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New generic recircumscription of the Loxocarpinae (Gesneriaceae), as inferred by phylogenetic and morphological data

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Abstract
The Loxocarpinae, also known as the “Boea group”, are the subtribe of Gesneriaceae which includes Boea and a number of segregated genera and close relatives. This group currently comprises over 200 species in 15 genera. Here we present the most up-to-date phylogeny, covering all the genera known to belong to the group, based on Bayesian Inference and Parsimony of the nuclear ITS and the plastid regions trnL-trnF (intron and spacer) and ndhF-trnL¹⁴AG (spacers). The results show discrepancies between the current generic delimitation in the subtribe and the clades delineated by the phylogeny. As a result Boea, Damrongia, Streptocarpus and Paraboea are recircumscribed in an attempt to establish a more natural classification and a number of new combinations are made. The new genus Middletonia is described.

Keywords: Boea, Damrongia, Paraboea, Southeast Asia, Streptocarpus.

Phylogeny of Loxocarpinae.
Introduction

The Gesneriaceae are a large family with an estimated 3500 species in 147-150 genera (Stevens, 2001 onwards; Skogg & Boggan, 2007; Möller & Clark, 2013; Weber & al., 2013), distributed mostly in the tropics and subtropics, with some outliers in the temperate areas of both hemispheres (Weber, 2004). The subject of this investigation is the group formerly referred to informally as the “advanced Asiatic and Malesian Gesneriaceae with twisted fruit” (Möller & al., 2009) and now formally classified as the subtribe Loxocarpinae, part of the tribe Trichosporeae, subfamily Didymocarpoideae (Weber & al., 2013). It is also informally known as the ‘Boea group’ (Möller & al., 2009; Puglisi, 2014).

The Loxocarpinae are found throughout Southeast Asia, reaching Australia and the Solomon Islands, and are characterised by having a predominantly twisted capsule. However, in some genera all the species have a ‘straight’ fruit, with valves not twisting (e.g. Loxocarpus R.Br.), and in other genera there are species with a twisted fruit and species with a straight fruit (e.g. Paraboea (C.B.Clarke) Ridl., Fig. 1).

The first genus in the subtribe described to accommodate twisted-fruited species was Boea Comm. ex Lam. Boea grew larger, and became rather heterogeneous, as more species with the same fruit type were discovered and were described in the genus. Progressively, the majority of the species was then transferred to other genera, many of which were directly segregated from Boea, in order to establish morphologically distinct units.

Key publications, in which many species of Boea were described, are the works by Brown (1839, 1840) and Clarke (1883). The most important recent work on the Boea group is that by Burtt (1984), in which the generic concepts formed around a twisted-fruited Boea and a straight-fruited Paraboea were profoundly modified with new generic concepts based on indumentum type rather than on the fruit twisting. This change led to a large number of species being transferred from Boea to Paraboea.

Later, the new genera Kaisupeea B.L.Burtt (Burtt, 2001), Senyumia Kiew, A.Weber & B.L.Burtt, Spelaeanthus Kiew, A.Weber & B.L.Burtt and Emarhendia Kiew, A.Weber
& B.L.Burtt (Kiew & al., 1997), were created to accommodate the few remaining doubtful species of Boea and Paraboea.

The study by Möller & al. (2009) was the first phylogenetic investigation focusing on the tribe Trichosporeae (then referred to as Didymocarpoid Gesneriaceae). In a subsequent study, Möller & al. (2011) expanded their sampling and confirmed that the straight-fruited genera Chirita Buch.-Ham. and Henckelia Spreng. were both polyphyletic and that taxa of both were to be found in the Boea group. Weber & al. (2011), Yao (2012) and Middleton & al. (2013) focused on these problematic genera and, as a result, Chirita was split into five genera, including Damrongia Kerr ex Craib within the Boea group, comprising a few species from the former Chirita sect. Chirita. Likewise, Henckelia was split into three genera, including Loxocarpus in the Boea group (formerly Henckelia sect. Loxocarpus (R.Br.) A.Weber & B.L.Burtt). Puglisi & al. (2011a) also examined relationships within the Boea group, focusing on the genera Paraboea, Trisepalum C.B.Clarke and Phylloboea Benth., resulting in them all being synonymised under Paraboea (following conservation of the name by Middleton & al., 2010), by far the largest genus in the Loxocarpinae.

The aims of this new study are to reconstruct the molecular phylogenetic tree for the entire subtribe Loxocarpinae, to test whether the current classification is in agreement with the phylogenetic structure of the group, to identify robust phylogenetic entities suitable for a redefinition of the generic limits, and to propose a new generic classification accordingly.

**Materials and methods**

In this study, 140 ingroup accessions belonging to 110 taxa of Loxocarpinae were sequenced, and all the genera recognised in the subtribe were included. The outgroup consisted of six accessions of the closely related subtribes Didissandrinae, Didymocarpinae and Streptocarpinae (Weber & al., 2013), represented by two taxa of Didissandra C.B.Clarke, Codonoboea Ridl. and African Streptocarpus Lindl. respectively. The material used in the analyses consisted of silica gel-dried leaves samples with the exception of the sample called “Boea sp.”, which was taken from a herbarium specimen
(Hoogland 5129, CANB herbarium). Generic types have been included for all the genera with the exception of Boea and Ornithoboea Parish ex C.B.Clarke, for which no material suitable for DNA extraction was available. Information on the accessions, including collection data, repository of the vouchers and GenBank numbers, is available as supplemental material.

Total genomic DNA was extracted following a modified version of the cetyltrimethyl ammonium bromide (CTAB) method by Doyle & Doyle (1987), with no further purification.

The markers used in the phylogenetic analyses were chosen based on previous work on the Didymocarpoideae (e.g. Atkins & al., 2001; Bramley & al., 2004; Clark & al., 2009; Möller & al., 2009, 2011; Puglisi & al., 2011a, 2011b; Puglisi, 2014). These were the nuclear ITS and the plastid regions trnL-trnF (including both the trnL intron and the trnL-trnF spacer) and ndhF-trnL\textsuperscript{UAG} (ndhF-rpl32 and rpl32-trnL\textsuperscript{UAG} spacers).

The PCR of the ITS and trnL-trnF regions followed an optimised recipe already tested in previous studies (Puglisi & al., 2011a, 2011b). The 20 µl reaction contained 2 µl 2 mM dNTPs, 2 µl 10x NH\textsubscript{4} buffer, 0.6 µl 25 mM MgCl\textsubscript{2}, 2 µl each 10 µM forward and reverse primer, 0.4 µl 0.4% BSA, 0.4 µl Biotaq polymerase (Bioline), 1 µl DNA template and 9.6 µl dH\textsubscript{2}O. In some cases, TBT-PAR was employed as recommended by Samarakoon & al. (2013) and it seemed to have a positive effect on problematic PCRs. The 20 µl PCR reaction mix containing TBT-PAR was: 2 µl 2 mM dNTPs, 2 µl 10x NH\textsubscript{4} buffer, 0.6 µl 25 mM MgCl\textsubscript{2}, 2 µl each 10 µM forward and reverse primer, 4 µl 5x TBT-PAR, 0.4 µl Biotaq polymerase (Bioline), 1 µl DNA template and 6 µl dH\textsubscript{2}O. The primers used to amplify the ITS were 5P and 8P (Möller & Cronk, 1997). Occasionally, the internal primers 2G and 3P (Möller & Cronk, 1997) were used when the sequencing signal strength was low. The thermocycle settings used in the PCR were: 94°C for 3’, 30× [94°C for 1’, 55°C for 1’, 72°C for 1.5’], 72°C for 5’, 10°C forever. The trnL-trnF region was amplified using the universal primers c, d, e and f (Taberlet & al., 1991).

