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Phylogenetic study of *Plectranthus*, *Coleus* and Allies (Lamiaceae): taxonomy, distribution, and medicinal use.

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Abstract

Subtribe Plectranthinae is a palaeotropical group of just over 450 species with mainly zygomorphic flowers and stamens which are contiguous at the point of insertion at the base of the lower corolla lip. It contains the medicinally and horticulturally important genus *Plectranthus*, which currently includes the formerly recognized *Coleus* and *Solenostemon*. A phylogenetic analysis of the group is presented based on *rps16*, *trnL-F* and *trnS-G* regions of the chloroplast genome. *Plectranthus* as currently recognized is paraphyletic, a clade containing the type of *Coleus* and including *Solenostemon*, *Pycnostachys* and *Anisochilus* is sister to the rest of the group. Three endemic and monotypic Madagascan genera, *Dauphinea*, *Madlabium*, *Perrierastrum* and the Madagascan *Capitanopsis* all belong to a single clade and are recognized under *Capitanopsis,* and the new combinations are made here. *Plectranthus s.s.* is sister to a clade comprising *Thorncroftia* and *Tetradenia*,. *Tetradenia,* unlike any other members of the Plectranthinae, has actinomorphic corollas and is usually dioecious. A group of other species previously recognized as *Plectranthus* form a clade separate from *Plectranthus s.s.* and is recognized as *Equilabium* gen. nov. Estimates of clade age suggest that the genera begin to diversify from the mid to late Miocene . The Plectranthinae are found in dry woodlands, montane grasslands and evergreen forest margins. Shifts between habitats occur in most clades, though significantly fewer than if the changes were random. The distribution of the clades in the major habitats is examined. Migration in the Plectranthinae was from Africa to Madagascar and Asia, and there is no evidence of migration back to Africa. The phylogenetic pattern of medicinal use within Plectranthinae is weak and issues surrounding this are discussed.

ADDITIONAL KEYWORDS: *Solenostemon*; *Capitanopsis*; *Tetradenia*; *Thorncroftia, Pycnostachys, Anisochilus*; *Equilabium,* Africa; Madagascar; endemic; medicinal uses; habitat shifts;

INTRODUCTION

Subtribe Plectranthinae Endlicher (Tribe Ocimeae, Lamiaceae) is a paleotropical group of 11 genera and just over 450 species (Harley *et al*., 2004). It is the largest of seven subtribes that belong to the pantropical tribe Ocimeae Dumort, (Zhong *et al.*, 2010; Pastore *et al.*, 2011). The largest genus *Plectranthus* L'Hér., is a widely used medicinal and horticultural genus including over 320 species (Rice *et al*., 2011; Lukhoba *et al.* 2006). *Plectranthus* incorporates the currently synonymized genera *Coleus* Lour. and *Solenostemon* Thonn. (Harley *et al.* 2004) names which are frequently still used in medicine and horticulture (e.g. Vanaja & Annadurai, 2013; Shepherd & Maybry, 2016; Cubey 2017). The aim of this paper is to provide an updated molecular phylogenetic analysis of the Plectranthinae to identify major clades and examine congruence with existing generic limits, to use the phylogenetic analysis to examine geographic and ecological distribution of the clades, and to build upon previous studies to further explore the distribution of medicinal use of the group (Lukhoba *et al.*, 2006) to facilitate further targeted medicinal and economic research.

Both Tribe Ocimeae and the Subtribe Plectranthinae have been shown to be monophyletic based on a molecular phylogeny using the *trnL* intron, *trnL-trnF* intergenic spacer and *rps16* intron of cpDNA (Paton *et al*., 2004). Paton *et al.* (2004) found the Plectranthinae to be sister to the subtribe Ociminae including *Ocimum*, but the relationships to the other subtribes are unresolved. Although the Plectranthinae cannot be diagnosed unambiguously by a single unifying morphological character, all members apart from *Tetradenia* Benth. have all their stamens inserted at the base of the lower (anterior) lip of the corolla, a character which is not found elsewhere in the Ocimeae (Paton *et al*., 2004).

Within the monophyletic Plectranthinae, Paton *et al*. (2004), and Lukhoba *et al.* (2006) recognized two clades: the *Coleus* Clade and the *Plectranthus* Clade. The *Coleus* Clade was strongly supported while the *Plectranthus* Clade had less than 50% Bootstrap support (Paton *et al.*, 2004). Neither of these clades could be unambiguously diagnosed by morphological characters. The *Plectranthus* Clade comprised the currently recognised genera of *Tetradenia*, *Thorncroftia* N.E.Br., *Aeollanthus* Spreng., *Capitanopsis* S.Moore. and *Dauphinea* Hedge (Harley *et al.*, 2004) and part of *Plectranthus* including the type species, *P. fruticosus.* Similarly the *Coleus* Clade comprised the remaining species of *Plectranthus*, (including *P. amboinicus* the type of *Coleus*) the currently recognized *Anisochilus* Benth. and *Pycnostachys* Hook., and some genera recently placed in synonymy: *Leocus* A.Chev., *Neohyptis* J.K.Morton, *Englerastrum* Briq., *Isodictyophorus* Briq. and *Holostylon* Robyns & Lebrun (Paton *et al*., 2009; 2013). Paton *et al.* (2004) recommended a further analysis with increased taxon sampling of the Plectranthinae to clarify the best way of dividing the subtribe into monophyletic, communicable groups. Floristic treatments of the genus *Plectranthus* in eastern and southern tropical Africa (Paton *et al.*, 2009, 2013) suggested that species of *Plectranthus* in the *Coleus* Clade have a larger anterior and reduced posterior (upper) corolla lip and calyces with a pedicel attaching opposite the posterior lip of the calyx; whereas those in the *Plectranthus* Clade have ± equal anterior and posterior corolla lobes and calyces with a centrally fixed pedicel.

Most species of the Plectranthinae are found in Africa, but the group also has species in Tropical Asia and Australia, and a few species are naturalised in the New World. The Plectranthinae occupy a variety of habitats including evergreen forest margins, seasonally dry woodlands, and montane grassland, some of the latter being seasonally flooded. Species are generally restricted to one of these habitat types, and can be broadly distributed, or narrowly endemic (Codd, 1985; Hedge *et al.*, 1998; Paton *et al.*, 2009, 2013; Suddee *et al.* 2005; Forster, 1992, 1994, 2011).

Lukhoba *et al.* (2006) reviewed the ethnobotanical uses of the 62 species of *Plectranthus* cited with medicinal use in the literature. Medicinal use was mapped to the Paton *et al.* (2004) phylogenetic analysis. This work suggested that 70% of medicinal *Plectranthus* species belonged to the *Coleus* Clade and that medicinal usage tended to be concentrated in particular clades across the phylogeny. The study presented here seeks to provide greater resolution to the phylogeny of *Plectranthus* and the occurrence of medicinal use by increasing the number of species of *Plectranthus* sampled, including medicinal species not included in the previous analyses and including records of medicinal use in Plectranthinae genera not considered by Lukhoba *et al.* (2006).

As a result of the analyses presented here, several changes to current generic delimitation are proposed and the nomenclatural changes necessary to recognize these generic level changes are made. However, the bulk of name changes affecting the names of species currently placed in *Plectranthus* sens. lat., will be published elsewhere, providing a conspectus of all names published in *Plectranthus* and *Coleus*.

MATERIAL AND METHODS

SAMPLING

A survey of herbarium collections was undertaken to guide sampling of geographical and morphological diversity within the Plectranthinae and clades within *Plectranthus* recognised in Paton *et al*. (2004, 2009, 2013) and Lukhoba *et al.* (2006). Recent floristic accounts and the *World Checklist of Selected Plant Families* were also consulted (Govaerts *et al.*, 2016; Paton *et al*., 2009, 2013; Suddee *et al.*, 2005; Codd, 1985; Hedge *et al.*, 1998; Forster, 1992, 1994, 2011 and references within). All genera of the Plectranthinae recognized by Harley *et al.* (2004) were included in the analysis except *Madlabium* Hedge as DNA extractions from available herbarium material of this genus failed to amplify. Two species of *Callicarpa*, two *Prostanthera* one each of *Gmelina*, *Vitex*, *Congea* and *Tectona* were selected as outgroups to represent early diverging lineages of the family. Nine members of Tribe Mentheae and one Esholtzieae were used to represent subfamily Nepetoideae excluding the Ocimeae. Two species of *Ocimum* and *Orthosiphon* (Ociminae), one species of *Hyptis* (Hyptidinae) and one of *Isodon* were chosen to represent the remaining subtribes of the Tribe Ocimeae. Living plant material was sourced from the wild and voucher specimens deposited at RNG (Mwanyambo, 2008). Herbarium material was sourced from K and RNG (Acronyms following Thiers, 2018). The study sample includes 123 species including 97 of the 455 species of Plectranthinae (Table 1) building on the 31 used by Paton *et al.* (2004) and includes a greater range of narrowly endemic and broadly distributed species, medicinal species and representatives of groups of *Plectranthus* identified in Lukhoba *et al.* (2006). A list of accessions used is provided in Appendix 1.

