The generic concept of *Lotononis* (Crotalarieae, Fabaceae): Reinstatement of the genera *Euchlora*, *Leobordea* and *Listia* and the new genus *Ezoloba*

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**Abstract** *Lotononis* is a large and taxonomically complex genus of the tribe Crotalarieae, with the majority of its species occurring within southern Africa. In this study, sequence and morphological data of the rare *Lotononis macrocarpa* was added to existing data matrices for the Crotalarieae and these were re-analyzed using both parsimony and model-based (Bayesian) analyses. Molecular systematic data (nrITS and *rbcl*) indicated that *Lotononis* is polyphyletic, with *Lotononis sect. Euchlora* as sister to *Bolusia* and *Crotalaria* and with *L. macrocarpa* close to the ‘Cape’ group of the Crotalarieae. As a result, the genus *Euchlora* is here reinstated and the new genus *Ezoloba* is described herein to accommodate the anomalous *L. macrocarpa*. *Ezoloba* is distinct in its 5+5 anther configuration, exceptionally large fruit, paired stipules, minutely serrate bracts and the presence of bracteoles. Within the remaining species of *Lotononis*, both combined and separate analyses of the morphological and sequence datasets revealed three strongly supported clades corresponding largely to clades recovered in previous cladistic analyses based on morphological, chemical and cytological data. These are here recognised at the generic level as *Lotononis* s.str., *Leobordea* and *Listia* and the necessary new name combinations are formalised. Characters supporting these generic re-assignments are discussed and a key to the 16 genera now recognised in the tribe Crotalarieae is presented.

**Keywords** Crotalarieae; *Euchlora*; *Ezoloba*; Fabaceae; *Listia*; *Leobordea*; *Lotononis*; southern Africa; taxonomy

**Supplementary Material** Figures S1–S2 and Table S1 are available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

## INTRODUCTION

*Lotononis* (DC.) Eckl. & Zeyh. (tribe Crotalarieae) is a large and complex genus of 151 species (one recently described from Namibia by Van Wyk & Kolberg, 2008), ca. 144 of which occur in southern Africa (Van Wyk, 1991a). The classification system of the genus has been somewhat volatile since the original concept was established by Candolle in 1825, published as *Ononis* sect. *Lotononis* DC. This section was thereafter raised to generic level by Ecklon & Zeyher (1836), proposed numerous other genera to accommodate the species of what is today *Lotononis*. The genus *Euchlora* Eckl. & Zeyh. was described by Ecklon & Zeyher (1836) to accommodate an anomalous plant that was placed in the genus *Ononis* L. by Thunberg (1800) and in *Crotalaria* L. and later *Microtropis* E. Mey. by Meyer (1832, 1836). Bentham (1843) expanded the concept of *Lotononis* to include the genera described by Ecklon & Zeyher (1836) and Meyer (1836), with the exception of *Euchlora*, and a sectional classification was proposed which Harvey (1862) also followed in the treatment of the genus in the *Flora capensis*. Dahlgren (1864) discussed the taxonomic history and synonymy of *Euchlora* and mentioned the similarities between it and *Lotononis*, such as trifoliolate, stipulate leaves (in some forms of *Lotononis hirsuta* (Thunb.) D. Dietr.), a similar hair type and the warty upper suture of the pod. *Euchlora serpens* (E. Mey.) Eckl. & Zeyh. was subsequently transferred to *Lotononis* (as *L. serpens*) by Dahlgren (1964). The most recent revision of *Lotononis* by Van Wyk (1991a) follows Bentham (1843) to some extent but expanded the generic concept to include *Buchenroedera* Eckl. & Zeyh. Based on cladistic analyses of morphological, cytological and chemical data, a detailed infrageneric classification system was proposed, comprising 15 sections (Van Wyk, 1991a). This study, published as a series of papers and as a synopsis in 1991, represents the most rigorous treatment of the genus to date, and provided a framework for future studies on *Lotononis*.