While this intron-spacer region is generally amplifiable with just the external primers c and f, a number of samples proved problematic and needed several adjustments to the PCR reaction and thermocycle settings. However, none of these variations appeared optimal or widely applicable. The following PCR thermocycle settings were used: 94°C
for 4', 35× [94°C for 45", 55°C for 45", 72°C for 3']], 72°C for 10’, 10°C forever. The
primers used for the amplification of the ndhF-trnL \textsuperscript{UAG} were ndhF, rpl32F, rpl32R and
trnL \textsuperscript{UAG} (Shaw & al., 2007).

The two spacers ndhF-rpl32 and rpl32-trnL \textsuperscript{UAG} were tentatively co-amplified or, when
necessary, treated individually. The thermocycle settings used in the PCR of the entire
region were 80°C for 5’, 30× [95°C for 1’, 50°C for 1’, 65°C for 1’ 7” with ramp of 0.3
C/sec, 72°C for 2’], 72°C for 5’, 4°C forever. Individual spacers, instead, followed the
thermocycle 80°C for 5’, 30× [95°C for 1’, 50°C for 1’, 65°C for 1”7” with ramp of 0.3
C/sec, 65°C for 4’], 65°C for 5’, 4°C forever.

PCR products were stained with SYBR Safe (Invitrogen) and checked by
electrophoresis on 1% agarose gel. Successful PCR products were purified with
ExoSAP-IT (Affymetrix), following the manufacturer’s protocol. Sequencing PCRs
were 1/8 reactions with BigDye Terminator v.3.1 (Applied Biosystems). The
thermocycle was: 25× [95°C for 30”, 50°C for 20”, 60°C for 4’], 4°C forever.

Sequencing products were processed at the GenePool laboratory of the University of
Edinburgh on an ABI3730 DNA Analyser (Applied Biosystems). Sequences were
edited in Sequencher v.4.7 (Gene Code Corporation) and aligned manually in Mesquite
v.2.74 and v.2.75 (Maddison & Maddison, 2010, 2011).

Given the relatively low number of sequences available for the ndhF-trnL \textsuperscript{UAG} plastid
DNA region, two different datasets were analysed: the “2-markers” dataset with 142
accessions, including only ITS and trnL-trnF data, and the “3-markers” dataset with 68
accessions, but with the additional contribution of the ndhF-trnL \textsuperscript{UAG} region (Table 1).
Four of the accessions included in the 3-markers dataset were not analysed in the larger
2-markers dataset, due to the low quality of some sequences, especially the trnL-trnF,
and the simultaneous presence in the matrix of other accessions of the same species
with more reliable sequences.

Parsimony analyses were run in PAUP* v.4.0b10 (Swofford, 2003) on unordered and
unweighted characters with the following settings: heuristic search running over
100,000 stepwise random addition replicates, with two trees held at each step; tree
bisection reconnection (TBR) branch swapping algorithm with steepest descent and
MulTrees options enabled; MaxTrees setting fixed at 1,000,000. The resulting
parsimonious trees were filtered to retain the ‘best score’ trees only. Topological
support for the phylogenies was estimated by bootstrap analyses. These were run with
10,000 pseudo-replicate samples, following the parsimony criterion and the following
heuristic search settings: stepwise random addition, one replicate and TBR on; steepest
descent and MulTrees options disabled.
Evolution models for Bayesian Inference were inferred in jmodeltest2 (Guindon &
Gascuel, 2003; Darriba & al., 2012) according to the Akaike Information Criterion (AIC
- Akaike, 1974). While the plastid markers trnL-trnF and ndhF-trnL_{UAG} were not
partitioned, thus assuming a uniform evolutionary rate across the regions, two distinct
elements were identified within the nuclear ITS: the highly conserved 5.8S gene and the
combined, highly variable spacers ITS1 and ITS2. Sequences and models were analysed
for Bayesian Inference in MrBayes v.3.2.2 (Ronquist & Huelsenbeck, 2003; Ronquist &
al., 2011). Preliminary tests were run to help choose the most suitable parameter
settings. The number of generations was fixed at 10 million, with a sample frequency of
1000 and a burn-in of 2000, for both matrices. The Bayesian analyses were run on the
CIPRES Science Gateway V 3.3 (Miller & al., 2010). The output trees were edited in
FigTree v.1.3.1 (Rambaut & Drummond, 2009).

Results
The combinability of the different partitions was assessed through preliminary
individual Bayesian analyses (not shown). Overall, the resulting trees did not highlight
any topological conflict, with the exception of minor discrepancies generated by the low
resolution at the backbone of the trees and among the branches subtending the genera
Loxocarpus, Emarhendia and Orchadocarpa Ridl. However, since the clades defined
by the phylogenies remained consistent, the partitions were combined for analysis.
The outputs of the Parsimony and Bayesian Inference of the two datasets, 2-markers
and 3-markers, have been summarized in four consensus trees (strict for Parsimony,
50% majority rule for Bayesian Inference, all presented as electronic supplement). The
trees do not have fully matching topologies especially towards the backbone, but
consistently outline the same well-defined seven clades (Fig. 2), which are the focus of
this study. These clades all receive maximum support in the Bayesian 3-markers
analysis, as do all except clade 3 in the Bayesian 2-markers analysis (0.64 posterior
probability). Clade 3 has no support in either Parsimony analysis. Of the other groups,
all but clade 4 receive 100% bootstrap support in the Parsimony 3-markers analysis,
whereas in the Parsimony 2-markers analysis clades 4 and 6 receive less than 95%
support (Table 2).

The first clade to diverge within the ingroup is clade 1 (Fig. 2), formed by a group of
species ascribed to *Paraboea*, specifically *P. monticola* Triboun & D.J.Middleton which
is sister to *P. evrardii* (Pellegr.) B.L.Burtt and *P. multiflora* (R.Br.) B.L.Burtt. The
position of this group, with respect to the remaining ingroup taxa and the core of
*Paraboea*, is consistent in all the trees generated in this study, although is statistically
supported only by the Bayesian analyses.

With an increased sampling since Puglisi & al. (2011a), all the remaining species of
*Paraboea* form a strongly supported monophyletic group (hereafter referred to as
*Paraboea sensu stricto*), i.e., clade 7.

Similarly, *Boea* is polyphyletic, with species spread across clades 2, 3 and 6. Clade 2
comprises *B. geoffrayi* Pellegr., *B. hygrometrica* (Bunge) R.Br., *B. philippensis*
C.B.Clarke and a new species (*Boea* sp. nov.). The relative position of *Boea geoffrayi* is
not stable, as it appears as either sister to the new species (2-markers dataset) or to *B.
philippensis* (3-markers dataset).