An additional attempt was made to include all the species of a group of similar, and presumed closely related species, to allow study of habitat changes at species level within a set of taxa which are known to occur across a range of habitat types. Following preliminary analysis (Mwanyambo, 2008), a monophyletic group of mainly African species with non-saccate, sigmoid corolla tubes within the *Plectranthus* Clade was chosen for this detailed analysis (representing Clade 2 groups 6, B and E of Lukhoba *et al.* (2006); *Plectranthus* species 4–33 in Paton *et al.* (2009); species 10–31 in Paton *et al.* (2013)). 25 out of 38 species in this group were included, DNA from several species being unobtainable.

DNA EXTRACTION, PCR & SEQUENCING

Total genomic DNA was extracted from dried leaf and some floral material, where good quality leaf material was not available. The majority of extractions used a 2xCTAB method following protocols in use at Biological Sciences, University of Reading (Mwanyambo, 2008) or the Jodrell Laboratory, Kew, both based on Doyle & Doyle (1987). DNA extracted at the Jodrell was further purified through a caesium chloride gradient. Supplementary extractions were conducted at the Botanical Garden laboratories, University of Oslo using a DNeasy Plant Mini Kit following the manufacturer's instructions. The extracted DNA was assessed for quality by visual inspection of an ethidium bromide (0.35µg/ml) stained TAE pH 8.0 agarose gel and was then stored in water or TE buffer at -20°C.

Double-stranded DNA was amplified by the polymerase chain reaction (PCR) on an AB GeneAmp PCR System 2700 or 9700 thermocycler, in a 25 µl reaction volume. Final concentrations of: x1 NH4 reaction buffer (Bioline), 3mM $MgCl_2$, 200µM each dNTP, 0.1-0.2µM each primer, 4UTaqPolymerase, and 0.1mg/ml BSA or 0.9M Betaine (Sigma, B0300) were added where necessary.

Three cpDNA markers: *rps16* & *trnL-F* (Paton *et al.* 2004) and *trnS-G* (Shaw *et al.* 2005) were amplified as summarised in (Table S1). Amplification and sequencing of other markers was attempted; including *trnS*^{GCU}-*trnG*^{UUC}-*trnG*^{UUC}, *trnC*^{GCA}-*ycf6-psbM, ycf6-psbM-trnD*^{GUC}, *rps4-*

trnT^{UGU}-*trnL*^{UAA}, *trnD*^{GUC}-*trnT*^{GGU}, *rpoB*-*trnC*^{GCA}, *psbM*-*trnD*^{GUC}, *trnT*^{UGU}-*trnL*^{UAA}, *trnH*^{GUG}-*psbA* (all cpDNA), and nuclear G3pdh and ITS. Due to lack of, or patchy, amplification of products (Mwanyambo, 2008), these regions were not surveyed widely or used in this phylogeny reconstruction.

To improve amplification of difficult templates bovine serum albumin (BSA) and Betaine were used extensively. To further purify some difficult templates the following kits were employed: MF-Millipore membrane filter, VSWP02500 for drop dialysis; SureClean (Bioline), and Micropure-EZ Enzyme Removers (Millipore). While BioTaq worked well for the majority of samples, other enzymes used were Restorase DNA Polymerase (Sigma) and Phusion DNA Polymerase (New England Biolabs) for challenging samples. The details of primers used and thermocycling conditions are given in Table S1. All PCR products were purified for subsequent cycle sequencing using a Qiagen QIAquick PCR Purification Kit following the manufacturer's instructions with slight modifications. PE buffer was added and left to stand for 5-15min during the washing step to increase yield. DNA cycle sequencing was performed on the cleaned products in 10µl reaction volumes. Reaction components were BigDye Terminator v3.1 (4µl: ThermoFisher), 1µM primers (as used for PCR, 1.6µl), Nanopure water (2.4µl) and DNA template (3µl). Thermocycling parameters were 25 iterations of: 10s at 96°C; 5s at 50°C; 4min at 60°C. The products were run on an ABI Prism 3100 Genetic Analyser (with 50cm 16 capillary array). Due to sequencing difficulties, the outgroups and three *Plectranthus* species: *P. lactiflorus*, *P. ecklonii* and *P. amboinicus*, were not sequenced for the trnS-G region.

SEQUENCE ASSEMBLY AND ALIGNMENT

Trace data were assembled, checked for trace quality and edited in Seqman II (DNAstar Inc.) and the resulting consensus sequence files exported to Megalign (DNAstar Inc.) for initial alignment. FASTA alignments were later exported to BioEdit 7.2.5 (Hall, 2013). Aligned sequence lengths are: rps16, total length 1102 (1-691, 698-1102 used in analysis); *trnL-F*, total length 1009 (all used in analysis), *trnS-G*, total length 1088 (1-477, 561-1088 used in analysis) and sequences are deposited in

Genbank/EBI/DDBJ (Appendix A). Aligned files were exported in NEXUS for analysis using MrBayes (v3.2). The beginning and end of each alignment where fewer than 80% of the DNA sequences were available were excluded from analysis using the EXCLUDE command. Further short regions with microsatellite-like or other characteristics that prevented unambiguous alignments were also excluded.

PHYLOGENETIC ANALYSIS

Each DNA region was explored using MrModeltest v.2.3 (Nylander, 2004) and all best fitted the GTR + I + G model based on the Akaike information criterion (Akaike, 1974). For combined data analysis, congruence between cpDNA markers was previously tested and reported (Mwanyambo, 2008). Bayesian Inference analyses were performed in Mr Bayes v.3.2.6 (Ronquist et al. 2012). Gaps were treated as missing data. All the analyses were conducted with two separate runs each of four chains for 10 million iterations. Burn-in was established using Tracer v1.6 (Rambaut *et al.* 2014) and trees were sampled every 10000th generation based on tests for autocorrelation of treelength using the excel 'corr' function. The first 1 million trees were discarded on this basis. Combinable component consensus trees generated in Bayes trees (Pagel *et al.* 2004) were used in subsequent investigations because these show the best supported clades (including those with low (<50%) support).

CHARACTER CODING OF NON-MOLECULAR DATA

For phylogenetic character mapping and ancestral-state reconstruction, species of Plectranthinae were coded for habitat type, geographic distribution and medicinal use. The habitat of each species was coded from herbarium sheets, in-depth revisions (Paton *et al.*, 2009, 2013; Suddee *et al.*, 2005; Hedge *et al.*, 1998; Codd, 1985; Forster, 1992, 1994 and 2011), and field observations. It was related to one of four broad categories: evergreen forest margins; seasonally dry woodland such as African *Brachystegia* woodland or Asian dry Dipterocarp woodland, montane grassland which often burns in dry seasons; and seasonally flooded grassland or marsh. A few species are found in rocky areas, but these also usually occur within one of the main habitats and were scored under the relevant habitat type or scored as polymorphic for habitat if found in more than one. Occurrence in four major geographical regions: Sub-Saharan Africa, Madagascar, Tropical Asia and Australia was recorded. The medicinal uses of *Plectranthus* reported in Lukhoba *et al.* (2006) were also mapped onto the phylogeny, both as individual classes of use following Cook (1995) and combined into an 'any medicinal use' category. Post 2006 literature was scanned for any more recently recorded uses of Plectranthinae species included in the sample and these are listed in Appendix 2. Plectranthinae species were coded for: distribution by continent; habitat, and medicinal use category (Treebase http://purl.org/phylo/treebase/phylows/study/TB2:S22332). Continent and habitat were multistate characters and medicinal use categories were binary. The states were optimised over all trees to give a character state frequency per node on the consensus tree. A reduced taxon set of outgroups was used for optimisation analyses, rooted on *Orthosiphon* and *Ocimum.*

The pattern of distribution of the characters on the consensus tree was explored using randomization tests in Mesquite v3.04 (Maddison & Maddison, 2015) using the Reshuffle Character option following the protocol in Bytebier *et al.* (2011). Each coded character was subjected to reshuffling 100,000 times to generate a frequency graph of treelengths. Characters whose actual steps on the consensus tree fell outside the 95th percentile of the randomized distribution were considered significantly different from random, either by being clustered (>95 percentile of shortest tree lengths) or over-dispersed (>95 percentile of longest treelengths). Tests were conducted for distribution, habitat and medicinal use characters.