*Lotononis* shares similarities with many of the genera in the tribe Crotalarieae (viz., *Crotalaria*, *Lebeckia* Thunb., *Peasonia* Dümmer, *Rothia* Pers.) and a sister relationship with *Crotalaria* was suggested by Van Wyk (1991b) based on the presence of macrocyclic pyrrolizidine alkaloids and rugose seeds.

Data presented by Boatwright & al. (2008a) showed that *Lotononis* is polyphyletic, through the analysis of nrITS and plastid *rbcl* sequence data which included 52 species of *Lotononis* representing all the currently recognized sections of the genus. However, the analysis of morphological characters in combination with the sequence data indicated that *Lotononis* was in fact weakly supported to be monophyletic if *L. hirsuta* (*L.* sect. *Euchlora* (Eckl. & Zeyh.) B.-E. van Wyk) was excluded. However, an anomalous species, *Lotononis macrocarpa* Eckl. & Zeyh.,
could not be included in this study seeing that it is extremely rare and several attempts to recollect the plant have failed. *Lotononis macrocarpa* possesses a unique combination of characters: paired stipules that are equal in size, a 5+5 anther arrangement, minutely serrate bracts, presence of bracteoles and large fruit. Subsequent to the study of Boatwright & al. (2008a) material of the species was obtained from a herbarium specimen of the only recent collection. The new sequence data, in combination with available data from Boatwright & al. (2008a) has allowed for a re-evaluation of the generic circumscription of *Lotononis*.

This paper is aimed at presenting a new generic classification system for *Lotononis* s.l. based on molecular and morphological evidence. A discussion of critical characters is presented along with systematic data on the placement of the anomalous *Lotononis macrocarpa*. The necessary new name combinations are made for 54 species. A key to the genera of Crotalarieae is also presented in which the changes at generic level formalised in this paper are incorporated.

**MATERIALS AND METHODS**

**DNA sequencing and phylogenetic analyses.** — Subsequent to a phylogenetic study of tribe Crotalarieae by Boatwright & al. (2008a), material of the rare and unusual *Lotononis macrocarpa*, a species which is pivotal in studying relationships within the genus *Lotononis*, was obtained from the Compton Herbarium, South Africa (voucher specimen: Helme 2076, NBG). The combined *rbcL/ITS/morphology* dataset of Boatwright & al. (2008a), with and without the morphological data, was used to evaluate relationships within *Lotononis* and assess the position of *L. macrocarpa*. DNA of this anomalous species was extracted using a DNeasy Plant Minikit (Qiagen Inc., Hilden, Germany) and sequenced following the procedures outlined in Boatwright & al. (2008a). These sequences of ITS (GenBank accessions FM875935 and FM875936) and *rbcL* (GenBank accession FM875934) were added to the matrices of Boatwright & al. (2008a; available on TreeBASE, study number S2070), which required minimal adjustments to the original alignment and the 31 morphological characters included in the latter study were also polarized for *L. macrocarpa* for inclusion in the morphological matrix. Parsimony (MP) analyses were conducted in PAUP* using a heuristic search with 1000 random sequence additions, tree-bisection-reconnection (TBR) branch swapping and the MULTREES option in effect, keeping only 10 trees per replicate. Character transformations were treated as unordered and with equal weighting (Fitch parsimony, Fitch, 1971). Trees collected in the 1000 replicates were used as starting trees for another similar search, but without a tree limit, to test whether the shortest trees were obtained in the first search. Delayed transformation character optimization (DELTRAN) was used to calculate branch lengths. Internal support was estimated with 1000 bootstrap replicates (Felsenstein, 1985) using TBR and holding 10 trees per replicate. The following scale was used to evaluate bootstrap support percentages (BP): 50%–74%, low; 75%–84%, moderate; 85%–100%, strong. Congruence of the separate datasets was evaluated through visual inspection of the individual bootstrap consensus trees as well as incongruence length difference tests (ILD) as described in Boatwright & al. (2008a). Although this test indicated significant difference between the datasets (*P* = 0.001 for the combined molecular dataset and *P* = 0.002 for the combined *rbcL/ITS/morphology* dataset) visual inspection indicated no ‘hard’ incongruence. Following suggestions of Seelanan & al. (1997) and Wiens (1998), together with indications of the possible unreliability of the ILD test (Reeves & al., 2001; Yoder & al., 2001), the datasets were combined directly. Bayesian MCMC analysis (BI; Yang & Rannala, 1997; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; MrBayes v.3.1.2.) was performed using the GTR + I + G model for the molecular data as selected by Modeltest v.3.06 (Posada & Crandall, 1998) using the corrected Akaike information criterion (Akaike, 1974) and a total of three million generations with a sampling frequency of 100. The ‘standard’ model (default parameters) was used for the morphological data (Lewis, 2001). The analysis was terminated after the standard deviation of split frequencies fell below 0.01. One fourth of the resulting trees were discarded as the burn-in and a majority-rule consensus tree produced from the remaining trees to illustrate the posterior probabilities (PP) of all observed bipartitions. The following scale was used to evaluate the PPs: 0.50–0.84, low; 0.85–0.94, moderate; 0.95–1.0, strong. The GenBank accession numbers for all the taxa of Crotalarieae used are listed in Boatwright & al. (2008a).