Clade 3 contains all the examined species of *Loxocarpus*, nested within which are
*Emarhendia* and *Orchadocarpa*, plus a well-supported subclade (bootstrap 97-100%,
posterior probability 1) comprising *Senyumia*, *Spelaeanthus* and the Australasian
species of *Boea*. In *Boea*, the Australian *B. hygroscopica* F.Muell. is sister to the
accessions from Papua New Guinea, *B. lawesii* H.O.Forbes and *Boea* sp. Sister to this
subclade in most analyses (the 2-marker parsimony analysis is equivocal) is a clade of
*Loxocarpus* which includes *L. rufescens* (C.B.Clarke) B.L.Burtt, *L. sericiflavus* (Kiew
& Banka) T.L.Yao, *L. holttumii* M.R.Hend. and related species. A second clade of
*Loxocarpus* is formed by the accessions of *L. incanus* R.Br. and is most closely related
to *Orchadocarpa*. The remaining accessions of *Loxocarpus*, *L. argenteus* B.L.Burtt, *L.
violoides* (C.B.Clarke) T.L.Yao, *L. verbeniflos* (C.B.Clarke) B.L.Burtt and *L. repens*
B.L.Burtt, form a further, well supported clade. The affinities of *Emarhendia* are not
entirely clear.
The remaining Boea species, *B. clarkeana* Hemsl., is nested within *Damrongia* in clade 6 in all the analyses, and is sister to *D. trisepala* (Barnett) D.J.Middleton & A.Weber and *D. cyanantha* Triboun. Likewise, *Streptocarpus sumatranus* B.L.Burtt is consistently nested inside *Damrongia* and is likely related to *D. lacunosa* (Hook.f.) D.J.Middleton & A.Weber or *D. fulva* (Barnett) D.J.Middleton. All these species together form a well-supported clade, which is sister to *Streptocarpus orientalis* Craib, completing clade 6. Clade 6 is strongly supported as sister to clade 5, which corresponds to a clearly monophyletic *Somrania* D.J.Middleton. Within *Somrania*, *S. albiflora* D.J.Middleton is sister to *S. lineata* D.J.Middleton & Triboun plus *S. flavida* D.J.Middleton & Triboun.

**Discussion**

Our analyses reveal that the Loxocarpinae comprise a number of well-supported clades, with the exception of clade 3, and also reveal that many genera are not monophyletic (*Boea, Damrongia, Loxocarpus, Paraboea* and *Streptocarpus*). Among the genera with more than one species, only the monophyly of *Ornithoboea, Somrania* and *Kaisupeea* is supported. However, relationships between many of these clades are poorly resolved or supported, providing only limited information about higher level relationships within the subtribe. Relationships involving *Loxocarpus, Emarhendia* and *Orchadocarpa* within clade 3 are particularly complex.

**Boea.** This study confirms the polyphyly of *Boea* already shown by Möller & al. (2009, 2011). Six out of 14 species were included in the analyses. They are found in three different clades (2, 3 and 6): *Boea clarkeana* (Fig. 3: 6c) is nested in *Damrongia* (clade 6), whereas *B. hygroscopica* (Fig. 3: 3f), *B. lawesii* and an unnamed species form a clade with *Senyumia* (Fig. 3: 3d) and *Spelaeanthus* (Fig. 3: 3e), nested within *Loxocarpus* (clade 3). The remaining four species examined form a clade on their own
(clade 2, Fig. 3: 2). These results indicate that *Boea*, already greatly reduced in size by
the removal of several segregate genera and the realignment with *Paraboea*, is not
tenable in its current delimitation and should be split. Morphological characters, such as
the shape of the corolla, also support a formal separation. The type species of *Boea*, *B.
magellanica* Comm. ex Lam., from Papua New Guinea and the Solomon Islands, was
not examined here, but has the same corolla morphology as the other members of the
Australasian group, especially *Boea lawesii*: the flower has a flat-faced, unevenly
coloured corolla and the stamens are exserted; the filaments are bent and they appear
bright yellow and fleshy. Conversely, the corolla of all the species forming clade 2 is
uniformly lilac to blue, obliquely campanulate with a ventricose tube, reflexed upper
lobes and a broad throat; the stamens are included in the throat, have slender filaments
and the anthers are erect (Fig. 3: 2). Under the new circumscription suggested here, the
name *Boea* remains with the Australasian group (*B. magellanica*, *B. hygroscopica*, *B.
lawesii*, etc.), whereas clade 2 acquires the resurrected name *Dorcoceras* Bunge (1832),
coincd for *Dorcoceras hygrometricum* Bunge. The hitherto inclusion of the species of
*Dorcoceras* within *Boea* is a relic of the very broad generic concept adopted by Clarke
(1883) which neither Schlechter (1923) nor Burtt (1984) effectively resolved. The
resurrected *Dorcoceras* includes the four Southeast Asian species of *Boea* with a
campanulate corolla: *B. geoffrayi*, *B. hygrometrica*, *B. philippensis* and *B. wallichii*
R.Br. The new combinations are provided below.

*Boea clarkeana*, instead, is transferred to *Damrongia*, based on the results of the
phylogenetic analysis and its morphological similarity to *D. trisepala* (Fig. 3: 6b). There
are also substantial differences between *Boea clarkeana* and the existing species of
*Damrongia*, the most obvious of which are in the fruit. *Boea clarkeana* has an
orthocarpic, twisted capsule that bears little resemblance to the plagiocarpic, straight
fruit of the other *Damrongia* species. However, *Paraboea* (Puglisi & al., 2011a and see
below) and *Ornithoboea* (Scott & Middleton, 2014) also contain species with twisted
and species with non-twisted capsules, indicating that this character is variable within
genera, and hence not a good argument against transferring *Boea clarkeana* to
*Damrongia*. 
Paraboea.—Paraboea was found to be non-monophyletic, with clade 1 forming a
group separate from all the other species (Fig. 2). Paraboea s.s. (clade 7, Fig. 3: 7) is
monophyletic with high statistical support and the same overall structure as found by
Puglisi & al. (2011a). The first subclade to diverge includes all species with a calyx
divided into five equal parts that are found north of the Isthmus of Kra in the Thai
Peninsula. Of the two other sister subclades, one comprises species formerly placed in
Trisepalum and Phylloboea, and is characterised morphologically by a strongly
bilabiate calyx; the other, instead, comprises species with a calyx divided into five equal
parts and distributed south of the Isthmus of Kra and in Malesia.

Clade 1 possesses characters typical of Paraboea, such as the interwoven indumentum
on the lower surface of the leaves, the flat-faced corolla and the twisted capsules.
However, these plants also have stamens with free, erect anthers opening upwards, with
the apices of the anthers parallel to the axis of the flower. The species of Paraboea s.s.,
conversely, have anthers with the apex rotated towards the gynoecium, coherent,
divergent and opening along the median line. The gynoecia also differ, as in clade 1
there is an indumentum of minute white, greenish or yellow glands on the ovary and the
capsule which is absent in clade 7. The clear phylogenetic and morphological
distinction of this group from the rest of Paraboea supports the segregation of a new
genus, Middletonia C.Puglisi (Fig. 3: 1).

