. ebracteolatus*, *P. mannii*, *P. sapinii*, *P. semsei*, *P.* sp. A) are sister to *Coffea* spp. from the Lower Guinea/Congolian region [BP (bootstrap percentage value; Felsenstein, 1985) 53; *b* (Bremer support value/decay value; Bremer, 1988, 1994; Källersjö *et al*., 1992) = 1]; African *Psilanthus* (BP 85; $b = 2$) and Indian *Psilanthus* (*P. bridsoniae*, *P. travancorensis*) (BP 100; $b = 7$) are both well supported, although the latter was unresolved at the base of the tree. The ITS analyses provides less information on relationships for *Psilanthus* spp.: the two species of Indian *Psilanthus* are well supported as a clade (BP 93; $b = 4$), but the relationships for the other species are unresolved. Their combined plastid–ITS analysis shows that Indian *Psilanthus* (BP 100; $b = 12$) and African *Psilanthus* (BP 96; $b = 5$) form well-supported clades. *Psilanthus* subgenus *Psilanthus* (*P. mannii*, *P. sapinii*) was well supported (BP 99; $b = 5$), but the monophyly of *Psilanthus* subgenus *Afrocoffea* was not substantiated. *Coffea rhamnifolia* was placed with the two species of Indian *Psilanthus*, but this relationship was weakly supported (BP 57; $b = 1$). As in the combined plastid analysis and ITS analysis, the relationship between *Coffea* and *Psilanthus* was largely unresolved because of low levels of sequence divergence. Maurin *et al*. (2007) concluded that: 'The robust morphological (Robbrecht & Puff, 1986; Davis *et al*., 2005) and molecular support for *Coffea* plus *Psilanthus* (Davis *et al*., 2007), low sequence diversity between these genera (Davis *et al*., 2007, fig. 4) and indications of paraphyly (Davis *et al*., 2007, figs 2 and 4), may be taken as evidence for accepting *Coffea* and *Psilanthus* as a single genus (Lashermes *et al*., 1997; Cros *et al*., 1998). However, it is believed that further molecular data are needed to resolve fully the relationship between *Coffea* and *Psilanthus*, and in particular sequence data are required for *P. melanocarpus* and other species of *Psilanthus*'.

In an appraisal of tribe Coffeeae, Davis *et al*. (2007) concluded that *Coffea* and *Psilanthus* formed a well-supported clade, based on combined molecular data (BP 100, $b = 9$) and combined molecular–morphological data (BP 100, $b = 13$), which was positioned in a sister relationship relative to the rest of the tribe. Morphologically, the *Coffea* and *Psilanthus* clade was supported by the apparent loss of secondary pollen presentation and the presence of a hard (horny/crustaceous) endocarp, seeds with a deep ventral groove and a seed coat consisting of crushed endotestal cells and more or less isolated fibres ('coffee bean' morphology).

The study of the genera of Coffeeae by Tosh *et al*. (2009) supported many of the findings of Davis *et al*. (2007), but provided a much clearer indication of intergeneric sequence divergence within the tribe. Tosh *et al*. (2009) showed that well-established, easily circumscribed genera of Coffeeae have substantially longer branch lengths supporting the genera relative to the branch lengths within these genera. The only obvious exceptions are the clades *Argocoffeopsis* Lebrun + *Calycosiphonia* Pierre ex Robbr. (BP 99, branch length $(bl) = 15$) and *Coffea* + *Psilanthus* (BP 100, $bl = 17$). Like *Coffea* and *Psilanthus*, the generic delimitation of *Argocoffeopsis* and *Calycosiphonia* is problematic and it is likely that these genera need to be combined as a single entity (Davis & Sonké, 2008).

Recently, Anthony *et al*. (2010) used plastid sequences from *trnL–F*, *trnT–L* and *atpB–rbcL* IGS from 24 *Coffea* taxa and two *Psilanthus* spp. (*P. mannii* and *P. ebracteolatus*), but were unable to offer any new insights because of low levels of sequence variation.

Hybridization and cytogenetic studies

Couturon, Lashermes & Charrier (1998) produced intergeneric hybrids between *C. arabica* L. ($2n = 44$) and tetraploid genotypes of *P. ebracteolatus* ($2n = 22$). Forty-one plants were obtained, with nine plants surviving after 5 months in a nursery. Hybrid status was confirmed by means of cytological, molecular and morphological analysis. Couturon *et al*. (1998) posited that the mean production of two surviving hybrids per 100 pollinated flowers, and their fertility, were comparable with those reported for intrageneric crosses between *Coffea* spp. Both the capacity of *C. arabica* to hybridize with *P. ebracteolatus* and the fertility of the resultant hybrids appear high enough to envisage intergeneric gene transfer from *P. ebracteolatus* into *C. arabica*. Even though the *P. ebracteolatus–C. arabica* hybrid was made under laboratory conditions, with isolating barriers overcome by chemical and physical manipulation, Couturon *et al*. (1998) argued that the successful production of the hybrids demonstrates that intergeneric hybridization is not strongly affected by genome incompatibility and that their results did not support the separation of *Coffea* and *Psilanthus* at the generic level.

Cytogenetic studies of *Coffea* (*C. brevipes* Hiern, *C. racemosa* Lour.) and *Psilanthus* [*P. ebracteolatus*, *P. benghalensis* and *P. travancorensis*] were undertaken by Lombello & Pinto-Maglio (2003, 2004), using chromomycin A3/4′,6-diamidino-2-phenylindole (CMA/DAPI) and fluorescence *in situ* hybridization (FISH) cytogenetic markers. Their analysis enabled karyological characterization of these species, but their main finding was the remarkable cytological similarity between the species and the two genera.

The present study

In this contribution we use the molecular markers *trnL–F* intron, *trnL–F* intergenic spacer (IGS), *rpl16* intron and *accD–psa1* IGS) and the ITS region (ITS1/5.8S/ITS2), as used by Maurin *et al*. (2007), to further elucidate the relationships between *Coffea* and *Psilanthus*. We examine ten species of *Psilanthus* (16 samples; 50% of the total species diversity) from across its natural range. This represents respectable taxonomic and geographical coverage for *Psilanthus*, considering that the Asian and Australasian species (13 species in total; four sampled here) are likely to be monophyletic, given their close morphological similarity (Davis *et al*., 2005; Davis, 2010). An assumption of monophyly is supported by a molecular [random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers] study of four *Psilanthus* spp. from peninsula India, which showed statistically high values of genetic similarity (Kumar, Sudisha & Sreenath, 2008). Our study includes the morphologically incongruent *P. melanocarpus*, a species identified as critical for resolving the issue of delimitation and systematic placement of *Psilanthus* (Andreasen & Bremer, 1996, 2000; Davis *et al*., 2005; Maurin *et al*., 2007).

MATERIAL AND METHODS

Taxon sampling and plant material

We used a broad sampling of 45 *Coffea* spp., based on the study of Maurin *et al*. (2007), with all major lineages included. Notably, Madagascan species were reduced to nine species, as taxa from this island are largely unresolved (Maurin *et al*., 2007) based on the markers used. A further sample of *C. rhamnifolia* is included and we add *C. charrieriana* Stoff. & F.Anthony (Stoffelen *et al*., 2008), which was sampled by Anthony *et al*. (2010) but not by Maurin *et al*. (2007). For *Psilanthus*, we examine ten species (16 samples; 50% of the total species diversity). This sample includes a good representation of both subgenera: *Psilanthus* subgenus *Psilanthus* (all species) and *Psilanthus* subgenus *Afrocoffea* (eight species); and species from across the geographical range of the genus (Africa, India, Thailand and Australia). Four species not previously sampled in other molecular analyses are included here: *P. lebrunianus* (two samples), *P. brassii* (J.-F.Leroy) A.P.Davis (two samples), *P. merguensis* (Ridl.) J.-F.Leroy (one sample) and, crucially (see Introduction), *P. melanocarpus* (one sample). Further samples of *P. mannii* (two samples), and *P. ebracteolatus* (one sample) were newly sequenced. Three species of *Tricalysia* A.Rich. ex DC., a genus belonging to Coffeeae (Davis *et al*., 2007; Tosh *et al*., 2009), were used as the outgroup. Adding further members of Coffeeae and other Rubiaceae does not influence the ingroup topology, so further outgroups were not required; the systematic limits of Coffeae are well established (Davis *et al*., 2007; Tosh *et al*., 2009). Accession details and GenBank accession numbers for all samples are given in Table 1.

Taxonomic details and geographical range for all taxa (below generic rank) used or mentioned in this study follow the *World Rubiaceae Checklist* (Govaerts *et al*., 2011; http://www.kew.org/wcsp/rubiaceae); more specific information for *Coffea* is given in Davis *et al*. (2006). Details of the subgeneric classification of *Coffea* and *Psilanthus*, including synonymy, is given in Davis (2003) and Davis *et al*. (2005).

Map construction and use of phytogeographical areas

Figure 4 is based on the distribution of individual specimens for each species, as recorded in a *Coffea* specimen database (approximately 4100 records; A. Davis, S. Dawson and P. Stoffelen, unpubl. data) and Madagascan/Mascarene *Coffea* specimen database (approximately 1100 records; A. Davis and S. Dawson, unpubl. data). A species distribution map was plotted and then a generalized map was drawn by hand.

The terminology for area-based clades follows Maurin *et al*. (2007): Upper Guinea (UG) clade, Lower Guinea/Congolian (LG/C) clade, East–Central Africa (EC-Afr) clade, East Africa (EA) clade and Mascarenes (MAS) clade (see Fig. 3). We adopt the following abbreviations for the African/Indian Ocean clade (A/IO), and Indian Ocean clade (IO). The humid West and Central African forests are contained within the Guineo-Congolian Regional Centre of Endemism (White, 1983). Within this major region there are three subcentres of endemism for humid forest species: (1) Upper Guinea; (2) Lower Guinea; and (3) Congolian (White, 1979). For practical purposes, subcentres (2) and (3) are often put together as the Lower Guinean/Congolian region and this convention is followed here.