DATING

Phylogenetic and divergence time analysis for the combined data set was performed using Beast v 2.4.6 (Bouckaert *et al.*, 2014), following file processing using BEAUti. A trial analysis visualised in Tracer v1.6 (Rambaut *et al.*, 2014) showed there was no requirement to partition the data. Rate constancy was rejected for all partitions, so we used the relaxed clock model. The GTR+G+I, birth death model was implemented. All partitions fit the same optimal model measured by AIC and were combined before analysis. Two separate BEAST analyses were conducted and then subsequently combined to ensure a run did not stall at a local optimum. Rooting follows the MrBayes analysis. There is no fossil evidence to constrain the dates of clades within Tribe Ocimeae. The sampling of the analysis is heavily skewed towards the Plectranthinae, and thus the priors used by Drew & Sytsma (2012) for dating the Mentheae would be inappropriate. Therefore, the most recent common ancestor of the Ocimeae/Elshlotziae taken from Drew & Systma (2012) was used as a calibration point. This node was constrained at a mean of 56MYA and SD of 5% with normal distribution. A Yule tree prior was used given that we were sampling individuals from a wide range of species. Tree building ran for 40 million generations sampled every 10000th generation. Stationarity was established by the 4 millionth generation. Clock models were unlinked. Resulting trees were explored in TreeAnnotator v1.6.1 prior to visualization in the program FigTree v1.3.1.

RESULTS

TREE TOPOLOGY AND DATING

In both the Bayesian analysis using MrBayes and the Bayesian analysis using Beast the Plectranthinae is retrieved as monophyletic with a posterior probability of 1.00; within that, two sister groups , the *Plectranthus* Clade and *Coleus* Clade, recognized by Paton *et al.* (2004) and Lukhoba *et al*. (2006) both also have a p.p. of 1.00 (Fig. 1 A and B). The dated tree produced in the Beast analysis is topologically the same as that produced by MrBayes for all nodes with a posterior probability greater than 50% (Fig S1). At the base of the *Plectranthus* Clade (Fig 1A) *Alvesia* forms the first branch with a p.p. of 1.00 and the remainder of the *Plectranthus* Clade is also supported as monophyletic (0.82). Five clades within this group are strongly supported: *Aeollanthus* (1.00); Clade I - a clade comprising *Tetradenia* and *Thorncroftia* (0.96), supported as sister (1.00) to Clade II - *Plectranthus s.s.*, a clade of African and Madagascan species morphologically similar to the type species of *Plectranthus*, *P. fruticosus L' Hér.* (1.00); Clade III - a clade comprising the Madagascan endemic genera *Capitanopsis*, *Dauphinea*, and *Plectranthus bipinnatus* (previously recognized as the monotypic *Perrierastrum*) (1.00); and Clade IV a group of African *Plectranthus* with one Asian member *P. mollis* (1.00). Clade IV is more extensively sampled and internal branches are generally shorter and less well supported than in other clades. The backbone of the tree supporting the relationships between Clades I & II, Clade III and Clade IV is not strongly supported (<0.50) and the MrBayes and the dated Beast analyses differ in the topological ordering of these clades. Within the *Coleus* Clade, two clades with a p.p. of 1.00 are recovered (Fig. 1B). The *Coleus* Clade A contains species of *Pycnostachys* and *Anisochilus* in addition to species of the currently recognised *Plectranthus,* and *Coleus* Clade B contains the type species of *Coleus*, *P. amboinicus*.

Dating analysis supports that the Plectranthinae + Ociminae clade diversified from around 24.65 MYA; the Plectranthinae diversified from around 21.6 MYA and the *Plectranthus* and *Coleus* clades diversified from around 19.2 and 18.0 MYA respectively. *Alvesia* & *Aeollanthus* and Clades I-IV, diversified between 4.2 MYA (Clade III -*Capitanopsis*) and 10.75 MYA (Clade IV) in the *Plectranthus* Clade, with the Coleus A and B clades diversifying from around 16.9 and 14.4 MYA respectively. Species level divergence times vary from 0.5 to 8 MYA in the more densely sampled Clade IV. The mean dates of the crown nodes of all clades named above and the 95% Highest Posterior Density (HPD) intervals are given in Table 2 and Fig. S1.

OPTIMISATION OF GEOGRAPHY, HABITAT AND MEDICINAL USE

GEOGRAPHY

Results demonstrated that all migrations occurred from Africa to other continents. When distribution states were mapped onto the phylogenetic results, the majority of internal nodes are optimised as African (Fig. S2). Character optimisation shows nine continental migrations, significantly fewer (P=<0.01) moves between continents than that expected from a randomized distribution (P=0.99, >17-<21 steps). Four clades are non-African: the Australian *Plectranthus congestus – P. parviflorus* clade which contains all the Australian species, and the Asian *P. glabratus – P. parishii* clade both within the *Coleus* Clade A; the Madagascan endemic Clade III, *Capitanopsis*; and Madagascan species of *Tetradenia* within Clade I (Fig. 1, Fig. S2). Of the clades identified in the previous section only

Alvesia and *Aeollanthus* are restricted to tropical Africa, although one species of *Aeollanthus* is naturalized in Brazil. Isolated terminal taxa with extra-African distributions not included in the clades just described are mostly also found in Africa except *P. emirensis* Clade II in Madacascar, *P. mollis* (Clade IV) and *P. scutellarioides* (*Coleus* Clade B) in Asia (Fig S2).

In *Plectranthus* Clade I, *Tetradenia* is split between Africa and Madagascar, with no species common to both regions. The Madagascan species of *Tetradenia* may form a monophyletic group, although the sample of this genus is small. The other genus in Clade I, *Thorncroftia*, has 6 species all restricted to Southern Africa. *Plectranthus* Clade II (*Plectranthus- sensu stricto*) is found in Africa and Madagascar, although the Madagascan members of this clade are only represented in the analysis by one species. *Plectranthus* Clade IV is mainly African with only one species included in the analysis, *P. mollis*, which is found in Asia. One species, *P. flaccidus* occurs both in Africa and Madagascar, but there are no endemic Madagascan species of this clade.

HABITATS

The *Coleus* Clades A and B and Clade IV of the *Plectranthus* Clade all have representatives in seasonally dry woodland, evergreen forest margins and montane grassland although there is noticeable grouping of related species within similar habitats. Thirty-one changes of habitat are recorded within the Plectranthinae which is significantly fewer than random ($P < 0.01$ ($P=0.99$, >39-<51 steps), Fig S3). *Aeollanthus, Tetradenia* and *Thorncroftia*, can also be found in these three habitats when all species including those not in the analysis, are considered. *Alvesia* and the Madagascan Clade III are only found in dry woodland, whereas Clade II, *Plectranthus sensu stricto*, is found mainly in evergreen forest margins or forested gorges (Fig. S3). The flooded grassland habitat is only recorded in *Coleus* Clade A.

MEDICINAL USE

Several instances of medicinal use not recorded in Lukhoba, *et al.* (2006) are reported in Appendix 2. Thirty species represented in the phylogeny have recorded medicinal use. This broad category of

Medicinal use has 24 steps, just significantly fewer than random (P=0.05, >24, <31, Fig. S4). None of the individual classes of medicinal use reported in Lukhoba, *et al*. (2006), were distributed across the phylogeny in a pattern significantly different from randomized data. Of the named clades, *Coleus* Clade B has the most recorded medicinal use with 13 of the 22 sampled species being used. Internal nodes are only optimised for medicinal use in the following groups: *P. lasianthus – P. lactiflorus* clade, the *P. scutellarioides – P. shirensis* clade, the *P. alpinus – P. diversus* clade, all within *Coleus* Clade B; and within Clade A, the *Pycnostachys reticulata* - *Pycnostachys urticifolia* clade (Fig. 1B Fig. S4).

DISCUSSION

PHYLOGENY AND TAXONOMY

The phylogenetic analysis presented here supports the monophyly of the Plectranthinae, but *Plectranthus*, as currently recognized, is paraphyletic. The division of the Plectranthinae into *Plectranthus* and *Coleus* Clades as suggested by Paton *et al.* (2004) and Lukhoba *et al.* (2006) is supported, with support values much increased from these previous analyses.