It should be noted that the sample of Paraboea multiflora in Puglisi & al. (2011a),
which formed a monophyletic clade with the core Paraboea species, was misidentified.
The voucher (Wen 2010-01, collected in Guangdong, China) could not be located but a
new specimen said to be from the same locality and of the same species by the original
collector has been identified as Paraboea cf. dictyoneura (Hance) B.L.Burtt, which is
morphologically similar to the species in its clade and not to P. multiflora. The sample
was omitted from the analyses presented here.

Streptocarpus.—Streptocarpus is an Afro-Madagascan genus with c. 140 species, first
described in 1828. Due to the presence of a twisted capsule, Franchet (1899), Craib
(1911, 1919) and Burtt (1962) decided to ascribe to this genus some Asian plants which
did not have a better alternative placement. There are currently three species of
Streptocarpus in Asia: S. burmanicus Craib from Burma, S. orientalis from Thailand
and *S. sumatranus* from West Sumatra (Indonesia). Despite the carpological similarity, Möller & al. (2009), Puglisi (2014) and the present study all show that *Streptocarpus orientalis* does not form a monophyletic group with the other species of the genus (represented by the African *S. rexii* and *S. glandulosissimus* in the present study). Puglisi (2014) and the present study additionally show that *Streptocarpus sumatranus* does not form a monophyletic group either with the African species or with *S. orientalis*.

In the present study, *Streptocarpus sumatranus* is nested within *Damrongia*. When he described it, Burtt (1962) was unable to place it in any existing Southeast Asian genus of Gesneriaceae and opted, cautiously, for *Streptocarpus* because of its caulescent habit, the narrowly campanulate corolla and the twisted capsule. Moving *Streptocarpus sumatranus* into *Damrongia* is currently the best option, or at least the only viable option, given its current, untenable position as a species of *Streptocarpus*. This inclusion deeply alters the morphological characterisation of *Damrongia*, especially through the introduction of the caulescent habit in the genus. A twisted fruit has already been introduced into *Damrongia* by the inclusion of *Boea clarkeana*, incidentally a species also formerly ascribed to *Streptocarpus* (Hilliard & Burtt, 1971).

*Streptocarpus orientalis* is sister to *Damrongia* (incl. *Boea clarkeana* and *Streptocarpus sumatranus*, Fig. 3D). Its inclusion in *Streptocarpus* is clearly erroneous and the species requires a more appropriate generic placement. As the expanded *Damrongia*, including *Boea clarkeana* and *Streptocarpus sumatranus*, already possesses morphological characters such as the twisted capsule, caulescent habit and chiritoid stigma (two-lipped stigma with the upper lip strongly reduced and the lower bilobed), which are characters also present in *S. orientalis*, the most appropriate course of action is to place *S. orientalis* in *Damrongia*, rather than in a separate genus. Although no sample of *Streptocarpus burmanicus* was available for DNA extraction, its morphology suggests this species to be very closely related to *S. orientalis*. With these changes, the distribution of *Damrongia* becomes much wider, from China to Sumatra.

**Loxocarpus.**--- *Loxocarpus* was found to be non-monophyletic in this and previous phylogenies (Yao, 2012; Puglisi, 2014), forming three distinct, well-supported clades.
Mixed in with these, and together forming clade 3, are Orchadocarpa, Emarhendia and the Boea/Spelaeanthus/Senyumia subclade, but relationships among these lineages are not fully resolved.

One Loxocarpus clade contains all the accessions of the type species of the genus, Loxocarpus incanus, including L. incanus var. sekayensis (Banka & Kiew) T.L.Yao.

In the 2-markers trees, the separation of Loxocarpus incanus var. sekayensis from L. incanus var. incanus does not receive strong support. Further investigation of this species is needed as perhaps the identifications were not accurate at the varietal level. A second clade of Loxocarpus, including e.g. L. violoides (C.B.Clarke) T.L.Yao, is entirely composed of species from Borneo and is morphologically heterogeneous in that it includes one species, L. argenteus B.L.Burtt, with a campanulate corolla, in contrast to the other members of the group, all with flat-faced corollas (Yao, 2012). The third Loxocarpus clade is entirely made of species with a campanulate corolla, comprising L. rufescens (C.B.Clarke) B.L.Burtt from Borneo and species from the Malay Peninsula. This clade is sister to the Senyumia/Spelaeanthus/Boea alliance in all the analyses, but this relationship receives significant support only in the 3-markers Bayesian Inference. Nevertheless, Loxocarpus is clearly paraphyletic and perhaps best split into three different genera, since the morphologies of Boea, Senyumia and Spelaeanthus are too different to encourage the synonymisation into a large genus encompassing the entire clade 3 (Fig. 3: 3). However, the geographical and morphological ranges present in Loxocarpus remain relatively poorly sampled, and these, plus the incomplete resolution of clade 3, make the proposition of formal taxonomic changes premature. Hence Loxocarpus is left unaltered, until further studies provide more data.

Patterns of diversity.—Gesneriaceae present several fruit types: fleshy or dry berries and capsules, these orthocarpic or plagiocarpic, cylindrical or conical and varying greatly in length and mode of dehiscence (Weber, 2004). In the Loxocarpinae only dry capsules are found. The most common shape is cylindrical, with longitudinal dehiscence into two valves (Fig. 1A-1B). Most twisted capsules have this structure, or, less frequently, are conical, as in the group of Paraboea species with a bilabiate calyx. Straight fruit types need to be further categorised. Most straight capsules, like the twisted ones, dehisce into two valves along two suture lines. The straight fruits of
Orchadocarpa and Paraboea (except for *P. incudicarpa* B.L.Burtt) have this morphology, and ortho- and plagiocarpic forms exist. A variation of this morphology is seen in *Loxocarpus*, where the short, conical and plagiocarpic capsule has two dorso-ventral valves which are strongly unequal and whose dehiscence results in a cup for splash dispersal (Fig. 1C). *Paraboea incudicarpa*, *Somrania* and *Emarhendia*, instead, produce capsules that are plagiocarpic and cylindrical but, unlike those of e.g. *Orchadocarpa*, dehisce only along the upper suture line (Fig. 1D).

While most genera of Loxocarpinae have exclusively twisted or straight fruits, few have both types represented among their species. Genera with an exclusively straight fruit are *Emarhendia*, *Loxocarpus*, *Orchadocarpa* and *Somrania*, although all with substantially different types of capsules. Genera with an exclusively twisted fruit are *Dorcoceras*, *Rhabdothamnopsis*, *Senyumia* and *Spelaeanthus*. Finally, genera where both states are present are *Boea*, *Damrongia*, *Kaisupeea*, *Middletonia*, *Ornithoboea* and *Paraboea*.

Despite the obvious difference in fruit morphologies, there does not appear to be any clear pattern across the phylogenetic trees. A lack of consistency was already inferred by Burtt (1984), when he modified the generic boundaries of *Boea* and *Paraboea* hitherto based on the fruit twisting. In our phylogeny, the position of *Middletonia* and *Dorcoceras* suggests that a twisted fruit is the ancestral condition, and that straight fruits have evolved several times in the Loxocarpinae. This mirrors the evolution of the other subtribe of Gesneriaceae with a predominantly twisted fruit, the Streptocarpinae, where multiple independent losses of fruit twist have been inferred by Nishii et al. (in press). In order to make further progress in our understanding of the evolution of the fruit in the Loxocarpinae, however, a more in-depth carpological study is necessary.