DNA extraction, amplification and sequencing

DNA extraction and PCR amplification and sequencing protocols followed Maurin *et al*. (2007).

Data matrix composition and phylogenetic analyses

DNA sequence assembly followed the methods of Maurin *et al*. (2007). Newly generated sequences were added to the matrices of Maurin *et al*. (2007) and aligned using MUSCLE (Edgar, 2004), with subsequent manual editing performed in MacClade (Maddison & Maddison, 2002).

Maximum parsimony was implemented to analyse: (1) *trnL–F*; (2) *rpl16*; (3) *accD–psa1*; (4) combined plastid data; (5) ITS and (6) combined sequence data, using PAUP*. In all analyses, gaps were treated as missing data and characters were equally weighted and unordered (Fitch, 1971). All data sets were analysed separately and examined by eye in order to identify topological conflict, i.e. moderate to strong support for placement of a taxon in different clades. Tree searches were conducted using 10 000 replicates of random taxon sequence addition, retaining ten trees at each step, with tree–bisection–reconnection (TBR) branch swapping, delayed transformation (DELTRAN) optimization, MulTrees in effect and saving a maximum of ten trees per replicate. Support for clades in all analyses was estimated using bootstrap analysis (Felsenstein, 1985), with 10 000 replicates of full heuristic search, simple sequence addition, TBR swapping, with MulTrees in effect and saving a maximum of ten trees per replicate. Bootstrap support values (BP) are described as well supported (85–100%), moderate (75–84%) or low/weak (50–74%).

Bayesian analyses were implemented in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001), using the University of Oslo Bioportal (http://www.bioportal.uio.no). DNA substitution models for each data partition were determined using Modeltest ver. 3.06 (Posada & Crandall, 1998) under the Akaike information criterion (AIC). For each data set, two independent Bayesian analyses, each with four chains and starting from random trees, were run for 5 000 000 generations, sampling trees every 1000 generations. TRACER ver. 1.4 (Rambaut & Drummond, 2007) was used to check that each parameter had an effective sample size (ESS) > 100. The initial 1250 trees (25%) from each Bayesian run were dis-

Table 1. Taxon accession data. Herbarium abbreviations after Holmgren *et al*. (1990). Where several internal transcribed spacer (ITS) types were isolated these are listed below with multiple GenBank accesion numbers

Taxon	Voucher (and duplcates)	Source	*accd-psa1*	*rpl16*	*trnL-F*	*ITS*
Coffea ambongensis J.-F.Leroy ex A.P.Davis & Rakotonas.	Davis 2509 (K)	Madagascar	DQ153419	DQ153668	DQ153786	DQ153539/DQ153540/DQ153541
Coffea anthonyi Stoff. & F. Anthony	IRD-Montpelier OE 53 (K)	Congo-Brazzaville	DQ153489	DQ153738	DQ153856	DQ153620
Coffea arabica L.	Jaufeerally-Fakim 29 (K)	Mascarenes (introduced)	DQ153478	DQ153727	DQ153845	DQ153609
Coffea bakossi Cheek & Bridson	Lane 361 (BR, K)	Cameroon	DQ153468	DQ153717	DQ153835	DQ153599
Coffea boinensis A.P.Davis & Rakotonas.	Davis 2502 (K)	Madagascar	DQ153408	DQ153657	DQ153775	DQ153528
Coffea brevipes Heirn	Maurin 8 (K)	Cameroon	DQ153460	DQ153709	DQ153827	DQ153591
Coffea bridsoniae A.P.Davis & Mvungi	Davis 2904 (BR, K)	Tanzania	DQ153455	DQ153704	DQ153822	DQ153584/DQ153585/DQ153586
Coffea campaniensis J.-F.Leroy*	Leroy 55 (K)	Mascarenes (Mauritius)	DQ153470	DQ153719	DQ153837	DQ153601
Coffea canephora Pierre ex A.Froehner	Maurin 21 (BR, K)	Cameroon (cultivated)	DQ153462	DQ153711	DQ153829	DQ153593
Coffea charrieriana Stoff. & F.Anthony	Anthony *s.n.* (BR)	Cameroon	FR828682	FR828690	FR832857	FR832869
Coffea congensis A.Froehner	Harris & Fay 1507 (BR, K, MO)	Cameroon	DQ153467	DQ153716	DQ153834	DQ153598
Coffea costatifructa Bridson	ORSTOM 08 117 (K)	Tanzania	DQ153473	DQ153722	DQ153840	DQ153604
Coffea eugenioides S.Moore	Harley 9332 (BR, K)	Tanzania	DQ153457	DQ153706	DQ153824	DQ153588
Coffea fadenii Bridson	Mvungi 9 (DSM, K)	Tanzania	DQ153446	DQ153695	DQ153813	DQ153574
Coffea grevei Drake ex A.Chev.	Davis 2566 (K)	Madagascar	DQ153414	DQ153663	DQ153781	DQ153534
Coffea heterocalyx Stoff.	Maurin 23 (BR, K)	Cameroon	DQ153463	DQ153712	DQ153830	DQ153594
Coffea humbertii J.-F.Leroy	Rakotonasolo 50 (BR, K,TAN)	Madagascar	DQ153437	DQ153686	DQ153804	DQ153565
Coffea humilis A.Chev.	Bamps 1967 (BR)	Ivory Coast	DQ153480	DQ153729	DQ153847	DQ153611
Coffea kapakata (A.Chev.) Bridson	Hepper & Maley 7723 (K)	Angola	DQ153465	DQ153714	DQ153832	DQ153596
Coffea kianjavatensis J.-F.Leroy	Davis 2313 (K)	Madagascar	DQ153482	DQ153731	DQ153849	DQ153613
Coffea kihansiensis A.P.Davis & Mvungi	Mvungi 21 (DSM, K)	Tanzania	DQ153454	DQ153703	DQ153821	DQ153583
Coffea kimbozensis Bridson	Mvungi 6 (DSM, K)	Tanzania	DQ153447	DQ153696	DQ153814	DQ153575
Coffea kivuensis Lebrun	Lebrun 5539 (BR)	DR Congo	DQ153481	DQ153730	DQ153848	DQ153612
Coffea pterocarpa A.P.Davis & Rakotonas.	Davis 2519 (K)	Madagascar	DQ153425	DQ153674	DQ153792	DQ153550
Coffea labatii A.P.Davis & Rakotonas.	Davis 3069 (K)	Madagascar	DQ153499	DQ153748	DQ153866	DQ153630
Coffea liberica var. *liberica* Bull. ex Hiern	Van Caekenbergh 442 (BR)	DR Congo	DQ153479	DQ153728	DQ153846	DQ153610
Coffea liberica var. *dewerei* (De Wild. & T.Durand) Lebrun	Hepper & Maley 7729 (BR, K, MO)	Central African Republic	DQ153472	DQ153721	DQ153839	DQ153603
Coffea lulandoensis Bridson	Mvungi 2 (DSM, K)	Tanzania	DQ153452	DQ153701	DQ153819	DQ153580
Coffea macrocarpa A.Rich.	Gueho 18555 (K)	Mascarenes (Mauritius)	DQ153471	DQ153720	DQ153838	DQ153602
Coffea mapiana Sonké, Nguembou & A.P.Davis	Sonké 3694 (K, YA)	Cameroon	DQ153509	DQ153758	DQ153876	DQ153640
Coffea mauritiana Lam.	Friedmann 1267 (K)	Mascarenes (Reunion)	DQ153469	DQ153718	DQ153836	DQ153600
Coffea mayombensis A.Chev.	Maurin 16 (K)	Cameroon	DQ153461	DQ153710	DQ153828	DQ153592
Coffea millotii J.-F.Leroy	Davis 2306 (K)	Madagascar	DQ153409	DQ153658	DQ153776	DQ153529
Coffea mongensis Bridson	Mvungi 11 (DSM, K)	Tanzania	DQ153448	DQ153697	DQ153815	DQ153576
Coffea montekupensis Stoff.	Davis 3010 (K)	Cameroon	DQ153459	DQ153708	DQ153826	DQ153590
Coffea mufindiensis Hutch. ex Bridson	Mvungi 19 (DSM, K)	Tanzania	DQ153449	DQ153698	DQ153816	DQ153577
Coffea myrtifolia (A.Rich. ex DC.) J.-F.Leroy	Jaufeerally-Fakim 022 (K)	Mascarenes (Mauritius)	DQ153477	DQ153726	DQ153844	DQ153608