There are several morphological features which can be used to diagnose the clades identified in the results, thus splitting *Plectranthus* into smaller monophyletic clades is the preferred option. *Tetradenia,* is morphologically very distinct from the rest of the Plectranthinae (Harley *et al.*, 2004; Paton *et al.*, 2004; Phillipson & Steyn, 2008). It has actinomorphic corollas and with anterior and posterior pairs of stamens separated by a clear gap at the point of insertion to the corolla, rather than having a strongly zygomorphic corolla and stamens contiguous at the base of the lower corolla lip as in all other Plectranthinae. These differences make morphological diagnoses of an enlarged *Plectranthus* comprising the whole of the Plectranthinae, including *Tetradenia*, impossible.

The genera recognized here are summarized in Table 3 and in Fig. 2. Although only chloroplast markers have been used, the fact that the strongly supported clades recognized at generic rank are either existing genera with morphological apomorphies, or have been previously informally recognized on the basis of morphology (*Plectranthus* Clade IV (Paton *et al.*, 2009, 2013)), or have strong morphological similarity (*Plectranthus* Clade III), suggests that the recognized groups themselves are robust, though the deeper relationships between them still needs to be fully resolved. The differences between the generic delimitation here and that proposed by Harley *et al.* (2004) and Paton *et al.* (2009, 2013) are: that *Coleus* is recognized at generic rank, corresponding to the *Coleus* Clade, with *Pycnostachys*, *Leocus*, *Solenostemon* and *Anisochilus* placed in synonymy; *Plectranthus s.s.* is restricted to species similar to the type of *Plectranthus*, *P. fruticosus* (*Plectranthus* Clade II); a new genus, *Equilabium* is created here to include species in Clade IV; *Capitanopsis* as currently circumscribed is paraphyletic as *P. bipinnatus* is included within it, therefore the Madagascan endemic and monotypic genera *Dauphinea*, *Madlabium*, *Perrierastrum* (the latter previously considered as *Plectranthus* in Harley *et al.*, 2004) are all placed in synonymy of the Madagascan endemic *Capitanopsis,* the earliest generic name (Clade III). Although *Madlabium* is not sampled in the analysis its corolla morphology with a truncate corolla throat with very small upper lobes is similar to the other taxa within the clade. The necessary new combinations in *Capitanopsis* and *Equilabium* are formally made at the end of this paper.

Coleus has previously been recognized as a separate genus and a good account of the taxonomic histories of *Coleus* and *Plectranthus* is provided by Codd (1975). Most treatments following Codd have merged *Coleus* into *Plectranthus*, though it was maintained as a genus in the *Flora of China* by Li & Hedge (1994). *Coleus* was diagnosed by having fused stamens (Bentham, 1832, Li and Hedge, 1994), but this character is homoplasious as shown by Paton *et al.* (2004), and the characters listed in Table 3 and used by Paton *et al.* (2009, 2013) to identify the *Coleus* Clade provide a more stable basis for diagnosis of *Coleus* as a genus. *Solenostemon* was maintained as a separate genus by Codd (1975), but *Solenostemon* here represented by *P. scutellarioides*, *P. shirensis*, *P.sigmoideus* and *P. schizophyllus* is paraphyletic (Fig. 1B). Recognition of *Solenostemon* as a genus would also render *Coleus* as a paraphyletic group and so it is not recognized at generic rank. Intermediates between *Coleus* and its synonyms previously recognized at generic rank by Harley *et al.* (2004) have been reported in the past: *Pycnostachys* (Paton *et al*., 2009, 2013); *Anisochilus* (Suddee *et al*, 2014), and *Leocus* (Pollard & Paton, 2009). In addition, none of the sections of *Coleus* or subgenera of *Plectranthus* recognized by Codd (1975) are monophyletic. Further work is required to identify morphological characters to diagnose *Coleus* Clades A and B, or other clades within those.

The merging of the Madagascan endemic and monotypic genera of Clade III into *Capitanopsis* has not been suggested before. Taxonomic over-splitting of Madagascan clades into several genera has been reported by Buerki, *et al.*, (2013) investigating the phylogenetic clustering of Madagascan endemic genera. These authors also identified recent radiations and extinctions as factors contributing the recognition of endemic genera. Both these factors might have contributed to the over-splitting of this clade. The variation in calyx form from funnel-shaped, expanded and membranous in *Capitanopsis* to tubular and non-membranous in *Dauphinea, Madlabium and Perrierastrum* (Hedge *et. al.*, 1998) might reflect recent rapid radiation within the Clade. The stem age of *Capitanopsis* Clade III is 14.3 MYA, but the extant species diversification occurs much later with the crown node at 4.2 MYA (Table 2), which might reflect a long period of stasis, but more likely extinction of earlier branches.

DATING AND CHARACTER OPTIMISATION

The clades recognized here in the *Plectranthus* Clade and suggested for generic recognition diversified in the late Miocene to Pliocene (10.75 MYA (*Equilabium*) 3.0 MYA (*Tetradenia*)) However, the *Coleus* clade has an earlier crown date (18.0 MYA) though morphologically identifiable subclades have not been recognized in this clade suggesting greater degree of morphological continuity of form in the *Coleus* clade as opposed to the *Plectranthus* Clade. Given the lack of available fossil evidence directly relevant to the Plectranthinae, the dated phylogeny should be regarded as a preliminary study and different sampling between the clades may result in different dating results being found.

There are few eco-phylogenetic studies of African plants, particularly of those occurring in seasonally dry woodland, wooded grassland or savanna floras (Bakker *et al.*, 2005, Holstein &

Renner, 2011, Linder, 2014) even though these habitats are widespread. This is in part due to the difficulties associated with species level sampling. An attempt was made to achieve this level of sampling in *Plectranthus* Clade IV (*Equilabium*), but the lack of well-preserved herbarium material and the difficulty of field collection in many different countries in a short time period made this very challenging, and only 62% of species were sampled in this clade (Table 4). For many studies of large, broadly distributed groups, comprehensive species level sampling is not possible and there is a need for better collection of material for DNA based research (Gaudeul & Rouhan, 2013). Due to the lack of species level sampling, conclusions based on character optimisation are preliminary and need to be tested with more in-depth sampling. The morphological features outlined in Table 3 were used to place all species of the Plectranthinae into one of the recognized clades. This information is used to examine whether patterns of character distribution observed in the analysis might be artefacts of incomplete sampling. The distribution of species between clades and geographical area is summarized in Table 4 below and the character optimisation studies are interpreted within this context.

Table 4 gives the total number of species found in each major clade from each of the geographical areas, using morphological characters to place species not included in the analysis. It has not been possible to give species numbers for the *Coleus* Clades A and B due to the difficulties of morphologically diagnosing these subclades as discussed above.

HABITATS

Shifts in habitat are significantly fewer than if habitat was randomly distributed on the phylogeny. The seasonally flooded habitat is only found in *Coleus* Clade A in the analysis (Fig. S3), though a few unsampled species of *Aeollanthus*, including *A. engleri* Briq., and *P. orbicularis* Gürke and *P. pulcherissimus* A.J.Paton of Clade IV also occur in this habitat. With the exception of these few species, habitat shifts have been between seasonally dry woodland, evergreen forest margins and montane grassland. Within the *Coleus* clade and *Plectranthus* Clade IV there are still several shifts in habitats, predominantly from dry woodland, to either montane grassland or forest. Similar habitat shifts were reported in *Coccinia* (Cucurbitaceae), moving between woodland, forests and arid habitats. (Holstein & Renner, 2011). As in *Coccinia,* the diversification of Plectranthinae clades date from the mid to late Miocene as the climate became cooler and drier habitats expanded and rainforests shrunk in range in Africa (Holstein & Renner, 2011; Hoetzel *et al.*, 2013; Pokorny *et al.*, 2015).

The habitats occupied by the Plectranthinae are contiguous. Dry woodlands are frequently found at mid altitudes, and mosaics of evergeen forest patches and montane grassland occur in adjacent higher altitude regions, particularly in eastern Africa. With variations in aridity, the boundaries between these habitats are likely to move, creating a dynamic landscape (Oliveras & Malhi, 2016; Fer *et al.*, 2017), influencing the spread and restriction of species distributions. Another parallel with *Coccinia* is that some forest species of the *Plectranthus* Clades II and IV and the *Coleus* clade have wide discontinuous distributions, perhaps reflecting forest expansion during the Pleistocene (Holstein & Renner, 2011). Such widespread species include *P. kamerunensis* Gürke and *P. laxiflorus* (Clade IV), *P. alboviolaceus* (Clade II) and *P. alpinus* (*Coleus* Clade). On the other hand, the disjunct distribution of *P. leptophyllus* (*Coleus* clade) found in mountains in Uganda, coastal Kenya and Tanzania, and in Eastern Zimbabwe and adjacent Mozambique, or the occurrence of *P. sylvestris* and *P. melleri* in Africa and Madagascar, probably reflect long distance dispersal as suggested for *C. schiebenii* (Holstein & Renner, 2011, Paton *et al.*, 2009, 2013).