While the variation in fruit type does not form a clear pattern in the tree topology, distribution data show a geographic line along the Isthmus of Kra in Peninsular Thailand. This is most remarkable in *Paraboea*, where two subclades (with e.g. *P. crassifolia* (Hemsl.) B.L.Burtt and *P. acutifolia* (Ridl.) B.L.Burtt) comprise species predominantly from south and north of the Isthmus, respectively. Species of the two groups are not as distinct morphologically as they are geographically and genetically. The same situation is present in the third subclade of *Paraboea* (with e.g. *P. subplana* (B.L.Burtt) C.Puglisi), sister to the species found south of Kra, where the two main
branches show a well-supported separation between species found on either side of the Isthmus.

In *Ornithoboea*, conversely, the three species found south of the Isthmus of Kra are morphologically distinct from their northern congenerics (Scott & Middleton, 2014), although the current phylogeny does not provide unambiguous support for a matching genetic differentiation.

*Emarhendia*, *Orchadocarpa*, *Senyumia*, *Somrania*, *Spelaeanthus* and all the species currently placed in *Loxocarpus* are entirely restricted to south of the Isthmus of Kra. *Boea* is only present much further east. *Middletonia* and *Damrongia* have species both south and north of the Isthmus of Kra, but the relationships between the species are not yet sufficiently resolved to test whether there is a significant biogeographical element to them. Additionally, *Damrongia trisepala* has a distribution that straddles the Isthmus of Kra, a rare occurrence in the Loxocarpinae.

*Dorcoceras* has a different distribution pattern from the other Loxocarpinae. Most species occur on the Asian continent, and one species, *D. philippense*, is broadly distributed in China, Laos, Vietnam, the Philippines and central Indonesia. This might suggest a migration from the continent to Indonesia through the Philippines, which differs from the pattern observed in the rest of the tribe Trichosporeae (Weber, 2004; Cronk & al., 2005; Möller & al., 2009, 2011), involving migration from China southwards, along the Thai/Malay Peninsula and then west to east across Malesia. The different route of migration of *Dorcoceras* does not touch the Isthmus of Kra and neither seems to cross any other discontinuity line to the east or the west.

**Taxonomic treatment**

*Boea* Comm. ex Lam., Encycl. 1: 401. 1785 – Type: *Boea magellanica* Comm. ex Lam. Fig. 3: 3f.

Ten species, distributed in Eastern Indonesia, Papua New Guinea, the Solomon Islands and Queensland (Australia). This is the only genus of the Loxocarpinae to have an Australasian distribution. *Boea* has a flat-faced corolla, exserted stamens, a twisted, orthocarpic capsule and a thin, simple indumentum on the lower surface of the leaf.

**Damrongia** Kerr ex Craib in Bull. Misc. Inform. Kew 1918(10): 364. 1918 – Type: *Damrongia purpureolineata* Kerr ex Craib. Fig. 3: 6a-6c.

With the inclusion of *Boea clarkeana* and the three Asian species of *Streptocarpus*, and with the synonymisation of *Damrongia cyanantha* into *D. trisepala*, *Damrongia* is now a genus of ten species, centred in Thailand and distributed from China to Sumatra. All species have an infundibuliform-tubular corolla and a chiritoid stigma, and the species for which a count is available, all have chromosome number 2n=18 (Christie et al., 2012; Möller & Pullan, 2015 onwards). The new circumscription has broadened the range of morphological variation of *Damrongia*, with the addition of characters such as the caulescent habit and the twisted, orthocarpic fruit.

The following are the new combinations in *Damrongia*.


montagnes a La-Kou, *Maire s.n.* (E barcode E00175310; isolectotype: G barcode G00303008).


Dorcoceras Bunge, Enum. Pl. Chin. Bor.: 54. 1832 (1833) – Type: Dorcoceras hygrometricum Bunge. Fig. 3: 2.

This genus is resurrected to accommodate the four species with a campanulate corolla excluded from Boea. Dorcoceras is found in China, Thailand, Cambodia, Vietnam, Philippines and Indonesia. Dorcoceras has a rosulate habit, simple indumentum, free calyx lobes and an obliquely campanulate, lilac corolla, with inserted stamens arising at the mouth. The new combinations needed are below.


Species list: Dorcoceras geoffrayi (Pellegr.) C.Puglisi, Dorcoceras hygrometricum Bunge, Dorcoceras philippense (C.B.Clarke) Schltr., Dorcoceras wallichii (R.Br.) C.Puglisi.


One species, endemic to Peninsular Malaysia, characterised by the plagiocarpic, straight fruit and the patch of glandular hairs between the two upper corolla lobes. Its relationships with Loxocarpus and Orchadocarpa are in need of further clarification.
Kaisupeea B.L.Burtt in Nordic J. Bot. 21(2): 115–119. 2001 – Type: Kaisupeea herbacea (C.B.Clarke) B.L.Burtt (= Boea herbacea C.B.Clarke). Fig. 3: 4a.

Three species from Burma and Thailand, characterised by the indumentum predominantly consisting of glandular hairs and the anthers hairy at the back. Kaisupeea is most closely related to Rhabdothamnopsis.

Species list: Kaisupeea cyanea B.L.Burtt, Kaisupeea herbacea (C.B.Clarke) B.L.Burtt, Kaisupeea orthocarpa B.L.Burtt.

Loxocarpus R.Br., Cyrtandreae: 120. 1839 – Type: Loxocarpus incanus R.Br. Fig. 3: 3a.

This recently revised genus (Yao, 2012) comprises 20–23 species, distributed in the Thai-Malay Peninsula, Sumatra and Borneo. Its most characteristic feature is the conical, plagiocarpic capsule, but it is otherwise highly variable in morphology. The phylogenetic analysis confirmed its non-monophyly and revealed the consistent presence of three distinct groups of species. Given the paucity of the material currently available for Loxocarpus, the genus is left untouched until further focused research, also involving the other Malaysian genera Emarhendia and Orchadocarpa.

Middletonia C.Puglisi, *gen. nov.* – Type: *Middletonia multiflora* (R.Br.) C.Puglisi. (≡ *Boea multiflora* R.Br.). Fig. 3: 1.

Similar to *Paraboea* (C.B.Clarke) Ridl. in having a matted indumentum on the abaxial side of the leaves but distinct by the farinose glandular indumentum on the ovary and the free and erect anthers.


Lithophytic, shortly caulescent, perennial herbs. Leaves opposite, those of a pair equal; lamina oblong to elliptic, apex obtuse to acute, base cuneate to obtuse, sometimes oblique, margin crenate or serrate, adaxial surface glabrescent, furfuraceous or pubescent, abaxial surface with a matted indumentum; veins raised beneath, more or less smooth above, tertiary veins reticulate and visible on the abaxial surface, especially in proximity to the leaf margin. Inflorescence an axillary cyme, many-flowered, densely tomentose; peduncles longer or shorter than the leaves; bracts inconspicuous. Calyx 5-mmerous, lobes divided to the base; lobes 1–3 mm long, narrowly ovate, glabrous or glandular inside, more or less tomentose outside. Corolla 5-merous, white, violet or blue, slightly bilabiate, 4–8(–10) mm long, 4–10 mm across, with or without an indumentum; tube 1–3 mm long; limb slightly 2-lipped, upper lip with 2 lobes 2–6(–9) × 1–6.5 mm, lower lip 3-lobed, lobes 2–6(–9) × 1–6.5 mm, all lobes spreading, flat.