Table 1. *Continued*

Taxon	Voucher (and duplcates)	Source	*accd-psa1*	*rpl16*	*trnL-F*	*ITS*
Coffea namorokensis A.P.Davis & Rakotonas.†	Davis 2537 (BR, K, P, MO, TAN, TEF)	Madagascar	DQ153429	DQ153678	DQ153796	DQ153556
Coffea pocsii Bridson	Mvungi 7 (DSM, K)	Tanzania	DQ153453	DQ153702	DQ153820	DQ153581/DQ153582
Coffea pseudozanguebariae Bridson	Mvungi 16 (DSM, K)	Tanzania	DQ153450	DQ153699	DQ153817	DQ153578
Coffea racemosa Lour.	Hepper & Maley 7717 (BR, K)	Mozambique	DQ153464	DQ153713	DQ153831	DQ153595
Coffea rhamnifolia (Chiov.) Bridson	Friis *et al*. 4908 (K, BR, P)	Somalia	DQ153458	DQ153707	DQ153825	DQ153589
Coffea rhamnifolia (Chiov.) Bridson	O'Brien 23 [+98] (K)	Somalia	FR828681	FR828691	FR832850	FR832860
Coffea schliebenii Bridson	Mbago 2256 (DSM)	Tanzania	DQ153456	DQ153705	DQ153823	DQ153587
Coffea sessiliflora Bridson	Mvungi 25 (DSM, K)	Tanzania	DQ153451	DQ153700	DQ153818	DQ153579
Coffea anthonyi Stoff. & F.Anthony	IRD-Montpelier OE 53 (K)	DR Congo	DQ153489	DQ153738	DQ153856	DQ153620
Coffea stenophylla G.Don	Hepper & Maley 7723 (K)	Ivory Coast	DQ153466	DQ153715	DQ153833	DQ153597
Coffea togoensis Jum. & H.Perrier	Hall & Abbins 43367 (K)	Togo	DQ153476	DQ153725	DQ153843	DQ153607
Coffea zanguebariae Lour.	Groenendijk 884 (K)	Mozambique	DQ153475	DQ153724	DQ153842	DQ153606
Psilanthus brassii (J.-F.Leroy) A.P.Davis	Nelder 3824 (BRI)	Australia	FR828683	FR828692	FR832853	FR832870
Psilanthus brassii (J.-F.Leroy) A.P.Davis	Fell & Mc Donald 4350 (BRI)	Australia	FR828684	FR828693	FR832854	FR832868
Psilanthus bridsoniae Sivar., Biju & P.Mathew	Biju & Sasi 44800 (K)	India	DQ153397	DQ153646	DQ153764	DQ153516
Psilanthus ebracteolatus Heirn	Davis 3008 (K)	Cameroon	DQ153392	DQ153641	DQ153759	DQ153510
Psilanthus ebracteolatus Heirn	(BR 197880061)	Upper Guinea	AM999392	AM999530	AM999471	FR832861 + FR832862
Psilanthus lebrunianus (Germ. & Kesler) Bridson	Breyne 2985 (BR)	DR Congo	FR828686	FR828695	FR832852	FR832864
Psilanthus lebrunianus (Germ. & Kesler) Bridson	Evrard 6322 (BR)	DR Congo	FR828685	FR828694	FR832851	FR832863
Psilanthus mannii Hook.f.	Maurin 1 (K)	Cameroon	DQ153393	DQ153642	DQ153760	DQ153511
Psilanthus mannii Hook.f.	Davis 3061 (K)	Cameroon	FR828687	FR828696	FR832856	FR832866
Psilanthus mannii Hook.f.	Harris 6958 (E)	Central African Republic	DQ180518	DQ180553	DQ180587	FR832865
Psilanthus melanocarpus (Welw. ex Hiern) J.-F.Leroy	Hallé 6469 (BR, K)	Angola	FR828688	FR828697	N/A	N/A
Psilanthus sapinii De Wild.	Sapin.*s.n.* (BR 0856914)	DR Congo	DQ153394	DQ153643	DQ153761	DQ153512
Psilanthus semsei Bridson	Kisera 1473 (K)	Tanzania	DQ153395	DQ153644	DQ153762	DQ153513
Psilanthus sp. 'A' (FTEA)	Luke 10197 (K)	Tanzania	DQ153399	DQ153648	DQ153766	DQ153518
Psilanthus merguensis (Ridl.) J.-F.Leroy	Gardner & Sidisunthorn 315 (K)	Thailand	FR828689	FR828698	FR832855	FR832867
Psilanthus travancorensis (Wight & Arn.) J.-F.Leroy	Biju *s.n.* (K)	India	DQ153398	DQ153647	DQ153765	DQ153517
Tricalysia cryptocalyx Baker	Davis 2173 [b] (BR, K)	Madagascar	DQ153400	DQ153649	DQ153767	DQ153519
Tricalysia perrieri Ranariv. & De Block subsp. *antsalovensis* Ranariv. & De Block	Davis 2325 (BR, K)	Madagascar	DQ180528	DQ180562	DQ180597	FR832858
Tricalysia dauphinensis Ranariv. & De Block	OKTAN 68 (K)	Madagascar	DQ180529	DQ180563	DQ180598	FR832859

Notes:
*Acceptance as a separate species pending. Considered a synonym of *C. mauritiana* by Davis *et al*. (2006).
†As *C. decaryana* J.-F. Leroy in Maurin *et al*. (2007).

carded as burn-in and the remainig trees were summarized in a 50% majority rule consensus tree using PAUP* ver. 4.0b10 to obtain posterior probabilities.

RESULTS

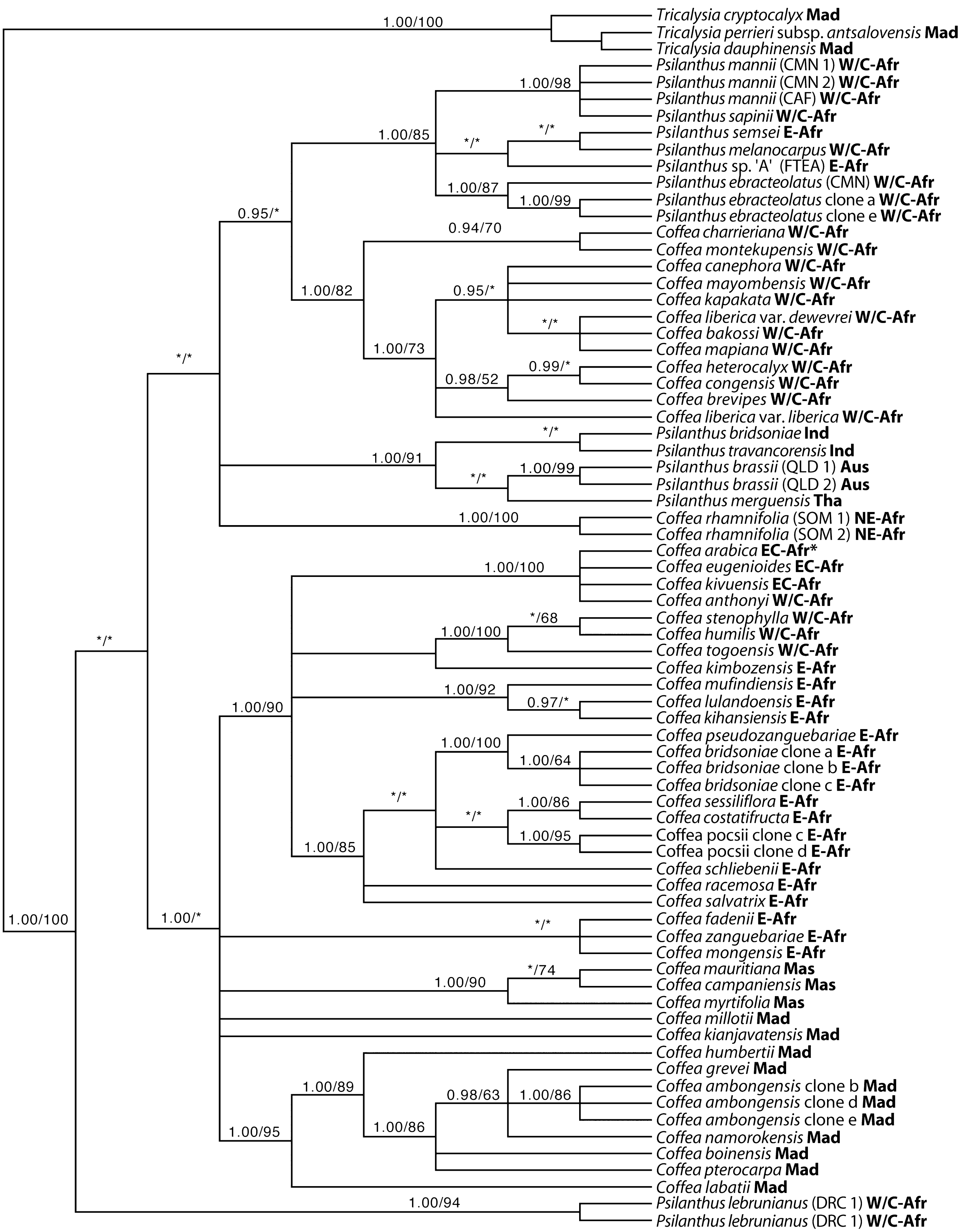
Single and combined plastid analyses

Individual plastid analyses, (1) *trnL–F*, (2) *rpl16* and (3) *accD–psa1*, were topologically consistent (negligible to zero incongruence) and so were combined and treated as a single analysis. The combined plastid data set contained a total of 2981 characters, of which 2719 were constant, 122 were variable but parsimony uninformative, and 140 were potentially parsimony informative. Using parsimony analysis, the combined data set produced 98 140 equally parsimonious trees. A 50% majority rule Bayesian consensus tree with bootstrap values is shown in Figure 1. The following clades (excluding species and two-species sister-pairs) are well supported [BP ≥ 85/Bayesian posterior probability (BPP) 1.0] under parsimony and Bayesian analysis (clade terminology follows Maurin *et al*., 2007; see above): *Coffea* and *Psilanthus* (all terminals in the analysis, less *Tricalysia*), BP 100/BPP 1.0; a group of African *Psilanthus* (*P. mannii*, *P. sapinii*, *P. semsei*, *P.* sp. 'A' (FTEA), *P. ebracteolatus* (both samples: Cameroon and Upper Guinea), *P. melanocarpus*), BP 85/BPP 1.0; *Psilanthus* subgenus *Psilanthus* (*P. mannii* and *P. sapinii*), BP 98/BPP 1.0; Asian and Australian *Psilanthus* species (*P. bridsonieae*, *P. travancorensis*, *P. merguensis*, *P. brassii*), BP 91/BPP 1.0; the LG/C clade [*C. charrieriana*, *C. montekupensis* Stoff., *C. canephora* Pierre ex A.Froehner, *C. heterocalyx* Stoff., *C. congensis* A.Froehner, *C. brevipes*, *C. mayombensis* A.Chev., *C. kapakata* (A.Chev.)Bridson, *C. liberica* Bull. ex Hiern (var. *liberica* and var. *dewevrei* (De Wild. & T.Durand) Lebrun), *C. bakossi* Cheek & Bridson, *C. mapiana* Sonké, Nguembou & A.P. Davis], BP 82/BPP 1.0; the EC-Afr clade (*C. anthonyi* Stoff. & F.Anthony, *C. eugenioides* S.Moore, *C. kivuensis* Lebrun), including *C. arabica*, BP 100/BPP 1.0; the UG clade (*C. stenophylla* G.Don, *C. humilis* A.Chev., *C. togoensis* A.Chev.), BP 100/BPP 1.0; a group of predominately lowland to mid-latitude East African coffee species (C. *pseudozanguebariae* Bridson, *C. bridsoniae* A.P.Davis & Mvungi, *C. sessiliflora* Bridson, *C. costatifructa* Bridson, *C. pocsii* Bridson, *C. schliebenii* Bridson, *C. racemosa*, *C. salvatrix* Swynn. & Philipson), BP 85/BPP 1.0; a group of species from the Udzungwa Mountains in Tanzania (*C. mufindiensis* Hutch. ex Bridson subsp. *mufindiensis*, *C. lulandoensis* Bridson, *C. kihansiensis* A.P.Davis & Mvungi) BP 92/BPP 1.0; the EC-Afr clade, the UG clade and East African species listed directly above form a larger clade, BP 90/BPP 1.0; the MAS clade (*C. mauritiana* Lam., *C. campaniensis* J.-F.Leroy, *C. macrocarpa* A.Rich., *C. myrtifolia* (A.Rich. ex DC.) J.-F.Leroy), BP 90/1.0; and the *Baracoffea* alliance (*C. labatii* A.P.Davis & Rakotonas., *C. humbertii* J.-F.Leroy, *C. grevei* Drake ex A.Chev., *C. ambongensis* A.P.Davis & Rakotonas., *C. boinensis* A.P.Davis & Rakotonas., *C. pterocarpa* A.P.Davis & Rakotonas., *C. namorokensis* A.P.Davis & Rakotonas.), BP 95/BPP 1.0, which has two other well-supported clades within the alliance.