Fire might influence the distribution of clades within the Plectranthinae. Clade IV and the *Coleus* Clade frequently inhabit dry woodland and montane grassland prone to burning, often having thick underground fire resistant rootstocks, a character rare in the other clades. In contrast, *Aeollanthus, Capitanopsis* (Clade III) and *Tetradenia* and *Thorncroftia* (Clade I) are most often associated with seasonally dry habitats and often have succulent or thick leaves and sometimes stems. These groups are mostly absent from evergreen forest margins and areas of montane grassland which are prone to burning, unless sheltered by rocks in these habitats. Clade II (*Plectranthus sensu stricto*) also occurs in naturally fire-free evergreen forest margins or forested gorges in the summer rainfall area of southern Africa and in Madagascar. The clade is largely absent from dry woodland, although around 10% of species in Africa and Madagascar have been recorded from that habitat, though these are often succulent and lack fire resistant rootstocks. Maurin *et al.* (2014) suggested a Pleistocene origin, less than 5.3 MYA, for the fire-resistant geoxylic life form seen in Clade IV and the *Coleus* Clade. This date is consistent with recent species and habitat diversification across habitats within these clades.

The Ociminae, sister group to the Plectranthinae has few evergreen forest margin species, there being around four times as many species of Plectranthinae than Ociminae in the forest margin habitat (Hedge *et al.*, 1998; Paton *et al*., 2009, 2013; Suddee *et al.*, 2005). More resolved relationships of the Ociminae are needed to investigate the pattern of migration and speciation and the relative importance of factors in explaining this difference between sister groups. However, as the relatively few forest species of Ocimineae occur in several different genera, including *Ocimum*, *Orthosiphon*, *Platostoma* and *Syncolostemon*, less speciation within the forest habitat in the Ocimineae is seen than in the Plectranthinae.

GEOGRAPHICAL DISTRIBUTION

Continental migrations are significantly fewer than expected from randomized distribution and there are no migrations into Africa, though some extant species occur both in Africa and Asia and are discussed below. Three historical migrations of the Plectranthinae to Asia from Africa are shown: *P. mollis* Clade IV; *P. scutellarioides* (*Coleus* Clade B); and *P.glabratus- P.parishii* clade (*Coleus* Clade A), (Fig. 1, Fig. S2). The Indian *P. gardneri* Twaites in Clade II (not sampled) is likely to represent another migration to Asia. Only one of these migrations shows speciation in Asia: the *P. glabratus – P. parishii* clade containing *Anisochilus* within *Coleus* clade A. However, Asian speciation in the *P. scutellarioides* and *P. shirensis* clade in *Coleus* clade B is also likely due to there being other species morphologically similar to *P. scutellarioides* in Asia; and the Indian *P. subincisus* Benth. is suggested to be closely related to the Asian *P. mollis* in Clade IV representing another possible Asian speciation (Smitha & Sunojkumar 2015). All Australian species, which are morphologically very similar, arose from a single migration event (1.6 MYA) from Asia, but increased sampling is necessary to confirm the monophyly of the Australian species.

There are three historic migration events to Madagascar: Clade I (*Tetradenia* and *Thorncroftia*), Clade II (*Plectranthus sensu stricto*), and Clade III (*Capitanopsis*) (Fig. S2). The migration events from Africa to Madagascar or to Asia in Clades I and III occurred in dry woodland clades, whereas those of Clade II were more likely through forest habitat species. Eleven species currently occur both in Africa and Madagascar and represent both forest and dry woodland species. This pattern of migration from Africa to Madagascar mainly through dry habitats but also with some through wetter forest is also seen in Apocynaceae subfamily Asclepiadoideae (Meve & Liede 2002), though unlike this group, there are no Madagscar to Africa migrations in the Plectranthinae.

A few species, all members of *Coleus* Clade B, are found in Africa, Madagascar, and Tropical Asia such as *P. barbatus*, *P. rotundifolius*, *P. amboinicus*, *P. hadiensis*, *P. montanus* and *P. caninus*. These species are all recorded as having medicinal uses so the broad distribution might reflect trade and human transport.

USES

In the Plectranthinae the pattern of distribution of any particular medicinal use as categorized by Lukhoba *et al.* (2006) is not significantly different from random, contrasting with previous studies suggesting that medicinal use is not randomly distributed across phylogenies (Saslis-Lagoudakis *et al.*, 2011 a; Douwes, *et al*. 2008; Rønsted, *et al*. 2008). However, when all medicinal uses are regarded together as a single character the pattern of distribution is just significant at the 0.05 level. Several instances of use have been recorded in *Tetradenia riparia* (Hochst.) Codd. This name was previously used to cover several tropical African species now separated as distinct species following Phillipson & Steyn (2008). It is possible that some of the recorded medicinal use attributed to *T. riparia* is actually *T. tanganyikae*. If that species is scored as having medicinal use, then the pattern of medicinal use is no longer significantly different from random across the phylogeny. A non-significant pattern of any medicinal use was also reported in *Pterocarpus* (Saslis-Lagoudakis *et al.*, 2011 b), though in that study some individual classes of medicinal use were significantly clustered on the phylogeny, unlike in the Plectranthinae. The lack of a clear phylogenetic pattern in medicinal use recorded here might be a consequence of relatively sparse and inconsistent depth of sampling across clades and/ or the equal scoring of non-homologous medicinally active compounds which may derive from different biosynthetic pathways. The lack of a strong pattern across the whole of the Plectranthinae is consistent with the findings of Kelly *et al.* (2014) who suggested that phylogenetic distance is correlated with feature similarity for only short distances along the tree; and those of Rønsted *et al.* (2012) who suggest that the strength of correlation is dependent on taxonomic scale.

Investigations into potential medicinal use could be focused on clades where interior nodes are optimised as showing this character. Clade B includes the highest number of medicinal species recorded in Lukhoba, *et al.* (2006) Several species within the *P. lasianthus – P. lactiflorus* clade (Clade B Fig. 1, Fig. S4) with medicinal use were placed by Codd (1975) in *Plectranthus* subgenus *Calceolanthus* Codd. The subgenus was diagnosed by having a calyx with a dense beard of hairs in the calyx throat, though the analysis presented here suggests not all species within this clade have this character, for example *P. puberulentus* and *P. lanuginosus*. None-the-less species with this character are likely to be closely related. Species unstudied for medicinal use and with dense hairs in the calyx throat include: *P. pentheri* (Gürke) van Jaarsv. & T.J.Edwards, *P. xylopodus* Lukhoba & A.J.Paton, *P. ornatus* Codd, *P. grandicalyx* (E.A.Bruce) J.K.Morton and *P. otostegioides* (Gürke) Ryding (Paton, 2009, 2013).

CONCLUSIONS

The major taxonomic conclusions for this medicinally and horticulturally important group are that *Coleus*, including *Solenostemon*, with over 270 species, is sister to the remainder of the Plectranthinae and merits generic recognition. *Plectranthus s.s.* (Clade II) is a group of only around 65 species and is sister to a clade comprising the morphologically different *Tetradenia* and *Throncroftia*. The species of Clade IV need to be moved from *Plectranthus* and placed into a separate genus, *Equilabium*. The Madagascan Clade III can be recognized as *Capitanopsis*, with three previously recognized monotypic genera moved into it.

Although changes in habitat are significantly fewer than would be expected if there were no phylogenetic pattern, shifts between habitats do occur. The lack of complete species level phylogenies, even when good taxonomic accounts exist, remains a barrier to understanding the details and frequencies of these changes. Herbaria represent an important resource for such studies and new analytical techniques might provide opportunities for creating such detailed phylogenies from degraded DNA from herbarium specimens (Dodsworth, 2015). However, such studies will still rely on good taxonomic accounts and well-curated herbaria.

Information on the medicinal use of plants remains fragmented and difficult to synthesise. Papers which deal with medicinal use tend to be regionally based and rarely present results on the underlying chemistry of the plant, whereas papers focusing on the biochemistry and medicinal use tend to deal with only a few species. Work such as the results presented here and those of Saslis-Lagoudakis *et al.,* 2011 b, provide a framework for further understanding of the relationship between the use of plants and shared biochemical pathways or properties.