Stamens 2; filaments straight; anthers with a minute, glandular indumentum, not coherent, opening towards the top; staminodes 2, reduced or aborted. Ovary syncarpous, 2-carpellate, ovoid, with a farinose glandular indumentum, 1–2.5 × c. 1 mm, ovules many; style glabrous, 2.5–3 mm long; stigma capitate. Fruit a capsule, to 1.3 cm long, straight or twisted, retaining the indumentum of the ovary. Seeds minute, compressed.

Distribution: India, Bangladesh, Bhutan, China, Burma, Thailand, Laos, Cambodia, Vietnam, Malaysia.

Habitat: limestone or granite.
This new genus is segregated from *Paraboea* following the results of the phylogenetic study and the subsequent morphological investigation. The new combinations in *Middletonia* are given below.


Bangladesh, Pundua, Sylhet Mt., *De Silva in Wallich 793* (BM barcode BM000797995; isolectotype: K barcode K001111906).


*Orchadocarpa* Ridl. in J. Straits Branch Roy. Asiat. Soc. 44: 78. 1905 – Type: *Orchadocarpa lilacina* Ridl. Fig. 3: 3c.

Monotypic genus from Peninsular Malaysia. It is recognisable by the short fruit, completely enclosed by the calyx, and the flat-faced corolla with a lower lip longer than...
the upper. The placement of *Orchadocarpa* in the phylogeny remains, like that of
*Emarhendia*, unresolved but likely to be somewhat close to part of *Loxocarpus*.

**Ornithoboea** Parish ex C.B.Clarke in A.DC. & C.DC., Monogr. Phan. 5(1): 147. 1883
– Type: *Ornithoboea parishii* C.B.Clarke. Fig. 3: 4c.

This genus was revised recently (Scott & Middleton, 2014) and consists of 16 species
from China, Thailand, Burma, Vietnam, Laos and Malaysia. It is easily recognisable by
the palatal beard and the circlet of hairs around the corolla mouth. *Ornithoboea* was
found to be monophyletic and its closest relatives are *Rhabdothamnopsis* and
*Kaisupeea*.

Species list: *Ornithoboea arachnoidea* (Diels) Craib, *Ornithoboea barbanthera*
B.L.Burtt, *Ornithoboea calcicola* C.Y.Wu ex H.W.Li, *Ornithoboea emarginata*
D.J.Middleton & N.S.Lý, *Ornithoboea feddei* (H.Lév.) B.L.Burtt, *Ornithoboea flexuosa*
(Ridl.) B.L.Burtt, *Ornithoboea henryi* Craib, *Ornithoboea lacei* Craib, *Ornithoboea*
*maxwellii* S.M.Scott, *Ornithoboea multitorta* B.L.Burtt, *Ornithoboea obovata*
S.M.Scott, *Ornithoboea occulta* B.L.Burtt, *Ornithoboea parishii* C.B.Clarke,
*Ornithoboea pseudoflexuosa* B.L.Burtt, *Ornithoboea puglisiae* S.M.Scott, *Ornithoboea*
*wildeana* Craib.


The circumscription of *Paraboea*, recently modified by Puglisi & al. (2011a) is
modified again by the segregation of the new genus *Middletonia*. *Paraboea* now
consists of 127 species distributed throughout Southeast Asia. It is easily recognised by
the combination of a matted, interwoven indumentum on the lower side of the leaves,
the flat-faced to shortly campanulate corolla, the non-erect anthers and the lack of
sessile glands on the ovary.
Species list: Paraboea acaulis (Barnett) C.Puglisi, Paraboea acuta (C.B.Clarke)

Paraboea albida (Barnett) C.Puglisi, Paraboea amplexicaulis (Parish ex C.Puglisi)

manhaoensis Y.M. Shui & W.H. Chen, *Paraboea martini*ii (H.Lév.) B.L.Burtt,

Rhabdothamnopsis Hemsl. in J. Linn. Soc., Bot. 35(247): 517–518. 1903 – Type: Rhabdothamnopsis sinensis Hemsl. Fig. 3: 4b.

Monotypic genus from China whose closest relative is Kaisupeea. It is characterised by the solitary flowers with infundibuliform corollas and the twisted fruit.


This is another monotypic genus from Peninsular Malaysia. Its closest relatives are Boea and Spelaeanthus. It is the only twisted-fruited genus to have a resupinate flower.

Somrania D.J.Middleton in Thai For. Bull. (Bot.) 40: 10. 2012 – Type: Somrania albiflora D.J.Middleton. Fig. 3: 5.

Somrania is closely related to Damrongia and consists of three species endemic to southern Thailand. The genus is easily recognisable by its tubular corolla and the indumentum of branched hairs.

Species list: Somrania albiflora D.J.Middleton, Somrania flavida D.J.Middleton & Triboun, Somrania lineata D.J.Middleton & Triboun.


One species from Peninsular Malaysia, closely related to Senyumia and Boea. Spelaeanthus has a characteristic white, bowl-shaped corolla.
Acknowledgements

The PhD project which forms the basis of this work was funded by the BBSRC. The herbarium CANB is acknowledged for allowing destructive sampling from a specimen. All collectors are acknowledged for sharing leaf material. The field work for material collection by the authors of this paper was funded by the Davis Expedition Fund, the James and Eve Bennett Trust, the Weir Fund for Field Studies, the Royal Horticultural Society, the Royal Botanic Garden Edinburgh and the Leverhulme Trust. We gratefully acknowledge the assistance in the field of many staff members of the Forest Herbarium Bangkok (BKF), P. Triboun (then BK), S. Lindsay (then E), M. Hughes (E), K. Bransgrove (JCU), D. Girmansyah (BO), W. Ardi Handoyo (Kebun Raya Bogor), R. Rubite (University of the Philippines Manila), P. Boyce (University Sains Malaysia).

Some sequences of *Didissandra* were generated by L. Forrest (RBGE), some sequences of *Streptocarpus* by K. Nishii (RBGE). Support with the laboratory work came from M. Hollingsworth, L. Forrest, A. Forrest and J. Tosh.

Literature cited


Tables

Table 1: The ‘2-markers’ dataset comprises ITS and trnL-trnF sequences; the ‘3-markers’ dataset comprises ITS, trnL-trnF and ndhF-trnL_UAG sequences.

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<th>Included characters</th>
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Table 2: Statistical support for the main clades identified. Values are shown as ‘bootstrap/posterior probability’.