There is no substantial support for the positions of the *Psilanthus* clades and *Psilanthus* spp. in relation to *Coffea*; in the strict consensus tree (not shown) they are unresolved at the base of the ingroup. *Psilanthus melanocarpus* is placed confidently within the African *Psilanthus* clade (see Fig. 1 and above), with two species of *Psilanthus* from the Udzungwa Mountains in Tanzania [*P. semsei*, *P.* sp. 'A' (FTEA)], although this relationship in not well supported (BP 55/BPP 0.86).

ITS analysis

The ITS matrix contained a total of 788 characters, of which 600 were constant, 64 were variable but parsimony uninformative and 124 were potentially parsimony informative. Using parsimony analysis, the combined data set produced 78 608 equally parsimonious trees. A 50% majority-rule Bayesian consensus tree with bootstrap values is shown in Figure 2. The following clades (excluding species and two-species sister-pairs) are well supported (BP ≥ 85/BPP 1.0) under parsimony and Bayesian analysis (clade terminology follows Maurin *et al*., 2007; see above): *Coffea* and *Psilanthus* (all terminals in the analysis, less *Tricalysia*), BP 100/BPP 1.0; Asian and Australian *Psilanthus* spp. (*P. bridsonieae*, *P. travancorensis*, *P. merguensis*, *P. brassii*), BP 83/BPP 1.0; the EC-Afr clade (*C. anthonyi*, *C. eugenioides*, *C. kivuensis*), BP 88/BPP 1.0; a LG/C group of species (*C. canephora*, *C. heterocalyx*, *C. congensis*, *C. brevipes*, *C. mayombensis*, *C. kapakata*), including *C. arabica*, BP 93/BPP 1.0, but lacking five of the other LG/C species [*C. liberica* (var. *liberica* and var. *dewevrei*), *C. charrieriana*, *C. montekupensis*, *C. bakossi*, *C. mapiana*] present in the LG/C clade based on the combined plastid data; a group of species from the Udzungwa Mountains in Tanzania (*C. mufindiensis* subsp. *mufindiensis*, *C. lulandoensis*, *C. kihansiensis*), BP 86/BPP 0.98; and the MAS clade (*C. mauritiana*, *C. myrtifolia*, *C. campaniensis*), BP 98/BPP 1.0. For comparison with the combined plastid analysis, weak to moderately supported groups include: the UG clade (*C. stenophylla*, *C. humilis*, *C. togoensis*), BP 56/BPP 0.91; the UG clade is sister the LG/C species group enumerated above, BP 80/BPP 1.0; and the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*/68
1.00/100
1.00/92
0.97/*
1.00/90
1.00/100
1.00/64
/
1.00/86
/
1.00/95
1.00/85
/
1.00/100
1.00/*
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*/74
1.00/90
1.00/89
0.98/63
1.00/86
1.00/86
1.00/95
1.00/94
Tricalysia cryptocalyx **Mad**
Tricalysia perrieri subsp. *antsalovensis* **Mad**
Tricalysia dauphinensis **Mad**
Psilanthus mannii (CMN 1) **W/C-Afr**
Psilanthus mannii (CMN 2) **W/C-Afr**
Psilanthus mannii (CAF) **W/C-Afr**
Psilanthus sapinii **W/C-Afr**
Psilanthus semsei **E-Afr**
Psilanthus melanocarpus **W/C-Afr**
Psilanthus sp. 'A' (FTEA) **E-Afr**
Psilanthus ebracteolatus (CMN) **W/C-Afr**
Psilanthus ebracteolatus clone a **W/C-Afr**
Psilanthus ebracteolatus clone e **W/C-Afr**
Coffea charrieriana **W/C-Afr**
Coffea montekupensis **W/C-Afr**
Coffea canephora **W/C-Afr**
Coffea mayombensis **W/C-Afr**
Coffea kapakata **W/C-Afr**
Coffea liberica var. *dewevrei* **W/C-Afr**
Coffea bakossi **W/C-Afr**
Coffea mapiana **W/C-Afr**
Coffea heterocalyx **W/C-Afr**
Coffea congensis **W/C-Afr**
Coffea brevipes **W/C-Afr**
Coffea liberica var. *liberica* **W/C-Afr**
Psilanthus bridsoniae **Ind**
Psilanthus travancorensis **Ind**
Psilanthus brassii (QLD 1) **Aus**
Psilanthus brassii (QLD 2) **Aus**
Psilanthus merguensis **Tha**
Coffea rhamnifolia (SOM 1) **NE-Afr**
Coffea rhamnifolia (SOM 2) **NE-Afr**
Coffea arabica **EC-Afr***
Coffea eugenioides **EC-Afr**
Coffea kivuensis **EC-Afr**
Coffea anthonyi **W/C-Afr**
Coffea stenophylla **W/C-Afr**
Coffea humilis **W/C-Afr**
Coffea togoensis **W/C-Afr**
Coffea kimbozensis **E-Afr**
Coffea mufindiensis **E-Afr**
Coffea lulandoensis **E-Afr**
Coffea kihansiensis **E-Afr**
Coffea pseudozanguebariae **E-Afr**
Coffea bridsoniae clone a **E-Afr**
Coffea bridsoniae clone b **E-Afr**
Coffea bridsoniae clone c **E-Afr**
Coffea sessiliflora **E-Afr**
Coffea costatifructa **E-Afr**
Coffea pocsii clone c **E-Afr**
Coffea pocsii clone d **E-Afr**
Coffea schliebenii **E-Afr**
Coffea racemosa **E-Afr**
Coffea salvatrix **E-Afr**
Coffea fadenii **E-Afr**
Coffea zanguebariae **E-Afr**
Coffea mongensis **E-Afr**
Coffea mauritiana **Mas**
Coffea campaniensis **Mas**
Coffea myrtifolia **Mas**
Coffea millotii **Mad**
Coffea kianjavatensis **Mad**
Coffea humbertii **Mad**
Coffea grevei **Mad**
Coffea ambongensis clone b **Mad**
Coffea ambongensis clone d **Mad**
Coffea ambongensis clone e **Mad**
Coffea namorokensis **Mad**
Coffea boinensis **Mad**
Coffea pterocarpa **Mad**
Coffea labatii **Mad**
Psilanthus lebrunianus (DRC 1) **W/C-Afr**
Psilanthus lebrunianus (DRC 1) **W/C-Afr**

Figure 1. Plastid Bayesian majority rule consensus tree, based on 1500 trees. Bayesian posterior probabilities and bootstrap values > 50% are indicated above branches (BPP/BP); Asterisks denote Bayesian posterior probabilities < 0.95 and bootstrap values < 50%. See Table 1 for species authorities and provenance. Region and country abbreviations: Aus, Australia; CAF, Central African Republic; CMN, Cameroon; DRC, Democratic Republic of Congo; E-Afr, East Africa; EC-Afr, East–Central Africa; Ind, India; Mad, Madagascar; Mas, Mascarenes; NE-Afr, North-eastern Africa; QLD, Queensland (Australia); SOM, Somalia; Tha, Thailand; W/C-Afr, West and Central Africa (Guineo-Congolian Regional Centre of Endemism; White, 1983). Other abbreviation: FTEA (*Flora of Tropical East Africa*; Bridson, 1988a).

Baracoffea alliance (*C. humbertii*, *C. grevei*, *C. ambongensis*, *C. boinensis*, *C. namorokensis*, *C. labatii*), but without *C. pterocarpa*, BP 65/BPP 0.98.

There is no substantial support for the positions of the *Psilanthus* clades and *Psilanthus* species in relation to *Coffea*, although in the strict consensus tree (not shown) Asian and Australian *Psilanthus* spp. are sister to the rest of *Coffea* + *Psilanthus* (less *C. rhamnifolia* and *C. charrieriana*), and African *Psilanthus* spp. remain positioned within a clade of African *Coffea* species. Thus, there is evidence in the ITS analysis that *Coffea* is polyphyletic if *Psilanthus* is recognized as a separate genus. We could not produce a complete or usable sequence for *P. melanocarpus* and so this species was not included in the ITS analysis.