TAXONOMIC NOVELTIES

A conspectus of all species of *Coleus*, *Plectranthus* and *Equilabium* is being prepared separately, including for the first time the formal placing of *Anisochilus* and *Pynostachys* in synonymy of *Coleus*. *Equilabium* is described and the necessary combinations in *Capitanopsis* made below.

Equilabium Mwanyambo, A.J. Paton & Culham **gen. nov.**

urn:lsid:ipni.org:names:60475121-2

Holotype species: *Equilabium laxiflorum* (Benth.) Mwanyambo, A.J.Paton & Culham **comb. nov.**
urn:lsid:ipni.org:names:60475122-2

Plectranthus laxiflorus Benth. in E.H.F.Meyer, Comm. Pl. Afr. Austr.: 228 (1838), lectotype, chosen by Codd (1975): South Africa, KwaZulu-Natal, between Umzimkulu and Umkomaas Rivers, Drège 3586 (K!, lectotype).

Equilabium is similar to *Coleus* Lour. and *Plectranthus* L'Hér. in having a strongly zygomorphic corolla and a funnel-shaped calyx with a broad upper lip and a lower lip with four lanceolate teeth, and in having stamens contiguous at the point of insertion at the base of the lower corolla lip. Differing from *Coleus* in having equal corolla lips, rather than the upper lip being shorter than the lower, and in having a symmetrical attachment of the pedicel to the base of the calyx, rather than the pedicel attaching to the calyx asymmetrically, opposite the upper lip of the calyx. Differing form *Plectranthus* by having a strongly to shallowly sigmoid corolla tube which is parallel-sided at the base (slightly saccate in only one species), as opposed to a straight or downward-curved corolla tube which is usually gibbous or saccate at the base; and in having the lateral lobes of the calyx held midway between the upper lip and lowermost teeth of the lower lip, rather than having the lateral teeth much closer to the lowermost teeth than the upper lip.

Perennial or annual, sometimes succulent, subshrubs or herbs or geoxylic herbs, usually aromatic. Leaves simple, sometimes succulent, opposite. Inflorescence thyrsoid, with cymes sessile or pedunculate, bractate, very rarely bracteolate, 1–3(–7)-flowered; bracts caducous or persistent. Pedicel attaching to calyx symmetrically. Calyx funnel-shaped, straight, 2-lipped, 5-lobed; posterior lobe lanceolate to obovate, sometimes decurrent, usually broader than other lobes; lateral lobes lanceolate or deltoid, held between the posterior and anterior lobes; anterior lobes lanceolate; throat open, glabrous. Corolla strongly two-lipped, 5-lobed, white, blue, or purple; posterior lip, ascending or erect, 4-lobed, median lobes exceeding lateral; anterior lip horizontal, cucullate, enclosing stamens, sometimes frilled at apex, corolla-tube narrow, sigmoid, parallel-sided at base, dilating distally. Stamens 4, free at base; anthers ± orbicular. Style apex bifid with subulate lobes. Disc 4-lobed with anterior lobe larger. Nutlets, ovoid, glabrous, usually mucilaginous.

The new combinations in *Capitanopsis* are made below:

Capitanopsis brevilabra (Hedge) Mwanyambo, A.J.Paton & Culham **comb. nov.**

urn:lsid:ipni.org:names:77165451-1

Dauphinea brevilabra Hedge, Notes Roy. Bot. Gard. Edinburgh 41: 119 (1983). Type: Material cultivated in Edinburgh, originally collected in Madagascar, Dist. de Fort Dauphin, Ste-Luce, *Hardy & Rauh* 2876 (E, holotype).

Capitanopsis magentea (Hedge) Mwanyambo, A.J.Paton & Culham **comb. nov.**

urn:lsid:ipni.org:names:77165452-1

–*Madlabium magenteum* Hedge, Fl. Madag. 175: 261. 1998. Type: Madagascar, forêt d'Ampandra, 6 km au nord de Vohemar, sur la route vers Ambilobe, *Lavranos* 28995 (E, holotype P, isotype).

Capitanopsis oreophila (Guillaumin) Mwanyambo, A.J.Paton & Culham **comb. nov.**

urn:lsid:ipni.org:names:77165453-1

–*Perrierastrum oreophilum* Guillaumin, Bull. Mus. Natl. Hist. Nat., sér. 2, 2: 694. 1930. Type: Madagascar, massif de l'Andringitra, *Perrier de la Bâthie* 13729 (P, holotype).

–*Plectranthus bipinnatus* A.J. Paton, Kew Bull. 58: 488. 2003. Type as *Perrierastrum oreophilum*

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Figure Legends

Figure 1A. Compatible component consensus tree produced from Mr Bayes analysis showing the outgroups, *Plectranthus* Clade (P) with *Alvesia*, *Aeollanthus*. *Plectranthus* Clades I – IV and the position of the *Coleus* Clade (Fig. 1B). Posterior probabilities are given below the branches: blue if over 0.50 support, red if below 0.50

Figure 1B. Compatible component consensus tree produced from Mr Bayes analysis, *Coleus* Clade comprising *Coleus* Clades A & B. Posterior probabilities are given below the branches: blue if over 0.50 support, red if below 0.50

Figure 2. Summary diagram showing genera recognized within the Plectranthinae with species numbers and dates of major clades. Species featured from top to bottom are: *Alvesia rosmarinifolia* (Photo M. Finckh); *Aeollanthus buchnerianus* (Photo B. Wursten); *Tetradenia nervosa*, *Plectranthus saccatus* (both RBG Kew), *Capitanopsis angustifolia* (P. Lowry), *Plectranthus petiolaris* (Photo N. Couch); *Plectranthus barbatus* (RBG Kew).

Table Legends

Table 1. Summary of the taxonomic breadth of sampling and the species numbers sampled.

Table 2 Crown node ages of recognized clades (MYA). Columns give the mean age and the minimum and maximum of the 95 % HPD calculated in Beast.

Table 3 Genera recognized and diagnostic features

Table 4 Total number of species found in each major clade from each of the geographical areas

Appendices

Appendix 1. Species, voucher specimens and Genbank numbers of materials used in analysis. Sequences marked are being deposited in Genbank

Appendix 2. Medicinal use of sampled species not recorded in previous review of the medicinal uses of *Plectranthus* (Lukhoba *et al.* 2006)

Supplementary tables and figures

Table S1 Amplification of cpDNA markers: primers used and thermocycling conditions.

Fig. S1 Dated analyses produced by Beast. Bars indicate 95% highest posterior density interval (HPD). Numbers give mean ages for crown nodes of named clades. P- *Plectranthus* Clade comprising *Alvesia, Aeollanthus* and *Plectranthus* Clades I – IV; and C – *Coleus* Clade comprising *Coleus* Clades A & B

Fig. S2. Consensus tree with geographical distribution optimised: Sub-Saharan Africa (Blue); Madagascar (Green); Asia (Yellow); Australia (Black). Grey indicates proportion of trees in which optimisation is unresolved. Red indicates proportion in which node is absent. P- *Plectranthus* Clade comprising *Alvesia, Aeollanthus* and *Plectranthus* Clades I – IV; and C – *Coleus* Clade comprising *Coleus* Clades A & B

Fig. S3 Consensus tree with habitat optimised: Dry woodland (Green); Evergreen forest margins (Blue); Montane Grassland (Black); Seasonally flooded grassland (White). Grey indicates proportion of trees in which optimisation is unresolved. Red indicates proportion in which node is absent. P- *Plectranthus* Clade comprising *Alvesia, Aeollanthus* and *Plectranthus* Clades I – IV; and C – *Coleus* Clade comprising *Coleus* Clades A & B

Fig. S4 Consensus tree with medicinal use optimised: Medicinal use recorded (Black); Medicinal use not recorded (White). Grey indicates proportion of trees in which optimisation is unresolved. Red indicates proportion in which node is absent. P- *Plectranthus* Clade comprising *Alvesia, Aeollanthus* and *Plectranthus* Clades I – IV; and C – *Coleus* Clade comprising *Coleus* Clades A & B

Appendix 1. Species, voucher specimens and Genbank numbers of materials used in analysis. Sequences marked are being deposited in Genbank