**Evolution of morphological characters.** — The patterns of evolution of eight morphological, cytological and chemical characters (Appendix 1; polarisations included as Supplementary Appendix S1) traditionally regarded as apomorphies for *Lotononis* s.l. were examined by reconstructing these onto the majority rule consensus tree produced by BI for both the combined molecular and combined *rbcL/ITS/morphology* datasets. The character reconstructions were done using parsimony in Mesquite v.2.5 ( Maddison & Maddison, 2008).

**RESULTS**

**Combined ITS/rbcL dataset.** — The combined molecular dataset consisted of 1854 included characters, 1401 of which were constant, 453 variable and 276 parsimony-informative. A total of 560 trees were retrieved using MP with a tree length (TL) of 1056, consistency index (CI) of 0.55 and a retention index (RI) of 0.82. The MP and BI analyses produced similar topologies, except for some differences in the ‘Cape’ group (Fig. 1A). The topology of the BI phylogram is similar to that retrieved by Boatwright & al. (2008a), with the exception that *Lebeckia* was monophyletic, albeit with weak support (PP 0.50). The
‘Cape’ group, including *Lotononis macrocarpa*, was strongly supported (PP 0.98). In the MP analysis *Rafnia* was sister to the *Aspalathus-Wiborgia* clade followed subsequently by *Lebeckia* and *Calobota* with *Lotononis macrocarpa* in a position outside the ‘Cape’ group, but without support. In this analysis *Lebeckia* was not monophyletic as in Boatwright & al. (2008a) with *L. pauciflora* not included in the main *Lebeckia* clade. In both MP and BI analyses *Lotononis* was polyphyletic with *Lotononis* s.str. (L. sect. *Lotononis* and allies) sister to *Pearsonia*, *Robynsiophyton* R. Wilczek and *Rothia*, i.e., the *Pearsonia* clade (51 BP; PP 0.97). *Lotononis* s.str. was strongly supported to be monophyletic (100 BP; PP 1.0). The *Leobordea* Del. clade (*Lotononis* sect. *Leobordea* (Del.) Benth. and allies) was sister to the *Listia* E. Mey. clade (*Lotononis* sect. *Listia* (E. Mey.) B.-E. van Wyk excluding *L. macrocarpa*) with a BP of 75 and PP of 1.0, and both these groups were strongly supported as monophyletic (99 BP; PP 1.0 and 100 BP; PP 1.0, respectively). *Lotononis hirsuta* (sect. *Euchlora*) was strongly supported as sister to *Bolusia* Benth. and *Crotalaria* (100 BP; PP 1.0).