Combined total plastid–ITS analysis

The relationships retrieved in the combined plastid analysis vs. the ITS analysis are not in serious conflict, but there are two notable incongruencies. Firstly, *C. arabica*, which is known to be a hybrid between *C. canephora* and *C. eugenioides* (Lashermes *et al*., 1999; Maurin *et al*., 2007): in the ITS analysis it is sister to *C. canephora* (BP 68/BPP 1.0), within a clade of several LG/C species; and in the combined plastid analysis it is confidently placed (BP 100/BPP 1.0) in an unresolved position within the EC-Afr clade (*C. kivuensis*, *C. anthonyi*, *C. eugenioides*). *Coffea arabica* was removed from the combined total plastid–ITS analysis. The second obvious incongruence is the UG clade, which falls within a clade containing the EC-Afr clade and East African species in the combined plastid analysis (BP 90/BPP 1.0), but in contrast is retrieved as a sister group to several LG/C species in the ITS analysis (BP 80/BPP 1.0). As reported by Maurin *et al*. (2007), removal or retention of the UG clade in the combined total plastid–ITS analysis does not significantly influence the topology, and for this reason it was retained.

The total combined plastid–ITS analysis retrieved the following well-supported clades, as shown in Figure 3. The following clades (excluding species and two-species sister-pairs) are well supported (BP > 85/BPP 1.0) under parsimony and Bayesian analysis (clade terminology follows Maurin *et al*., 2007; see above): *Coffea* and *Psilanthus* (all terminals in the analysis, less *Tricalysia*), BP 100/BPP 1.0; a group of African *Psilanthus* spp. (*P. mannii*, *P. sapinii*, *P. melanocarpus*, *P. semsei*, *P.* sp. 'A' (FTEA), *P. ebracteolatus* (two samples), BP 82/BPP 1.0; *Psilanthus* subgenus *Psilanthus* (*P. mannii* and *P. sapinii*), BP 98/BPP 1.0; Asian and Australian *Psilanthus* species (*P. bridsonieae*, *P. travancorensis*, *P. merguensis*, *P. brassii*), BP 100/BPP 1.0; the LG/C clade (*C. charrieriana, C. canephora*, *C. heterocalyx*, *C. congensis*, *C. brevipes*, *C. mayombensis*, *C. kapakata*, *C. liberica* (var. *liberica* and var. *dewevrei*), *C. montekupensis*, *C. bakossi*, *C. mapiana*, BP 61/BPP 1.0 (without *C. charrieriana*, BP 76;BPP 1.0); within the LG/C clade, less *C. charrieriana*) there are two further well-supported clades: the *canephora* alliance (*C. canephora*, *C. heterocalyx*, *C. congensis*, *C. brevipes*, *C. kapakata, C. mayombensis*; BP 98/BPP 1.0) and a clade which we call the '*liberica* alliance' [*C. liberica* (var. *liberica* and var. *dewevrei*), *C. montekupensis*, *C. bakossi*, *C. mapiana*, BP 67/BPP 1.0]; the UG clade (*C. stenophylla*, *C. humilis*, *C. togoensis*), BP 100/BPP 1.0; the A/IO clade, BP <50; BPP 1.0; the EC-Afr clade (*C. eugenioides*, *C. kivuensis*, *C. anthonyi*), BP 100/BPP 1.0; a group of species from the Udzungwa Mountains in Tanzania (*C. mufindiensis* subsp. *mufindiensis*, *C. lulandoensis*, *C. kihansiensis*), BP 100/BPP 1.0; the EC-Afr clade and the UG clade are sister to two groups of lowland East African (EA) species: (1) *C. pseudozanguebariae*, *C. bridsoniae*, *C. schliebenii*, *C. salvatrix*, BP 79/BPP 0.72 and (2) *C. sessiliflora*, *C. costatifructa*, *C. pocsii*, *C. racemosa*, BP 97/BPP 1.0; the UG + EC-Afr + EA clade has support value of BP 64/BPP = 1.0; a group of three species from the Eastern Arc Mountains, referred to by Maurin *et al*. (2007), as the '*mongensis* alliance' (*C. fadenii* Bridson, *C. zanguebariae* Bridson, *C. mongensis* Bridson), BP 86/BPP 1.0; the MAS clade (*C. mauritiana*, *C. campaniensis*, *C. myrtifolia*), BP 100/BPP 1.0; the IO clade, BP 63/BPP 0.99; and the *Baracoffea* alliance (*C. labatii*, *C. humbertii*, *C. grevei*, *C. ambongensis*, *C. boinensis*, *C. pterocarpa*, *C. namorokensis*), BP 99/BPP 1.0.

In the strict consensus tree there is no resolution for the positions of the *Psilanthus* clades, although *C. rhamnifolia* (Somalia) is consistently retrieved as sister to the Asian and Australian *Psilanthus* clade (Figs 1, 3). *Psilanthus melanocarpus* is placed within

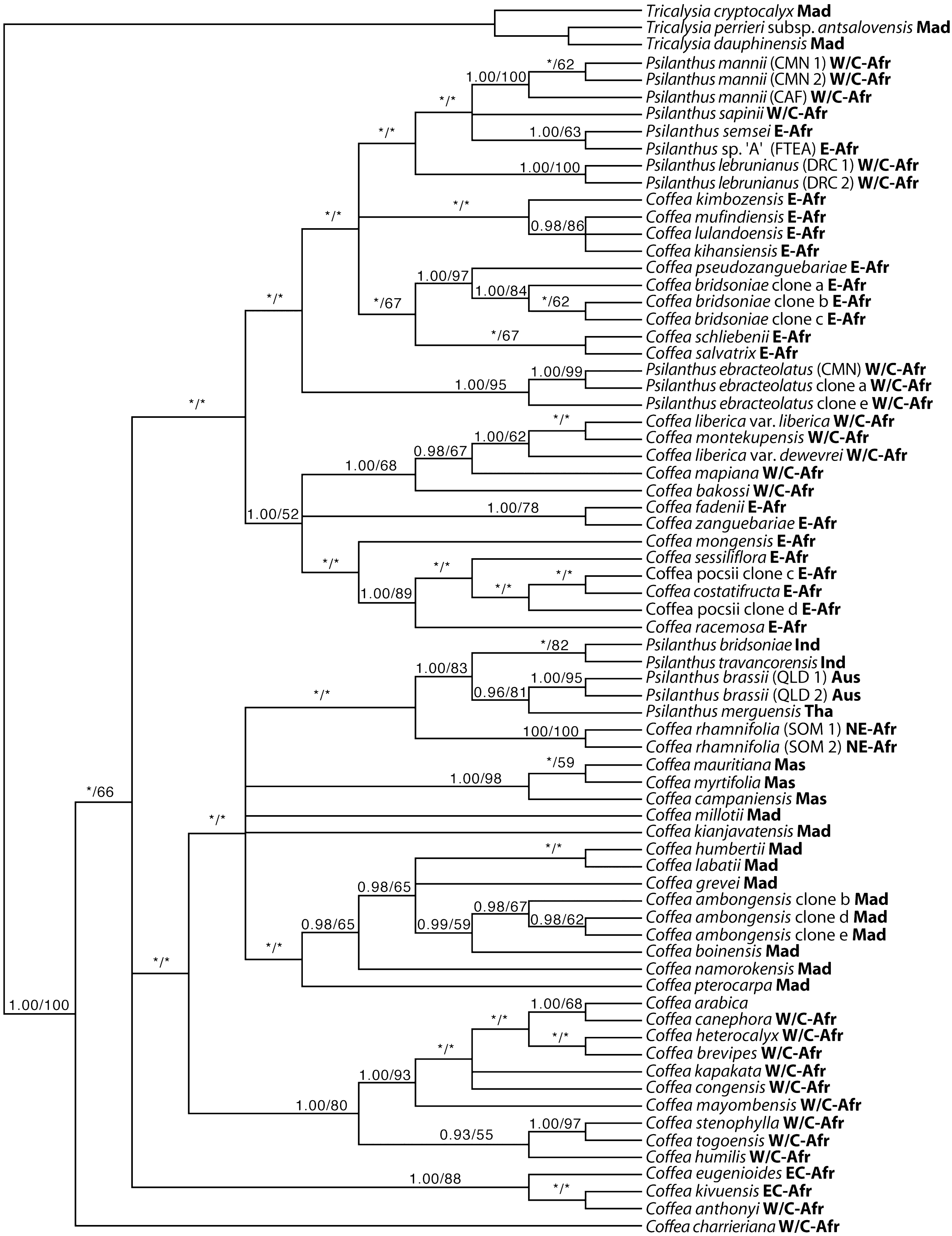
Tricalysia cryptocalyx Mad
Tricalysia perrieri subsp. antsalovensis Mad
Tricalysia dauphinensis Mad
*/62
1.00/100
Psilanthus mannii (CMN 1) W/C-Afr
Psilanthus mannii (CMN 2) W/C-Afr
/
Psilanthus mannii (CAF) W/C-Afr
Psilanthus sapinii W/C-Afr
/
1.00/63
Psilanthus semsei E-Afr
Psilanthus sp. 'A' (FTEA) E-Afr
1.00/100
Psilanthus lebrunianus (DRC 1) W/C-Afr
Psilanthus lebrunianus (DRC 2) W/C-Afr
/
/
Coffea kimbozensis E-Afr
0.98/86
Coffea mufindiensis E-Afr
Coffea lulandoensis E-Afr
Coffea kihansiensis E-Afr
1.00/97
Coffea pseudozanguebariae E-Afr
1.00/84
Coffea bridsoniae clone a E-Afr
/
*/67
*/62
Coffea bridsoniae clone b E-Afr
Coffea bridsoniae clone c E-Afr
*/67
Coffea schliebenii E-Afr
Coffea salvatrix E-Afr
1.00/99
Psilanthus ebracteolatus (CMN) W/C-Afr
1.00/95
Psilanthus ebracteolatus clone a W/C-Afr
/
Psilanthus ebracteolatus clone e W/C-Afr
/
Coffea liberica var. liberica W/C-Afr
1.00/62
Coffea montekupensis W/C-Afr
0.98/67
Coffea liberica var. dewevrei W/C-Afr
1.00/68
Coffea mapiana W/C-Afr
Coffea bakossi W/C-Afr
1.00/78
Coffea fadenii E-Afr
1.00/52
Coffea zanguebariae E-Afr
Coffea mongensis E-Afr
/
/
Coffea sessiliflora E-Afr
/
Coffea pocsii clone c E-Afr
/
Coffea costatifructa E-Afr
1.00/89
Coffea pocsii clone d E-Afr
Coffea racemosa E-Afr
*/82
Psilanthus bridsoniae Ind
1.00/83
Psilanthus travancorensis Ind
1.00/95
Psilanthus brassii (QLD 1) Aus
0.96/81
Psilanthus brassii (QLD 2) Aus
/
Psilanthus merguensis Tha
100/100
Coffea rhamnifolia (SOM 1) NE-Afr
Coffea rhamnifolia (SOM 2) NE-Afr
*/59
Coffea mauritiana Mas
1.00/98
Coffea myrtifolia Mas
*/66
Coffea campaniensis Mas
/
Coffea millotii Mad
Coffea kianjavatensis Mad
/
Coffea humbertii Mad
Coffea labatii Mad
0.98/65
Coffea grevei Mad
Coffea ambongensis clone b Mad
0.98/67
0.98/65
0.99/59
0.98/62
Coffea ambongensis clone d Mad
Coffea ambongensis clone e Mad
/
Coffea boinensis Mad
/
Coffea namorokensis Mad
Coffea pterocarpa Mad
1.00/100
1.00/68
Coffea arabica
/
Coffea canephora W/C-Afr
/
Coffea heterocalyx W/C-Afr
/
Coffea brevipes W/C-Afr
1.00/93
Coffea kapakata W/C-Afr
Coffea congensis W/C-Afr
1.00/80
Coffea mayombensis W/C-Afr
1.00/97
Coffea stenophylla W/C-Afr
0.93/55
Coffea togoensis W/C-Afr
Coffea humilis W/C-Afr
1.00/88
Coffea eugenioides EC-Afr
/
Coffea kivuensis EC-Afr
Coffea anthonyi W/C-Afr
Coffea charrieriana W/C-Afr