Taxon	**Country**	**Collector and Number**	**Herbarium**	***trnL-trnF* intron + 3'exon + spacer**	***rps16* intron**	***trnS-trnG* spacer**
Aeollanthus buchnerianus Briq.	Malawi	Brummitt 10401 (cultivated Kew 1970-2734)	K	AJ505434	AJ505327	√
Aeollanthus densiflorus Ryding	Kenya	Mathew 6137 (cultivated Kew 1970-3760)	K	AJ505435	AJ505328	√
Aeollanthus rhemanii Gürke	Malawi	M.L.Mwanyambo, *et al.* 746	MAL	√	√	√
Alvesia clerodendroides (T.C.E.Fr.) Mathew	Tanzania	Sally Bidgood, *et al.* 4547	K	√	√	√
Alvesia rosmarinifolia Welw.	Zambia	Harder, *et al.* 3634	K	AJ505436	AJ505329	√
Anisochilus harmandii Doan	Thailand	S. Suddee, *et al.* 775	BKF,K,TCD	AJ505437	AJ505330	√
Anisochilus pallidus Benth.	Thailand	S. Suddee *et al*. 1080	BKF,K,TCD	AJ505438	AJ505331	√
Callicarpa americana L.	Cultivated	Cult., Kew -0818400507	K	AJ505535	AJ505412	

Callicarpa japonica Thunb.	Cultivated	Cult., Kew K-1934-12904	K	AJ505536	AJ505413	
Capitanopsis albida (Baker) Hedge	Madagascar	P. Lowry 6255	P, MO. Silica K	√	√	√
Capitanopsis angustifolia (Moldenke) Capuron	Madagascar	Clement, *et al.* 2117	K	AJ505440	AJ505333	
Capitanopsis cloiselli S.Moore	Madagascar	R. Capuron 20.490-SF	K	√	√	√
Clinopodium myriantha	Malawi	M.L.Mwanyambo, *et al.* 771	MAL	√	√	√
Clinopodium vulgare L. subsp. arundanum (Boiss.) Nyman	Cultivated	Cult., Kew K-453-79-04649	K	AJ505547	AJ505426	
Congea tomentosa Roxb.	Cultivated	Wagstaff, s.n.	BHO	AJ505530	AJ505411	
Dauphinea brevilabra Hedge	Madagascar	Hardy & Rauh 2876 (cultivated Kew 1998-2417)	K	AJ505441	AJ505334	√
Elsholtzia stauntonii Benth.	Cultivated	Wagstaff 356	BHO	AJ505526	AJ505406	

Gmelina philippensis Cham [hystrix Kurz]	Cultivated	Cult., Kew K-381-74-02999	K	AJ505527	AJ505407	
Hyptis capitata Jacq.	Thailand	Wongprasert, *et al.* s.n.	BKF,K,TCD	AJ505449	AJ505337	√
Isodon lophanthoides (Buch.-Ham. ex D.Don) Kudô	Malaysia	H.J.M. Bowen 3993	RNG	√	√	√
Lavandula maroccana Murb.	Cultivated	Upson s.n.	RNG	AJ505461	AJ505347	
Lavandula minutolii Bolle	Cultivated	Upson s.n.	RNG	AJ505462	AJ505348	
Melissa officinalis L.	Cultivated	Wagstaff 88-09	BHO	AJ505529	AJ505410	
Mentha suaveolens Ledeb.	Cultivated	Cult. Kew K-1970-3169	K	AJ505541	AJ505418	
Nepeta racemosa Lam.	Iran	Z. Jamzad s.n.	TARI	AJ505432	AJ505325	
Ocimum filamentosum Forssk.	Kenya	Brummitt 18993	K	AJ505466	AJ505352	√
Ocimum tenuiflorum L.	Thailand	Suddee 893	K	AJ505473	AJ505358	

Origanum vulgare L.	Cultivated	Chase 13334 (Cultivated Kew K-000-69-19317)	K	AJ505543	AJ505422	
Orthosiphon rubicundus (D.Don) Benth.	Thailand	Suddee 809	K	AJ505477	AJ505360	
Orthosiphon schimperi Benth.	Malawi	M.L.Mwanyambo, *et al.* 769	MAL	√	√	√
Plectranthus acaulis Brummitt & Seyani	Zambia	M.L.Mwanyambo, *et al.* 742	MAL	√	√	√
Plectranthus adenophorus Gürke	Tanzania	K. & T. Pocs 87063	K	√	√	√
Plectranthus africanus (Scott-Elliot) A.J.Paton	Democratic Rebublic Congo	Masens da Musa Y. 632	K	√	√	√
Plectranthus agnewii Lukhoba & A.J. Paton	Kenya	Mrs. S.F. Polhill 327	K	√	√	√
Plectranthus albicalyx S.Suddee	Thailand	Suddee *et al*. 868	BKF, K, TCD	AJ505498	AJ505376	√
Plectranthus alboviolaceus Gürke	Malawi	M.L.Mwanyambo, *et al.* 762	MAL	√	√	√

Plectranthus alpinus (Vatke) O. Ryding	Kenya	Lukhoba, *et al.* 501	K	√	√	√
Plectranthus amboinicus (Lour.) Spreng.	Thailand	Suddee et al 869	BKF	AJ505499	AJ505377	
Plectranthus annuus A.J. Paton	Tanzania	S. Bidgood, *et al.* 1918	K	√	√	√
Plectranthus argentatus Blake	Cultivated	T.T. Aye s.n.	RNG	√	√	√
Plectranthus barbatus Andr.	Cultivated	Cult., Kew 1999-14	K	√	√	√
Plectranthus betonicifolius Baker	Tanzania	Sally Bidgood, *et al.* 3413	K	√	√	√
Plectranthus bipinnatus A.J.Paton	Madagascar	Cult., Kew K1988-3186	K	√	√	√
Plectranthus buchananii Baker	Cultivated	Chase 8514 (Cultivated K-1970-3559)	K	AJ505501	AJ505379	√
Plectranthus calycinus Benth.	S. Africa	Balkwill et al 10880	J, K	AJ505502	AJ505380	√
Plectranthus candelabriformis Launert	Tanzania	Sally Bidgood, *et al.* 3335	K	√	√	√

Plectranthus chimanimaniensis S. Moore	Zimbabwe	T. Müller 3592	K	√	√	√
Plectranthus ciliatus E.Mey	Cultivated	Chase 13336 (Cultivated K-1991-6)	K	AJ505532	AJ505409	√
Plectranthus coeruleus (Gurke) Agnew	Malawi	Cult. Kew. K1970-3233	K	√	√	√
Plectranthus congestus R.Br.	Australia	P.I.Forster PIF15180	K	√	√	√
Plectranthus crassus N.E.Br.	Malawi	Brummitt 9700 (Cultivated K-1991-6)	K	AJ505504	AJ505382	√
Plectranthus daviesii (E.A. Bruce) Mathew	Malawi	M.L.Mwanyambo *et al.* 792	MAL	√	√	√
Plectranthus dissectus Brenan	Malawi	J.D. & E.G. Chapman 7195	K	√	√	√
Plectranthus diversus S.T.Blake	Australia	J.R.Clarkson & V.J.Neldner 10114	K	√	√	√
Plectranthus djalonensis (A.Chev) A.J.Paton	Zambia	G. Pope, A-R. Smith & D. Goyder 2120	K	√	√	√

Plectranthus ecklonii Benth.	Cultivated	T.T. Aye s.n.	RNG		√	
Plectranthus elegans Britten	Malawi	J.D. Chapman 6065	K	√	√	√
Plectranthus emirnensis (Baker) Hedge	Madagascar	R.A.Clement, *et al.* 2057	K	√	√	√
Plectranthus esculentus N.E.Br.	Malawi	M.L.Mwanyambo *et al.* 707	MAL	√	√	√
Plectranthus glabratus (Benth.) Alston	India	J.Klackenberg & R.Lundin 176	K	√	√	√
Plectranthus glandulosus Hook.f.	Nigeria	J.D. Chapman 4015	K	√	√	√
Plectranthus goetzii Gürke	Malawi	M.L.Mwanyambo, *et al.* 765	MAL	√	√	√
Plectranthus gracilis Suesseng.	Malawi	E. Phillips 3636	K	√	√	√
Plectranthus gracillimus (T C E. Fr.) Hutch. & Dandy	Tanzania	S. Bidgood & K. Vollesen 3229	K	√	√	√