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<td>98/1</td>
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<tr>
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<td>100/1</td>
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</table>
Clade 2 (*Dorcoceras*)  
Clade 3  
(*Loxocarpus*/Orchadocarpa/Emarhendia/Senyumia/Spelaeanthus/Boea*)  
Clade 4 (*Ornithoboea*/Kaisupeea/Rhabdothamnopsis)  
Clade 5 (*Somrania*)  
Clade 6 (*Damrongia*)  
Clade 7 (*Paraboea*)

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<td><em>Somrania</em></td>
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<td><em>Paraboea</em></td>
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**Figure captions**

Figure 1: Capsule diversity in the Loxocarpinae. A: straight capsule with two longitudinal dehiscence lines. *Paraboea burttii* Z.R.Xu. B: twisted capsule with two longitudinal dehiscence lines. *Ornithoboea pugliasiae* S.M.Scott. C: splash cup with dorso-ventral dehiscence. *Loxocarpus incanus* R.Br.. D: straight capsule dehiscing only along the upper suture. *Paraboea incudicarpa* B.L.Burtt. Photo credits: D. Middleton (A, D), P. Karaket (B) and T. Phutthai (C)

Figure 2: Phylogeny of the Loxocarpinae. The overall structure of the subtribe is represented by this 50% majority rule consensus tree derived from the Bayesian analysis of the dataset available for the three markers ITS, *trnL-trnF* and *ndhF-trn*LUAG*. The numbers in bold font inside circles refer to the clade numbering used in the text. The numbers by the nodes are the posterior probabilities, followed by the bootstrap values returned by the Parsimony analysis of the same dataset. The bars at the right of the tree mark the placement of the recircumscribed genera. Asterisks mark accessions of the type species.

Figure 3: The seven clades of Loxocarpinae. 1: *Middletonia* C.Puglisi, a new genus including the species with a farinose indumentum on the ovary, segregated from *Paraboea* (C.B.Clarke) Ridl.. *Middletonia multifora* (R.Br.) C.Puglisi. Photos by D. Middleton. 2: *Dorcoceras* Bunge is resurrected to include the Southeast Asian species with a campanulate corolla previously ascribed to *Boea*. Left: *Dorcoceras* sp. nov.;

**Electronic supplements**

S1: Strict consensus of the six most parsimonious trees based on Parsimony analysis of the 2-markers dataset, including ITS and trnL-trnF sequences. Tree length=4049, CI=0.3779, HI=0.6221, RI=0.7310. The numbers inside the circles refer to the clades as treated in the text. The numbers above the branches represent bootstrap values. The bars at the right of the tree mark the placement of the recircumscribed genera. Asterisks mark accessions of the type species.

S2: 50% majority rule consensus tree based on Bayesian Inference of the 2-markers dataset, including ITS and trnL-trnF sequences. Number of generations: 10 mln, sample frequency: 1000, burn-in: 2000, average standard deviation: 0.002797. The numbers beside nodes represent posterior probabilities. The scalebar represents the number of substitutions per site. The numbers inside the circles refer to the clades as treated in the text. The bars at the right of the tree mark the placement of the recircumscribed genera.
Asterisks mark accessions of the type species.

S3: Strict consensus of the eight most parsimonious trees based on Parsimony analysis of the 3-markers dataset, including ITS, \textit{trnL-trnF} and \textit{ndhF-trnL}$_{UAG}$ sequences. Tree length=4271, CI=0.5273, HI=0.4727, RI=0.6824. The numbers inside the circles refer to the clades as treated in the text. The numbers above the branches represent bootstrap values. The bars at the right of the tree mark the placement of the recircumscribed genera. Asterisks mark accessions of the type species.

S4: 50% majority rule consensus tree based on Bayesian Inference of the 3-markers dataset, including ITS, \textit{trnL-trnF} and \textit{ndhF-trnL}$_{UAG}$ sequences. Number of generations: 10 mln, sample frequency: 1000, burn-in: 2000, average standard deviation: 0.001572. The numbers beside nodes represent posterior probabilities. The scalebar represents the number of substitutions per site. The numbers inside the circles refer to the clades as treated in the text. The bars at the right of the tree mark the placement of the recircumscribed genera. Asterisks mark accessions of the type species.

**Appendix**

**Appendix 1:** Accessions used in the phylogenetic study. Entries are in the format ‘\textit{voucher name}, updated taxon name, collection locality, collection date, \textit{collector}, \textit{number}, (herbarium). ITS Genbank accession, \textit{trnL-trnF} Genbank accession, \textit{ndhF-trnL}_{UAG} Genbank accession’. The asterisks indicate sequences newly submitted to Genbank.


*KU204000. Boea hygroscopica 2, Boea hygroscopica F.Muell., Australia, Queensland, Palmerston NP, A. Weber, 810808-1/1, (WU). ITS: xxxxxxxx; trnL-trnF:


**Kaisupeea herbacea 3, Kaisupeea herbacea** (C.B.Clarke) B.L.Burtt, Thailand,
Kanchanaburi, Sai Yok, 7 viii 2012, D.J. Middleton & al., 5282, (BK, BKF, E). ITS: *KU203831; trnL-trnF: *KU203926. **Kaisupeea orthocarpa 1, Kaisupeea orthocarpa**
Kaisupeea orthocarpa B.L.Burtt, Thailand, Surat Thani, Phanom, 7 ix 2008, D.J. Middleton & al., 4356, (BK, E). ITS: *KU203833; trnL-trnF: *KU203928; ndhF-trnL \( ^{UAG} \): *KU204002. **Loxocarpus angustifolius 1, Loxocarpus angustifolius** Ridl.,
Malaysia, FRIM staff, FRI 56313, (KEP). ITS: *KU203824; trnL-trnF: *KU203919. **Loxocarpus angustifolius 2, Loxocarpus angustifolius** Ridl., Malaysia, Pahang,
Gunung Tahan, T.L. Yao, FRI 65288, (KEP). ITS: *KU203825; trnL-trnF: *KU203920. **Loxocarpus argenteus, Loxocarpus argenteus** B.L.Burtt, Malaysia,
Loxocarpus incanus R.Br., Malaysia, D.J. Middleton, 4379, (E). ITS: *KU203810; trnL-trnF: *KU203905. **Loxocarpus incanus 5, Loxocarpus incanus** R.Br., Thailand,
Nakhon Si Thammarat, 23 ix 2010, D.J. Middleton & al., 5517, (BK, BKF, E). ITS: *KU203811; trnL-trnF: *KU203906. **Loxocarpus incanus var. sekayuensis 1,**
Loxocarpus incanus var. sekayuensis (Banka & Kiew) T.L.Yao, Malaysia, Terengganu,
**Loxocarpus incanus var. sekayuensis 2, Loxocarpus incanus var. sekayuensis** (Banka & Kiew) T.L.Yao, Malaysia, Terengganu, Lata Sekyu Recreational Forest, T.L. Yao, *FRI 65445*, (KEP). ITS: *KU203812; trnL-trnF: *KU203907; ndhF-trnL*\(^{UAG}\):

*KU204014. Loxocarpus repens, Loxocarpus repens* B.L.Burtt, Malaysia, Sabah, Crocker Range Park, T.L. Yao, *FRI 65457*, (KEP). ITS: *KU203820; trnL-trnF:


**Loxocarpus sericiflavus 1, Loxocarpus sericiflavus** (Banka & Kiew) T.L.Yao, Malaysia, Johor, Sungai Yong, T.L. Yao, *FRI 57986*, (KEP). ITS: *KU203826; trnL-trnF: *KU203921; ndhF-trnL*\(^{UAG}\). *KU204010. Loxocarpus sericiflavus 2,


*Orchadocarpa lilacina* Ridl., Malaysia, Pahang, R. Kiew, *5410*, (KEP). ITS:


*KU203944. Paraboëa brachycarpa, Paraboëa brachycarpa (Ridl.) B.L.Burtt, Malaysia, Pahang, Lipis distr., Gua Bama, A. Weber, 870508-2/6, (WU). ITS:


ITS: JN934757; trnL-trnF: JN934715. Paraboëa crassifolia 1, Paraboëa crassifolia (Hemsl.) B.L.Burtt, China, Guangxi, Ma Shan, 24 viii 2006, M. Möller & Y.G. Wei, MMO 06-804a, (E). ITS: *KU203841; trnL-trnF: *KU203936; ndhF-trnL\textsuperscript{UAG}.