**Combined ITS/rbcL/morphological dataset.** — The analysis of the combined molecular and morphological matrix included 1885 characters, 1401 of which were constant, 484 variable and 404 parsimony informative. The resulting 370 trees from the MP analysis were 1185 steps long, had a CI of 0.52 and a RI of 0.84. The MP and BI analyses produced similar topologies with some differences in the ‘Cape’ group (Fig. 1B). The topology differed slightly from that obtained by Boatwright & al. (2008a), largely within the ‘Cape’ group. *Aspalathus* L. and *Wiborgia* Thumb. were sister to each other and both monophyletic (98 BP; PP 1.0 and 94 BP; PP 1.0, respectively). *Wiborgiella inflata* and *W. mucronata* were not included in the main *Wiborgiella* Boatwr. & B.-E. van Wyk clade in the BI tree, although in the MP consensus tree these are included in *Wiborgiella*. Sister to the *Aspalathus-Wiborgia* clade in the BI tree is *Calobota* followed by *Wiborgiella*, *Rafnia* and *Lebeckia*. In the MP consensus tree *Wiborgiella* was sister to the *Aspalathus-Wiborgia* clade followed by *Lebeckia* and *Rafnia* which were sister without support and finally *Calobota*. The ‘Cape’ group was weakly supported (69 BP; PP 0.98) and *Lotononis macrocarpa* placed in a position sister to this group, but without bootstrap support and weak PP of 0.63. With the exclusion of *Lotononis hirsuta* and *L. macrocarpa*, the rest of *Lotononis* s.l. was weakly or moderately supported as monophyletic, as opposed to being polyphyletic in the combined molecular analysis (72 BP; PP 0.92). *Lotononis* s.str. (100 BP; PP 1.0), the *Leobordea* clade (100 BP; PP 1.0) and the *Listia* clade (100 BP; PP 1.0) were strongly supported, with the *Leobordea* and *Listia* clades strongly supported to be sister (86 BP; PP 1.0). *Lotononis hirsuta* (sect. *Euchlora*) was strongly supported as sister to *Bolusia* and *Crotalaria* (100 BP; PP 1.0).

**Evolution of morphological characters.** — The reconstructions of the eight morphological, cytological and chemical characters onto the BI trees from the combined molecular analysis are indicated in Figs. 2 and 3 and as supplementary data (Figs. S1–S2) for the combined molecular/morphological data. Stipules are largely absent from the ‘Cape’ group and only some taxa of the *Lotononis* and *Crotalaria* clades (character 1; Fig. 2; Fig. S1). Asymmetrical stipules are found mostly in *Lotononis* s.l. and reconstructed as an apomorphy for this group on the combined rbcL/ITS/morphology tree (Fig. S1), while on the tree from the combined molecular data the character is convergent between *Lotononis* s.str. and the *Leobordea* and *Listia* clades, with a reversal in *Lotononis* sect. *Aulacanthus* (E. Mey.) Benth. and sect. *Polylobium* (Eckl. & Zeyh.) Benth. in both analyses. Only one species of *Rothia*, *R. hirsuta* Baker, has single stipules. Loss of bracteoles (character 2; Fig. 2; Fig. S1) is convergent between *Lotononis hirsuta* and the other *Lotononis* clades, while the *Listia* clade is unique in having bracteoles. A zygomorphic calyx (character 3; Fig. 2; Fig. S1) is present in the *Lotononis* clades and *Pearsonia* with a reversal to an equal or subequal calyx in *Robynsiophyton* and *Rothia*. An anther arrangement (character 4; Fig. 2; Fig. S1) of 4+1+5 is most frequent in the ‘Cape’ group with *Lebeckia* and *Wiborgiella* species distinct in having 5+5 and 4+6 arrangements, respectively. This character is variable within *Lotononis* s.l. with only the *Listia* clade having an exclusively 4+1+5 arrangement. A verrucose upper suture of the pod (character 5; Fig. 2; Fig. S2) shows multiple convergences within the tribe and is present in species of all the *Lotononis* clades, except the *Listia* clade. The presence of long funicles (character 6; Fig. 2; Fig. S2) was reconstructed as an apomorphy for *Lotononis* s.l. in the combined rbcL/ITS/morphology analysis, but is also present in *Lotononis macrocarpa*. A chromosome base number (character 7; Fig. 3; Fig. S2) of x = 9 appears to be the plesiomorphic state in the *Crotalariaeae* with a reduction to x = 8 in *Crotalaria*, *Rafnia* and some *Aspalathus* species, while x = 7 is found in *Lotononis* s.str. and *Pearsonia*. Cyanogenesis (character 8; Fig. 3; Fig. S2) is unique to *Lotononis* s.str. with a reversal in *L. sect. Cleistogama* B.-E. van Wyk