Figure 2. Internal transcribed spacer (ITS) Bayesian majority rule consensus tree, based on 1500 trees. Bayesian posterior probabilities and bootstrap values > 50% are indicated above branches (BPP/BP); Asterisks denote Bayesian posterior probabilities < 0.95 and bootstrap values < 50%. See Table 1 for species authorities and provenance. Region and country abbreviations: Aus, Australia; CAF, Central African Republic; CMN, Cameroon; DRC, Democratic Republic of Congo; E-Afr, East Africa; EC-Afr, East–Central Africa; Ind, India; Mad, Madagascar; Mas, Mascarenes; NE-Afr, North-eastern Africa; QLD, Queensland (Australia); SOM, Somalia; Tha, Thailand; W/C-Afr, West and Central Africa (Guineo-Congolian Regional Centre of Endemism; White, 1983). Other abbreviation: FTEA (*Flora of Tropical East Africa*; Bridson, 1988a).

the African *Psilanthus* clade, within a clade (BP < 50/ BPP 0.99) containing two *Psilanthus* spp. from the Udzungwa Mountains in Tanzania [*P. semsei*, *P*. sp. 'A' (FTEA)].

DISCUSSION

Evidence supporting the inclusion of *Psilanthus* species within *Coffea*

To date, the most comprehensive molecular study into the relationships between *Coffea* and *Psilanthus* has been undertaken by Maurin *et al*. (2007). They examined 84 species of *Coffea* and seven species of *Psilanthus* (including *P*. sp. 'A' (FTEA). In the analyses presented here, we enlarge the sampling of *Psilanthus* with the addition of four newly sequenced species [*P. brassii* (Australia), *P. melanocarpus* (Angola), *P. lebrunianus* (West and Central Africa) and *P. merguensis* (Thailand)], in combination with a broad representation of *Coffea* spp. from the study of Maurin *et al*. (2007), and an additional species of *Coffea* (*C. charrieriana*). These additional species of *Psilanthus* provide a more comprehensive taxonomic and geographical sampling of the genus and include the problematic *P. melanocarpus*, a species that has been difficult to place based on morphological grounds (Leroy, 1980a, b; Andreasen & Bremer, 1996; Davis *et al*., 2005, 2007; Maurin *et al*., 2007).

Our results confirm previous studies (Lashermes *et al*., 1997; Cros *et al*., 1998; Davis *et al*., 2007; Maurin *et al*., 2007) showing that the level of sequence divergence between *Coffea* and *Psilanthus* spp. is negligible, particularly given the much longer branch lengths separating other genera of tribe Coffeeae (Davis *et al*., 2007; Tosh *et al*., 2009). There are also indications that *Psilanthus* is biphyletic, as inferred by the ITS data (Fig. 2). The combined plastid data shows that the morphologically incongruous *P. melanocarpus* falls within the ingroup, as part of a clade of Afican *Psilanthus* spp. (Fig. 1). In contrast to other species of *Psilanthus*, *P. melanocarpus* has short filaments and sub-medifixed anthers, as in *Coffea*, but an included style, as in *Psilanthus*. Now that we have demonstrated that *P. melanocarpus* falls within a group of African *Psilanthus*, only one character separates *Coffea* from *Psilanthus*: a long, emergent vs. a short, included style. As a character for generic delimitation, this is insubstantial, particularly as *Coffea* and *Psilanthus* are morphologically similar and share several synapomorphies (Davis *et al*., 2005, 2007). According to Davis *et al*. (2007), *Coffea* and *Psilanthus* are supported by the apparent loss of secondary pollen presentation, the presence of a hard (horny/crustaceous) endocarp (pyrene), seeds (and endocarp) with a deep ventral groove and a seed coat consisting of crushed endotestal cells and more or less isolated fibres. The hard crustaceous endocarp of the pyrene and the ventral excavation of pyrene and seed, in combination with shape and size, give the typical 'coffee bean' morphology of *Coffea* (including *Psilanthus* spp.). This synapomorphy is unambiguously unique in Coffeeae (Davis *et al*., 2007) and in Rubiaceae.

Given the above results, in combination with cytogenetic similarity (Lombello & Pinto-Maglio, 2003, 2004) and the ability to produce fertile intergeneric hybrids (Couturon *et al*., 1998), *Coffea* and *Psilanthus* should be treated as a single generic entity. The earliest published name is *Coffea* (Linnaeus, 1753), which predates *Psilanthus* (1873a) and has priority according to the *International Code of Botanical Nomenclature* (ICBN; McNeill *et al*., 2006). This decision is hardly controversial given the systematic evidence presented here, the convoluted taxonomic and systematic history of *Psilanthus* in relation to *Coffea* (see Introduction) and the fact that many workers already consider that coffee trees should belong to a single genus (e.g. Lashermes *et al*., 1997; Cros *et al*., 1998; Maurin *et al*., 2007). For many coffee researchers, this would make perfect sense, given that *Psilanthus* spp. have been used by local people and growers to make the beverage coffee (Cheney, 1925; Wellman, 1961; Burkill, 1997).

A new circumscription for *Coffea*: more species, an increase in geographical and ecological range and a broader morphological characterization

In line with the title of this contribution, the transfer of *Psilanthus* spp. to *Coffea* increases the number of species in that genus from 104 (Davis *et al*., 2006;