Plectranthus guerkii	Tanzania	L. Festo 723	K	√	√	√
Plectranthus hadiensis (Forssk.) Sprenger	Ethiopia	M.G.Gilbert, *et al.* 9265	K	√	√	√
Plectranthus hockii De Wild.	Tanzania	Goyder et al 3904	K	AJ505443	AJ505335	√
Plectranthus ignotus A.J.Paton	Tanzania	S.Bidgood, *et al.* 2544	K	√	√	√
Plectranthus lactiflorus (Vatke) Agnew	Ethiopia	I.Friis, *et al.* 8874	K	√	√	
Plectranthus lanuginosus (Benth.) Agnew	Tanzania	J.M.Grimshaw 9341	K	√	√	√
Plectranthus lasianthus (Gürke) Vollesen	Botswana	P.A.Smith 2090	K	√	√	√
Plectranthus laxiflorus Benth.	Ethiopia	M.G.Gilbert, Sylvia Phillips & Damtew Tefera 9245	K	√	√	√
Plectranthus leptophyllus (Baker) A.J.Paton	Tanzania	S. Bidgood, *et al.* 4207	K	√	√	√

Plectranthus longipes Baker	Tanzania	O.Hedberg 6825	K	√	√	√
Plectranthus masukensis Baker	Tanzania	S. Bidgood, *et al.* 792	K	√	√	√
Plectranthus melleri Baker	Cameroon	R. Letouzey 14998	K	√	√	√
Plectranthus modestus Baker	Zambia	M.L.Mwanyambo, *et al.* 752	MAL	√	√	√
Plectranthus mollis (Aiton) Spreng	India	J.Klackenberg & R.Lundin 260	K	√	√	√
Plectranthus montanus Benth.	Cultivated	cult., K-1996-1453; Chase 8518	K	AJ505538	AJ505383	√
Plectranthus oertendahlii T.C.E. Fr.	Cultivated	Chase 3380 (Cultivated, K-1969-5789)	K	AJ505534	√	√
Plectranthus parishii Prain	Thailand	Suddee 1144	BKF, K, TCD	AJ505511	AJ505390	√
Plectranthus parviflorus Willd.	Cultivated	T.T. Aye s.n.	RNG	√	√	√
Plectranthus parvus Oliv.	Kenya	M.G. Gilbert & Mesfin Tadessa 6713	K	√	√	√
Plectranthus pauciflorus Baker	Tanzania	Mrs. M. Richards 22953	K	√	√	√

Plectranthus petiolaris E. Mey. ex Benth..	S. Africa	Univ of Natal K-1996-2729	K	AJ505512	AJ505391	√
Plectranthus pinetorum A.J.Paton	Malawi	D.J.Goyder & A.J.Paton 3660	K	√	√	√
Plectranthus puberulentus K.Morton	Kenya	Mathew 6830 (Cultivated K-1970-3784)	K	AJ505507	AJ505386	√
Plectranthus pubescens Bak.	Malawi	M.L.Mwanyambo, *et al.* 778	voucher, MAL	√	√	√
Plectranthus punctatus subsp. edulis (Vatke) A.J. Paton	Kenya	Lukhoba, *et al.* 505	K	√	√	√
Plectranthus rungwensis A.J.Paton	Tanzania	L.B.Mwasumbi 16222	K	√	√	√
Plectranthus sallyae A.J. Paton	Tanzania	S. Bidgood, *et al.* 2661	K	√	√	√
Plectranthus sanguineus Britten	Malawi	Brummitt s.n. (Cultivated, K-1970-2072)	K	AJ505513	AJ505392	√
Plectranthus schizophyllus Baker	Malawi	M.L.Mwanyambo, *et al.* 796	MAL	√	√	√

Plectranthus scutellaroides (L.) R.Br.	Trinidad	Barnard, *et al.* 193	RNG	√	√	√
Plectranthus shirensis (Gürke) A.J. Paton	Tanzania	S. Bidgood, *et al.*4725	K	√	√	√
Plectranthus sigmoideus A.J. Paton	Malawi	E.G.Chapman 264	BM	√	√	√
Plectranthus stenophyllus Baker	Tanzania	S.Bidgood *et al.* 1951	K	√	√	√
Plectranthus stenosiphon Baker	Malawi	R.K.Brummitt, *et al.* 16050	MAL	√	√	√
Plectranthus stolzii Gilli	Tanzania	M. Richards 9880	K	√	√	√
Plectranthus sylvestris Gürke	Malawi	R.K.Brummitt 12332	K	√	√	√
Plectranthus tenuicaulis (Hook.f.) J.K.Morton	Angola	C.Henriques 323	K	√	√	√
Plectranthus termiticola A.J. Paton	Zimbambwe	Heany Teachers Training College H.69	K	√	√	√
Plectranthus thyrsoideus (Bak.) B.Matthew		Chase 13332 (Cultivated, K-5638704012)	K	AJ505533	AJ505405	√

Plectranthus triangularis A.J. Paton	Tanzania	J.A.Mlangwa 631	K	√	√	√
Plectranthus vesicularis A.J. Paton	Tanzania	R.I.Ludanga 1301	K	√	√	√
Plectranthus viphyensis subsp. zebrarum Brummitt & Seyani	Malawi	M.L.Mwanyambo & E.S.Kathumba 797	MAL	√	√	√
Plectranthus welwitschii (Briq.) Codd	Cultivated	Cult., K-1999-15;	K	AJ505505	AJ505384	√
Plectranthus xerophilus Codd	S.Africa	cult., K-1989-1322; Hardy 3966	K	AJ505515	AJ505394	√
Plectranthus zombensis Baker	Malawi	J.L. Balaka & K. Kaunda 378	K	√	√	√
Prostanthera petrophila B.J.Conn	Cultivated	Chase 6980		AJ505525	AJ505404	
Prosthanthera nivea Benth.	Cultivated	Chase 6975	K	AJ505524	AJ505403	
Pycnostachys reticulata (E.Mey.) Benth.	S. Africa	Cult. Kew K-1999-2425	K	AJ505516	AJ505395	√
Pycnostachys umbrosa Perkins	Kenya	Mathew 6067 (Cultivated K-1970-3755)	K	AJ505517	AJ505396	√

Pycnostachys urticifolia Hook.	S. Africa	Cult. Kew K 1999-2426	K	AJ505518	AJ505397	√
Rosmarinus officinalis L.	Cultivated	Cult. Kew K 1973 14217	K	AJ505546	AJ505425	
Salvia nilotica Juss. ex Jacq	Malawi	M.L.Mwanyambo, *et al.* 728	MAL	√	√	√
Tectona grandis L. f.	Cultivated	Waimea 73P172	BHO	AJ505528	AJ505408	
Tetradenia fruticosa Benth.	Madagascar	Hardy 2910B (Cultivated, K1989-1324)	K	AJ505519	AJ505398	√
Tetradenia nervosa Codd	Madagascar	Hardy 2910B (Cultivated, K1993-3116)	K	AJ505520	AJ505399	√
Tetradenia tanganyikae Phillipson	Malawi	M.L.Mwanyambo & E.S.Kathumba s.n.	MAL	√	√	√
Thorncroftia longifolia N.E.Br.	S. Africa	L.McDade LM1281	J	AJ505521	AJ505401	√
Thymus serpyllum L. var. citriodorum	Cultivated	Chase 13331 (Cultivated K -1975-1177)	K	AJ505544	AJ505423	
Vitex trifolia L.	Cultivated	Chase 8757 (TCMK 15;)		AJ505539	AJ505416	

Appendix 2. Medicinal use of sampled species not recorded in previous review of the medicinal uses of Plectranthus (Lukhoba *et al*. 2006)

Species	Use	Category (Cook 2005)	Reference
Aeollanthus buchnerianus	Cure for colds in children	infection/fever	Moteetee & Van Wyk (2011).
Aeollanthus densiflorus	Eye and skin diseases	skin	Awas & Demissew (2009).
Anisochilus harmandii	tonic – antimalarial & antimycobacterial properties	infection/fever	Lekphrom et al. (2010).
Plectranthus argentatus	stomach pain and inflammation	gastro urinary, infection fever, skin	Anthoney & Ngule (2013)
Plectranthus scutellarioides	Headache & coughs	Respiratory, Pain	Waruruai et al. (2011)
Plectranthus scutellarioides	Malaria, diarrhoea	Infection/fever, gastrourinary,	Kaou et al. (2008)
Plectranthus shirensis	Traditional Fever remedy	Infection/fever	Fowler (2006)
Plectranthus sylvestris	Anti-inflamatory	Infection /fever	Juch & Rüedi (1997).
Pycnostachys reticulata	Pain related ailments	Pain	Fawole et al. (2010)
Pycnostachys urticifolia	Mental diseases	Other- mental	Stafford et al. (2008)

Pycnostachys urticifolia	antibacterial	Infection/fever	Bascombe, K., & Gibbons, S. (2008)
Tetradenia riparia	Antimicrobial	Infection/fever	Njau et al. (2014)