**DISCUSSION**

**Evolution of characters.** — Polhill (1976) and Van Wyk & Schutte (1995) discussed the distribution of characters within *Crotalariaeae* and related tribes, but given the high incidence of convergence, some doubt has remained about the circumscription of certain genera, e.g., *Lebeckia* and *Lotononis*. The new insights into generic delimitations and relationships from this study and those of Boatwright & al. (2008a, 2009) allow for a reevaluation of diagnostic characters and apomorphic states.

**Stipules.** — A loss of stipules is autopomorphic for some species of the *Leobordea* clade, *Pearsonia* and *Crotalaria* where stipules are largely present. Asymmetrical stipules are unique to the lotononoid groups, i.e., *Lotononis* s.str., together with the *Leobordea* and *Listia* clades (Fig. 2; Fig. S1) and *Rothia hirsuta*, while *Lotononis hirsuta* and *Lotononis macrocarpa* are conspicuously different in having symmetrical stipules (when present in the former). In *Rothia hirsuta*, the stipules are single as opposed to being paired in *R. indica* (L.) Druce (Boatwright & al., 2008b). Asymmetrical stipules were reconstructed as an apomorphy for *Lotononis* s.l. in the combined molecular/morphological analysis, with a reversal in species from *Lotononis* sect. *Aulacanthus* and sect. *Polylobium*, but as a convergent character in the combined molecular analysis.
Fig. 2. Parsimony-based reconstructions of A stipule symmetry (character 1), B bracteole presence or absence (character 2), C calyx symmetry (character 3), and D anther arrangement (character 4) on the majority-rule consensus tree from the combined molecular analysis.
Fig. 3. Parsimony-based reconstructions of A verrucose upper suture presence or absence (character 5), B funicle length (character 6), C chromosome base number (character 7), and D presence or absence of cyanogenenic glucosides (character 8) on the majority-rule consensus tree from the combined molecular analysis.
Bracteole presence. — Bracteoles are present in all genera of the ‘Cape’ group, the Listia group, Lotononis macrocarpa, Pearsonia, Bolusia and Crotalaria. The loss of bracteoles appears to be a largely convergent character within the tribe and not unique to Lotononis s.l. Bracteoles are absent in Lotononis sect. Euchlora, the Leobordea clade and Lotononis s.str., as well as Rothia and Robynsiophyton (Van Wyk, 1991a; Boatwright & al., 2008b; Boatwright & Van Wyk, 2009) and this was reconstructed as convergences between these groups in the combined molecular and combined molecular/morphological analysis (Fig. 3). Only four species of the Leobordea clade have bracteoles present (large in two species of Lotononis sect. Lipozygis; very small or vestigial in two species of Lotononis sect. Leptis (E. Mey. ex Eckl. & Zeyh.)).

Calyx type. — Two calyx types are found within the Crotalarieae; the lebeckoid calyx type (equally or sub-equally lobed) and the lotoconoid calyx type (upper and/or lateral lobes on either side fused higher up than the lower lobe to varying degrees). Lotononis s.str., the Leobordea clade, the Listia clade and Pearsonia all have calyces of the lotoconoid type, while the remaining genera have the lebeckoid calyx type. The distinction of Lotononis hirsuta and L. macrocarpa from Lotononis s.l. based on this character is notable.