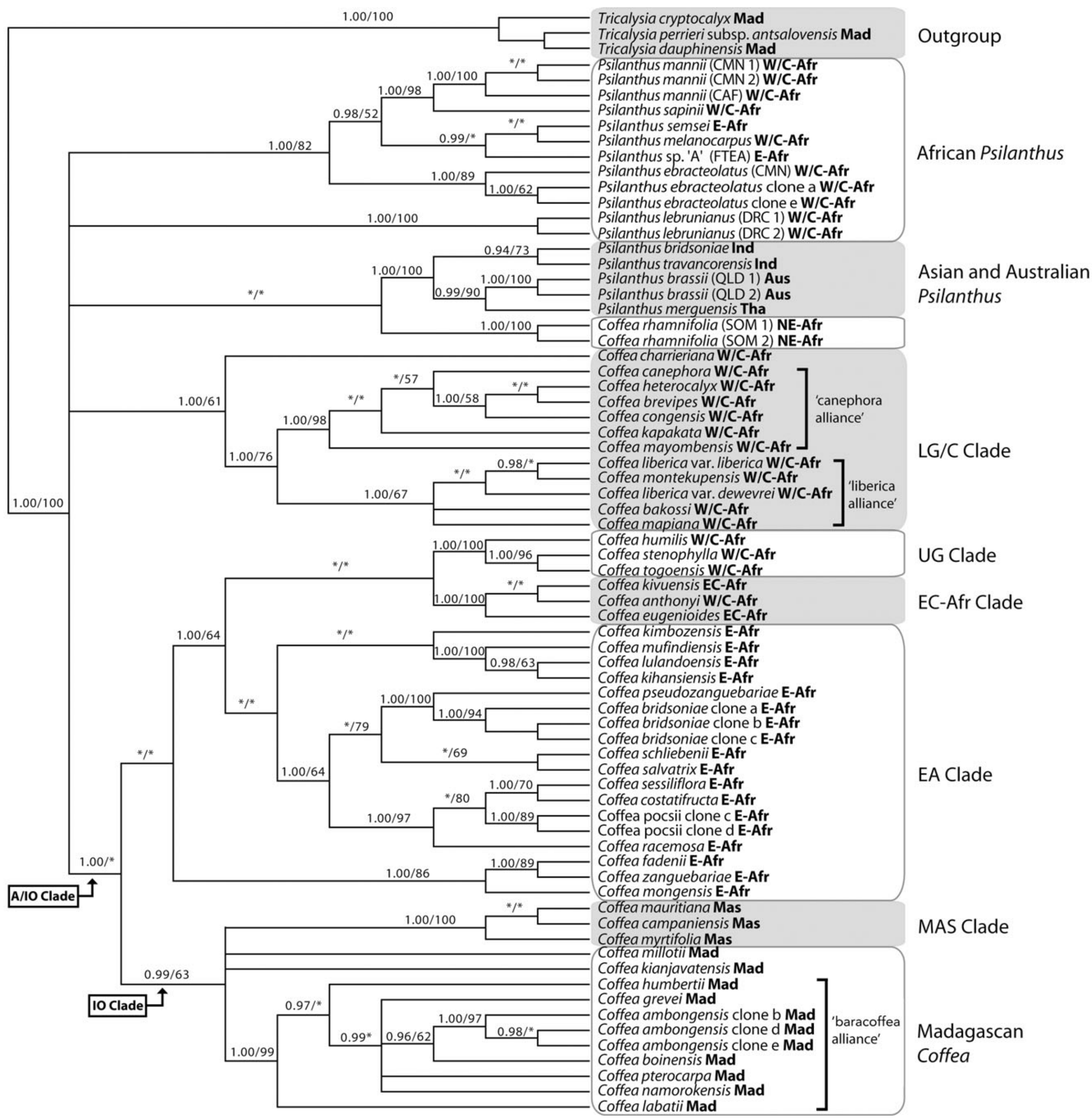

Figure 3. Combined plastid–internal transcribed spacer (ITS) Bayesian majority rule consensus tree, based on 1500 trees. Bayesian posterior probabilities and bootstrap values > 50% are indicated above branches (BPP/BP); Asterisks denote Bayesian posterior probabilities < 0.95 and bootstrap values < 50%. See Table 1 for species authorities and provenance. Clades: EA Clade, East African Clade; EC-Afr clade, East-Central African Clade; LG/C Clade, Lower Guinea/Congolian Clade; UG Clade, Upper Guinea Clade; A/IO Clade, Africa/Indian Ocean Clade; IO Clade, Indian Ocean Clade. MAS Clade, Mascarene Clade. Region and country abbreviations: Aus, Australia; CAF, Central African Republic; CMN, Cameroon; DRC, Democratic Republic of Congo; E-Afr, East Africa; EC-Afr, East-Central Africa; Ind, India; Mad, Madagascar; Mas, Mascarenes; NE-Afr, North-eastern Africa; QLD, Queensland (Australia); SOM, Somalia; Tha, Thailand; W/C-Afr, West and Central Africa (Guineo-Congolian Regional Centre of Endemism; White, 1983). Other abbreviation: FTEA (*Flora of Tropical East Africa*; Bridson, 1988a).

Davis, Rakotonasolo & De Block, 2010) to 124 species. The new combinations and new names necessary for the transfer of *Psilanthus* to *Coffea* have been made in anticipation of this contribution (Davis, 2010, 2011). Work in progress (A. Davis, pers. observ.) shows that, with the addition of new species from Africa and Madagascar, the number of *Coffea* spp. will soon reach 130.

The placement of *Psilanthus* spp. in *Coffea* considerably increases the geographical range of *Coffea*. Before the inclusion of *Psilanthus*, *Coffea* was restricted to tropical Africa, Madagascar, the Comoros and the Mascarenes. It now also occurs in southern Asia (Indian subcontinent), south tropical Asia (Cambodia, Myanmar, Thailand, Vietnam), south-eastern Asia (Java, Lesser Sunda Islands, Philippines, Papua New Guinea) and Australasia (Australia: Queensland). The ecological range of the genus is also extended, to include habitats previously not recorded for *Coffea*, including subtropical vegetation of higher latitudes (northern India, Nepal and Bhutan) and semi-deciduous microphyll vine forest (northern Queensland, Australia; Forster, 2004). A simplified distribution map for *Coffea* is presented in Figure 4.

The current morphological concept of *Coffea* (Bridson, 1988a, b, 2003; Stoffelen, 1998; Davis *et al*., 2005) also requires updating to include characters formerly restricted to *Psilanthus*: calyx lobes accrescent; anthers sessile or filaments short (0.5 mm long); anthers supramedifixed; anthers mostly or fully included; anthers with sterile appendages; style short (stigma lobes distinctly below anthers, often positioned at the base of the corolla tube); pollen apertures four or five. A new generic characterization of *Coffea*, plus an annotated enumeration of species formerly included in *Psilanthus*, following the format of Davis *et al*. (2006), is in preparation (A. Davis, P. Stoffelen & S. Dawson, unpubl. data). An overview of the currently accepted names of species formerly placed in *Psilanthus*, with their geographical distribution, is given in Appendix 1.

Our analyses show that the LG/C clade contains a group worthy of informal recognition, as the '*liberica* alliance', which, like the '*canephora* alliance', contains an assemblage of species that are closely related to a crop species (crop wild relatives: CWR), in this case *C. liberica* (see Fig. 3). In his treatment of *Coffea* spp. from the Congo, Lebrun (1941) recognized 'series Libericae', comprising a single species (*C. liberica*). Lebrun used morphological characteristics to distinguish 'series Libericae' from other groups of *Coffea* from the Lower Guninea/Congolian area. It would be worthwhile re-examining these morphological characters in order to assess their systematic and diagnostic value.

Given the apparent complicated evolutionary history of *Coffea* (including *Psilanthus*) and the lack of support for major groupings or previous classifications within the genus, it is not possible to propose a formal infrageneric classification at this time.

Implications for understanding the evolutionary history of *Coffea*

The inclusion of *Psilanthus* spp. in *Coffea* complicates our understanding of the evolutionary history of *Coffea*. Simplified hypotheses describing the evolutionary 'dispersal' of *Coffea* across and out of Africa (e.g. Leroy, 1982; Anthony *et al*., 2010, fig. 4) will now have to be reassessed. Firstly, our data show that the origin and diversification of *Coffea*, even within Africa, is more complicated than originally assumed (Maurin *et al*., 2007) and that there have been at least two separate dispersals out of Africa. The nested position of the Tanzanian *Psilanthus* spp., *P. semsei* and *P*. sp. 'A' (FTEA), within a West and Central African (Guineo-Congolian) clade and the incongruous position of the UG clade (i.e. in the plastid vs. ITS analyses) suggests interplay between West and Central Africa and East Africa on either side of the Albertine African Rift Valley. This can be explained either by dispersals across Africa or by some species having previously more extensive ranges (which was followed by fragmentation and reduction), or a mixture of both. Secondly, the position of the Asian *Psilanthus* clade (*P. bridsoniae*, *P. travancorensis, P. merguensis*, *P. brassii*) in our analyses suggests an origin in Africa (Figs 1–3), with a consistently retrieved but poorly supported sister relationship with *C. rhamnifolia* (Figs 2, 3). *Coffea rhamnifolia* is from dry, low altitude shrub lands in Somalia and north-east Kenya; Asian/Australasian *Psilanthus* spp. are from seasonal humid tropical or seasonal subtropical vegetation types, including deciduous forest types, in India, southern tropical Asia, south-east Asia, Papua New Guinea and Australia. This suggests an African dry biome as an ancestral refugia for *Coffea*, similar to those demonstrated by Schrire *et al*. (2009) in studies of the tribe Indigofereae Benth. (Fabaceae). In a recent study of *Livistona* R.Br. (Arecaceae), Crisp *et al*. (2010) show that *L. carinensis* (Chiov.) J.Dransf. & N.W.Uhl, from dry habitats in Somalia, Yemen and Djibouti, is sister to lineages in Asia and Australasia, mostly containing species from humid, tropical biomes, which is similar to the relationship retrieved for *C. rhamnifolia* and Asian/Australasian *Psilanthus*. White & Léonard (1991) clearly demonstrated strong phytochorial links between north-western Africa, Arabia and western India, which may have assisted the dispersal of *Coffea* from Africa to south-west Asia, although oceanic

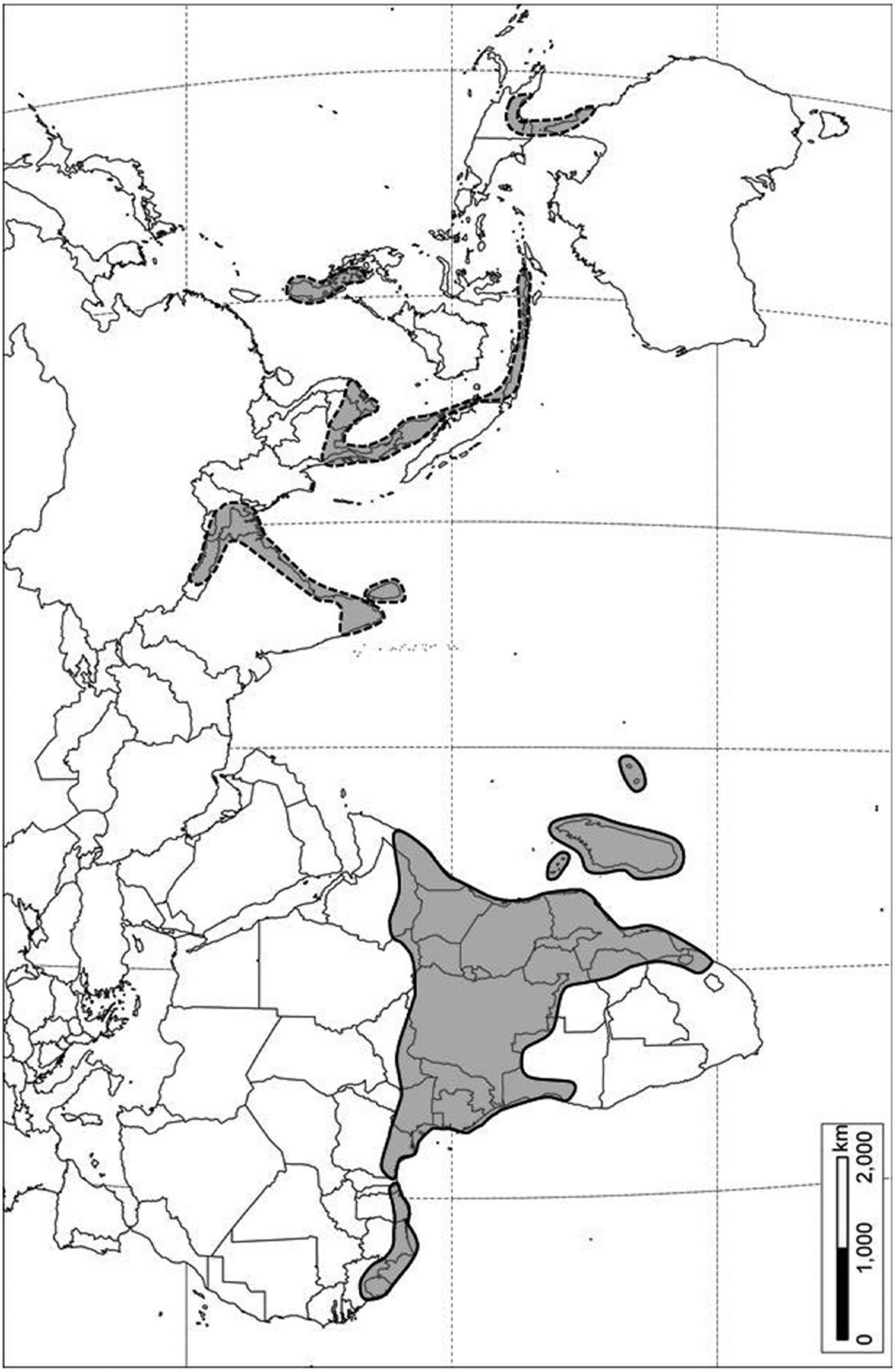

Figure 4. Generalized distribution map of *Coffea*. Dashed lines show new areas of distribution for *Coffea*, following the inclusion of Asian and Australasian *Psilanthus* spp.

dispersal from Africa to India is an equally plausible hypothesis with the present data at hand.