ITS: *KU203869; trnL-trnF: *KU203964. Paraboëa effusa, Paraboëa effusa
B.L.Burtt, Malaysia, Sarawak, Mulu, 14 viii 2010, C. Puglisi, CP 32, (E). ITS:
JN934760; trnL-trnF: JN934718. **Paraboea evrardii**, *Middletonia evrardii* (Pellegr.)

*KU203984. Paraboea ferruginea, Paraboea ferruginea* (Ridl.) Ridl., Malaysia,
Kedah, Pulau Langkawi, A. Weber, 860806, (WU). ITS: *KU203862; trnL-trnF:

*KU203957. Paraboea glabra, Paraboea glabra* (Ridl.) B.L.Burtt, Thailand, Krabi or
Phangnga, P. Triboun, s.n. (EDNA09_01765), (BK). ITS: JN934761; trnL-trnF:
JN934719; ndhF-trnL^UAG^: *KU204035. Paraboea glabrescens, Paraboea glabrescens*
(Barnett) C.Puglisi, Thailand, Kanchanaburi, Thong Pha Phum, 5 vii 2012, D.J.

**Paraboea glabrisepala, Paraboea glabrisepala** B.L.Burtt, Thailand, Chiang Mai, Doi
ITS: JN934762; trnL-trnF: JN934720. **Paraboea glanduliflora, Paraboea
glanduliflora** Barnett, Thailand, Chiang Rai, Fang, Doi Ang Khang, 21 ix 2008, D.J.

**Paraboea glandulosa, Paraboea glandulosa** (B.L.Burtt) C.Puglisi, Thailand,
Kanchanaburi, Thong Pha Phum, 28 x 2009, D.J. Middleton & P. Triboun, 5202G,
glutinosa, Paraboea glutinosa* (Hand.-Mazz.) K.Y.Pan, China, Guangxi, Xin Cheng,
23 viii 2006, M. Möller & Y.G. Wei, MMO 06-786a, (E). ITS: JN934764; trnL-trnF:
JN934722; ndhF-trnL^UAG^: *KU204025. Paraboea harrovia var. ovata, Paraboea
harrovia* ( Craib) Z.R.Xu var. ovata Z.R.Xu, Thailand, Prachuap Khiri Khan, Khao
trnL-trnF: JN934723; ndhF-trnL^UAG^: *KU204021. Paraboea havilandii, Paraboea
havilandii* (Ridl.) B.L.Burtt, Malaysia, Sarawak, Bau, Tai Ton, 21 vii 2010, C. Puglisi,
CP18, (E). ITS: JN934766; trnL-trnF: JN934724. **Paraboea hekouensis, Paraboea
hekouensis** Y.M. Shui & W.H. Chen, China, Yunnan, Hekou, ix 2012, Shui & al.,
94842, (KUN). ITS: *KU203843; trnL-trnF: *KU203938. **Paraboea incudicarpa,
Paraboea incudicarpa** B.L.Burtt, Thailand, Tak, Mae Sot, 11 ix 2009, D.J. Middleton
insularis, Paraboea insularis** Triboun, Thailand, Krabi, Ao Luk, P. Triboun, 3673,
1254 Paraboea trisepala W.H.Chen & Y.M.Shui, China, Guangxi Jing Xi, Y.M. Shui & al.,
1255 CH 153, (KIB). ITS: JN934778; trnL-trnF: JN934736. Paraboea umbellata,
1256 Paraboea umbellata (Drake) B.L.Burtt, China, Guangxi, Napo, 22 x 2001, M. Möller
1257 & Y.D. Qi, MMO 01-147/2, (E). ITS: JN934779; trnL-trnF: JN934737; ndhF-trnL^UAG.
1258 *KU204019. Paraboea variopila, Paraboea variopila Z.R.Xu & B.L.Burtt, Thailand,
1259 Nakhon Si Thaamarat, Thung Song, 11 ix 2010, D.J. Middleton & al., 5392, (BK,
1260 BKF, E). ITS: *KU203868; trnL-trnF: *KU203963 (partial); ndhF-trnL^UAG.
1261 *KU204029. Paraboea velutina, Paraboea velutina (W.T.Wang & C.Z.Gao)
1262 B.L.Burtt, China, Guangxi, Feng Shan, 4 vi 2007, M. Möller & Y.G. Wei, MMO 07-
1264 Paraboea verticillata, Paraboea verticillata (Ridl.) B.L.Burtt, Malaysia, Selangor,
1265 FRIM, FRI 48225, (KEP). ITS: JN934781; trnL-trnF: JN934739; ndhF-trnL^UAG;
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1267 Krabi, 11 ix 2008, D.J. Middleton & al., 4442, (E). ITS: JN934782; trnL-trnF:
1268 JN934740. Paraboea xylocaulis, Paraboea xylocaulis Triboun, Thailand, Krabi, Ao
1270 Rhabdothamnopsis sinensis 1, Rhabdothamnopsis sinensis Hemsl., China, Yunnan,
1272 trnL-trnF: *KU203924. Rhabdothamnopsis sinensis 2, Rhabdothamnopsis sinensis
1273 Hemsl., China, Sichuan, Luding, 17 viii 2009, M. Möller & P. Zhou, MMO 09-1613,
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1275 Rhabdothamnopsis sinensis Hemsl., China, ex cult. RBG Kew 19884866, (K). ITS:
1276 JN934794; trnL-trnF: AJ492302. Senyumia minutiflora, Senyumia minutiflora (Ridl.)
1277 Kiew, A.Weber & B.L.Burtt, Malaysia, Pahang, A.R. Rafidah, 55722, (KEP). ITS:
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1281 trnL^UAG: *KU203987. Somrania flava, Somrania flava D.J.Middleton & Triboun,
1282 Thailand, Surat Thani, Khao Sok, D.J. Middleton & al., 4324, (E). ITS: *KU203794;
1283 trnL-trnF: *KU203889; ndhF-trnL^UAG: *KU203988. Somrania lineata, Somrania
1284 lineata D.J.Middleton & Triboun, Thailand, Phangnga, Muang Phangnga, Tham Pha
1285 Phueng, 15 ix 2010, D.J. Middleton & al., 5434, (BK, E). ITS: *KU203793; trnL-