Anther arrangement. — Anther dimorphism and arrangement are important within Crotalarieae and informative at the generic level (Boatwright & al., 2008a, 2009). Pearsonia, Rothia and Robynsiophyton are unique within the tribe in having uniform anthers that are all similar in shape and size. The rest of the tribe have dimorphic anthers with alternating dorsifixed and basifixed anthers. The size, shape and attachment of the carinal anther is important and three arrangements are found: 5+5 (carinal anther resembles the long, basifixed anther) present in Bolusia, Crotalaria, Lebeckia and Lotononis macrocarpa; 4+1+5 (the carinal anther intermediate between the dorsifixed and basifixed ones) present in Aspalathus, Calobota, Lotononis s.str., the Listia clade, Rafnia and Wiborgia; 6+4 (carinal anther resembling the dorsifixed anthers) present in Lotononis hirsuta, the Leobordea clade, Lotononis s.str. and Wiborgiella. Anther arrangement is fairly diagnostic for the genera within the ‘Cape’ group, but the distinction within Lotononis s.l. is not as clear (Fig. 2; Fig. S1).

Fruit. — A verrucose upper suture of the pod was thought to be unique to Lotononis s.l., but this character is convergent between the Leobordea clade, Lotononis s.str., L. hirsuta (sect. Euchlora) and L. macrocarpa (Fig. 3; Fig. S2).

Exceptionally long funicles are only found in species of Lotononis s.l. In the combined molecular/morphological analysis it is reconstructed as an apomorphy for Lotononis s.l., but it is also present in L. macrocarpa (Fig. 3; Fig. S2).

Chromosome base number. — Goldblatt (1981) suggested a base number of x = 9 for the tribe Crotalarieae, which seems to be likely from the reconstructions presented here, showing a reduction to x = 8 and x = 7 in some lineages (Fig. 3; Fig. S2). Crotalaria, Rafnia and some Aspalathus species have a number of x = 8, while some species of Lotononis s.str., Pearsonia and Rothia have a base number of x = 7. It is possible that this pattern may become more complex as more counts become available if, for example, living material of L. macrocarpa (Ezoloba) can be found.

Chemistry. — Chemical data are of important systematic value in the Crotalarieae (Van Wyk, 2003) and provide additional insight into generic relationships. Cyanogenesis was reconstructed as an apomorphy for Lotononis s.str. and is absent from the Leobordea and Listia clades, Lotononis hirsuta and L. macrocarpa (Fig. 3; Fig. S2). The superficially similar genera Pearsonia, Rothia and Robynsiophyton are acyanogenic. The presence of prunasin and related cyanogenic glycosides therefore strongly supports the exclusion of the non-cyanogenic groups from Lotononis.

Quinolizidine alkaloids are present in most genera of the tribe, but absent from Lotononis s.str. and Crotalaria, where they are seemingly replaced by macrocyclic pyrrolizidine alkaloids. The presence of pyrrolizidine alkaloids in some sections of Lotononis s.l. was thought to suggest a sister relationship with Crotalaria that also produces such alkaloids (Van Wyk, 1991a). The presence of these alkaloids appears to be a convergence between Lotononis s.str. and Crotalaria.

Lotononis hirsuta. — Lotononis sect. Euchlora is only distantly related to the rest of Lotononis. It is placed within a clade comprising Crotalaria and Bolusia (100 BP; PP 1.0) and this clade represents the earliest diverging lineage within the tribe. The placement of Lotononis hirsuta in this clade is supported by the strongly inflated pods and trifoliolate leaves (if present) with paired stipules that are equal in size found in these genera. Dahlgren (1964) transferred the species to Lotononis where it was treated as a section by Van Wyk (1991a). This anomalous plant differs markedly from species of Lotononis s.str., the Leobordea clade and the Listia clade by the large underground tuber (geophytic habit), simple, sessile leaves (in some forms), large pods and the subequally lobed calyx. The enormous underground tuber may be an adaptation possibly to survive periods of aridity or unfavourable weather conditions. Species of Lotononis sect. Polylobium and sect. Lipozygis (E. Mey.) Benth. are also somewhat tuberous with annual shoots produced from a fleshy, carrot-like root, but the extensive underground system of Lotononis hirsuta is not found in any of these sections. In Lipozygis the tuberous habit could be an adaptation to recurrent fires in the grassland habitat where these plants occur (Van Wyk, 1991a). The position of Lotononis hirsuta renders two important generic apomorphies for Lotononis s.l., to be the result of convergence, namely the loss of bracteoles and the verrucose upper suture of the pod (Van Wyk, 1991a).