It is now clear that future phylogenetic study of *Coffea*, including investigation of early lineage origins and diversification, refugia theories and genus-wide phylogenetics, will have to sample species formerly included in *Psilanthus*. More inclusive studies are likely to alter pre-existing theories of relationships of *Coffea* spp. (e.g. Maurin *et al*., 2007), especially for Africa species. The challenge will now be to gather these extra samples and locate molecular markers (plastid and nuclear) that will provide further informative characters.

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APPENDIX 1

Generic synonymy* for *Coffea

COFFEA L., SP. PL. 172 (1753). TYPE: *COFFEA ARABICA* L.

Cafe Adans., *Fam. Pl.* **2:** 500 (1763).

Cafea Adans., *Fam. Pl.* **2:** 145 (1763).

Hexepta Raf., *Sylva Tellur.* 164 (1838).

Leiochilus Hook.f. in G.Bentham & J.D.Hooker, *Gen. Pl.* **2:** 116 (1873).

Psilanthus Hook.f., *Hooker's Icon. Pl.* **12:** t. 1129 (1873), **syn. nov.**

Pleurocoffea Baill., *Bull. Mens. Soc. Linn. Paris* **1:** 270 (1880).

Solenixora Baill., *Bull. Mens. Soc. Linn. Paris* **1:** 242 (1880).

Buseria T.Durand, *Index Gen. Phan.* 501 (1888).

Paolia Chiov., *Result. Sci.* Miss *Stefan.-Paoli Somal. Ital.* **1**: 93 (1916).

Psilanthopsis A.Chev., *Rev. Bot. Appl. Agric. Trop.* **19:** 403 (1939).

Nescidia A.Rich. ex DC., *Prodr.* **4:** 477 (Sept. 1830).

Cofeanthus A.Chev., *Cafeiers du Globe* **3:** 226 (1947).*

Paracoffea J.-F.Leroy, *J. Agric. Trop. Bot. Appl.* **14:** 276 (1967 publ. 1968).*

*Formerly synonyms of *Psilanthus*.

Number of species: 124.

Distrubtion: Tropical Old World: Africa, West Indian Ocean Islands (Madagascar, Comoros, Mascarenes), South Asia, south-east Asia, Papua New Guniea and Australia. TDWG: 22; 23; 24; 25; 26; 27; 29; 40; 41; 42; 43; 50. Figure 4.

Species of Coffea formerly included in Psilanthus

The accepted name of each species is given in bold, with the basionym or most recently replaced name in italics.

1. Coffea benghalensis B.Heyne ex Schult. in J.J.Roemer & J.A.Schultes, *Syst. Veg.* **5:** 200 (1819).

1a. var. **benghalensis**

Psilanthus benghalensis (B.Heyne ex Schult.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 252 (1981).

Distribution: – India, Nepal and Bhutan.

1b. var. **bababudanii** (Sivar., Biju & P.Mathew) A.P.Davis, *Phytotaxa* **10:** 42 (2010).

Psilanthus bababudanii Sivar., Biju & P.Mathew, *Bot. Bull. Acad. Sin., n.s.*, **33:** 212 (1992).

Distribution: – Western India.

2. Coffea brassii (J.-F.Leroy) A.P.Davis, *Phytotaxa* **10:** 42 (2010).

Psilanthus brassii (J.-F.Leroy) A.P.Davis, *Novon* **13:** 183 (2003).

Distribution: – Southern Papua New Guinea and north-eastern Australia (including Torres Strait Islands).

3. Coffea cochinchinensis Pierre ex Pit. in H.Lecomte, *Fl. Indo-Chine* **3:** 337 (1924).

Psilanthus cochinchinensis (Pierre ex Pit.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 256 (1981 publ. 1982).

Distribution: – Cambodia and Vietnam.

4. Coffea ebracteolata (Hiern) Brenan, *Kew Bull.* **8:** 115 (1953).

Psilanthus ebracteolatus Hiern in D.Oliver & auct. suc. (eds.), *Fl. Trop. Afr.* **3:** 186 (1877).

Distribution: – West Tropical Africa.

5. Coffea floresiana Boerl., *Handl. Fl. Ned. Ind.* **11:** 136 (1891).

Psilanthus floresianus (Boerl.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 256 (1981 publ. 1982).

Distribution: – Lesser Sunda Islands.

6. Coffea fragrans Wall. ex Hook.f., *Fl. Brit. India* **3:** 154 (1880).

Psilanthus fragrans (Wall. ex Hook.f.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 256 (1981 publ. 1982).

Distribution: – Bangladesh; reported to be in cultivation in India (Sivarajan *et al*., 1992).

7. Coffea horsfieldiana Miq., *Fl. Ned. Ind.* **2:** 308 (1857).

Psilanthus horsfieldianus (Miq.) J.-F.Leroy, *Ass. Sci. Internat. Café Colloque* **9:** 482 (1980).

Distribution: – Java.

8. Coffea malabarica (Sivar., Biju & P.Mathew) A.P.Davis, *Phytotaxa* **10:** 42 (2010).

Psilanthus malabaricus Sivar., Biju & P.Mathew *Bot. Bull. Acad. Sin., n.s.*, **33:** 219 (1992).

Distribution: – Western India.

9. Coffea lebruniana Germ. & Kesler, *Bull. Jard. Bot. État* **25:** 405 (1955).

Psilanthus lebrunianus (Germ. & Kesler) J.-F.Leroy ex Bridson, *Kew Bull.* **42:** 456 (1987).

Distribution: – West and Central Tropical Africa.

10. Coffea madurensis Teijsm. & Binn. ex Koord., *Teysmannia* **11:** 30 (1900).

Psilanthus madurensis (Teijsm. & Binn. ex Koord.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 256 (1981 publ. 1982).

Distribution: – Java (Madura Islands).

11. Coffea mabesae (Elmer) J.-F.Leroy, *J. Agric. Trop. Bot. Appl.* **9:** 419 (1962).

Psilanthus mabesae (Elmer) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 256 (1981 publ. 1982).

Distribution: – Philippines.

12. Coffea mannii (Hook.f.) A.P.Davis, *Nord. J. Bot.* 29: 471 (2011).

Psilanthus mannii Hook.f., *Hooker's Icon. Pl.* **12:** t. 1129 (1873).

Distribution: – West and Central Tropical Africa.

13. Coffea melanocarpa Welw. ex Hiern, *Trans. Linn. Soc. London, Bot.* **1:** 173 (1876).

Psilanthus melanocarpus (Welw. ex Hiern) J.-F.Leroy, *Ass. Sci. Internat. Café Colloque* **9:** 482 (1980).

Distribution: – Cabinda and Angola.

14. Coffea merguensis Ridl., *J. Fed. Malay States Mus.* **10:** 96 (1920).

Psilanthus merguensis (Ridl.) J.-F.Leroy, *Ass. Sci. Internat. Café Colloque* **9:** 482 (1980).

Distribution: – Myanmar (Burma), Thailand and Vietnam.

15. Coffea neobridsoniae A.P.Davis, *Phytotaxa* **10:** 43 (2010).

Psilanthus bridsoniae Sivar., Biju & P.Mathew, *Bot. Bull. Acad. Sin., n.s.*, **33:** 216 (1992).

Distribution: – India.

16. Coffea neoleroyi A.P.Davis, *Phytotaxa* **10:** 43 (2010).

Psilanthus leroyi Bridson, *Kew Bull.* **36:** 857 (1982).

Distribution: – Ethiopia and Uganda.

17. Coffea sapinii (De Wild) A.P.Davis, *Phytotaxa* **10:** 43 (2010).

Psilanthus sapinii De Wild., *Compagnie du Kasai*: 425 (1910).

Distribution: – Democratic Republic of Congo.

18. Coffea semsei (Bridson) A.P.Davis, *Phytotaxa* **10:** 43 (2010).

Psilanthus semsei Bridson, *Kew Bull.* **36:** 854 (1982).

Distribution: – Tanzania.

19. Coffea travancorensis Wight & Arn., *Prodr. Fl. Ind. Orient.* 435 (1834).

Psilanthus travancorensis (Wight & Arn.) J.-F.Leroy, *Ass. Sci. Internat. Café Colloque* **9:** 482 (1980).

Distribution: – Southern India and Sri Lanka.

20. Coffea wightiana Wall. ex Wight & Arn., *Prodr. Fl. Ind. Orient.* 436 (1834).

Psilanthus wightianus (Wall. ex Wight & Arn.) J.-F.Leroy, *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **3:** 252 (1981 publ. 1982).

Distribution: – Southern India and Sri Lanka.