Lotononis s.str. — With the exception of Lotononis sect. Oxydium, Lotononis s.str. is endemic to southern Africa. Two sections are mainly found in the eastern parts of southern Africa, L. sect. Krebsia and sect. Buchenroederia, while all the remaining sections are restricted to the Cape and Namaqualand. Lotononis sect. Monocarpa occurs in the north-western Cape, while sect. Lotononis, sect. Aulacanthus and sect. Polylobium are more or less restricted to the Western Cape Province. Lotononis sect. Cleistogama has a more eastern distribution in the Cape region (Van Wyk, 1991a). Lotononis s.str. is chemically distinct from the Leobordea and Listia clades in that its members are cyanogenic (except for L. sect. Cleistogama) and
accumulate macrocyclic pyrrolizidine alkaloids, the first of which is a synapomorphy for this group.

The Leobordea clade. — The Leobordea clade comprises Lotononis sect. Leobordea and its apparent relatives from Lotononis sect. Digitata B.-E. van Wyk, sect. Leptis, sect. Lipozygis, and sect. Synclistus. This clade is well-supported as sister to the Listia clade as was also shown by Van Wyk (1991a). The Leobordea clade shares with the Listia clade (and differs from Lotononis s.str. by) the rounded keel petals, acyanogenesis and a chromosome base number of $n = 9$, all plesiomorphic states. The two groups differ in the non-stoloniferous habit, single stipules that are sometimes similar to the leaflets, pubescent vegetative and reproductive parts, absence of bracteoles (except in four species) and 4+6 anther arrangement (very rarely 4+1+5) found in the Leobordea clade. The latter group is also characterised by its wide distribution range throughout southern and tropical Africa. Lotononis sect. Leptis and sect. Leobordea extend into the Mediterranean region of Africa, with L. genistoides (Fenzl) Benth. (L. sect. Leptis) extending into Europe and L. platycarpa (Viv.) Pic.-Serm. (L. sect. Leobordea) extending into Pakistan and the Cape Verde Islands. Lotononis sect. Synclistus and sect. Digitata both occur in the north-western Cape, while L. sect. Lipozygis occurs in the eastern parts of southern Africa (Van Wyk, 1991a).

The Listia clade. — The Listia clade is a very distinct group with a unique combination of characters: stoloniferous habit, paired stipules, presence of bracteoles and a 4+1+5 anther arrangement (Van Wyk, 1991a). The concept of Meyer’s (1836) monotypic genus Listia was broadened by Van Wyk (1991a) and included as a section of Lotononis. It is interesting to note that the species of Listia have lupinoid (sleeve-like) root nodules (as are also found in the genus Lupinus L.) and not the conventional types that are present in all other species of Lotononis (and indeed all Crotalarieae) hitherto investigated (Yates & al., 2007). The species of this group are distributed throughout southern and tropical Africa.

Lotononis macrocarpa. — Lotononis macrocarpa was included in Lotononis sect. Listia as a distinct subsection (L. subsect. Macarcpa B.-E. van Wyk) by Van Wyk (1991a) based mainly on the presence of bracteoles and superficial similarities with the other species in Listia that were taken at face value at the time. However, this anomalous species is unique in the 5+5 anther arrangement, equally lobed calyx, serrulate bracts and large fruit and seeds. The systematic placement close to the ‘Cape’ group of the Crotalarieae is demonstrated in this study. It is endemic to the south-western Cape and geographically isolated from the rest of the Listia group. Based on the unique combination of characters found in this plant and the fact that it allies with the ‘Cape’ group in the molecular study, it is here recognised as a monotypic genus.

Generic circumscription. — The close agreement of the relationships within Lotononis s.str. found by Boatwright & al. (2008a) and this study with those proposed by Van Wyk (1991a) is notable. The only difference is the inclusion of L. sect. Euchlora in Lotononis s.l. and the sister relationship proposed between L. sect. Oxydium and sects. Cleistogama and Monocarpa (Van Wyk, 1991a). Cladograms from Van Wyk’s (1991a) study, based on vegetative and reproductive morphology, cytology and chemical characters, also show Lotononis sect. Listia (the Listia clade) as sister to L. sects. Digitata, Leobordea, Leptis, Lipozygis, and Synclistus, i.e., the Leobordea clade. This clade is sister to L. sects. Aulacinthus, Buchenroedera, Cleistogama, Euchlora, Krebsia, Lotononis, Monocarpa, Oxydium and Polylobium, i.e., Lotononis s.str. (excluding sect. Euchlora).

Dahlgren (1970) discussed some examples of convergence and parallelisms in the tribe, but the results presented in this study have uncovered that the relationships within the Crotalarieae are even more complex (Boatwright & al., 2008a, 2009) and revealed more examples of convergent evolution of morphological and chemical characters. Characters thought to be unique to certain groups, especially Lotononis s.l., have proven to be shared by distantly related groups. Due to the extreme overlap of character states in the genera of the Crotalarieae, unique generic apomorphies are frequently not available and a combination of characters is necessary for generic circumscription. The original generic concept of Lotononis s.l. was shown to be polyphyletic (Boatwright & al., 2008a), largely due to the positions of L. macrocarpa and L. hirsuta and also the polyphylies of Lotononis s.l. shown by combined molecular evidence. Despite weak support for the monophyly of Lotononis s.l. in the combined molecular/morphological analysis, a narrower concept of Lotononis can be better circumscribed in light of the lack of generic apomorphies for Lotononis s.l. The recognition of smaller, monophyletic groups is now more practical and therefore the reinstatement of Euchlora, Leobordea and Listia is proposed and ‘Ezoloba’ described as a new genus to accommodate Lotononis macrocarpa. Unique combinations of characters are available to circumscribe these genera that are strongly supported as monophyletic in the phylogenetic analyses presented here.

TAXONOMIC TREATMENT

Key to the genera of the Crotalarieae

1. Stipules absent (if stipules rarely present then leaves acicular and keel spirally twisted) .................. 2
2. Calyx zygomorphic (upper and lateral lobes on either side fused higher up in pairs) ................. Lotononis (partly)
3. Style with 1–2 lines of hairs .................. Crotalaria
4. Style glabrous .......................... 3
5. Bracteoles absent, leaves (at least the basal ones) simple, flat and sessile; geophyte with large underground tuber
   4. Bracteoles present, leaves (simple and flat) not sessile; annuals, suffrutices or shrubs .......... 5
6. Leaves acicular, terete .......................... 6
7. Leaves digitate, unifoliolate or simple (flat, never terete) ....... Aspalathus
8. Ovary with more than 6 ovules, pods many-seeded ........ Lebeckia
7. Upper suture of pod asymmetrically convex ………………… 8
7. Upper suture of pod symmetrically convex ………………… 9
8. Plants glabrous except occasionally on bracts and bracteoles, usually turning black when dried ……………… Rafnia
8. Plants usually pubescent on all parts, if leaves glabrous then standard petal hairy and inner surface of calyx glabrous ………………. Aspalathus
9. Petals pubescent, if glabrous then plants strongly spinose-leaved shrubs; twigs green (bark formation late), leaves iso-bilateral ………… Calobota
9. Petals glabrous; twigs brown (bark formation early), if twigs rarely green then plant an annual fireweed; leaves dorsiventral ……………… 10
10. Fruits winged, indehiscent; carinal anther intermediate (anthers 4+1+5) ………………… Wiborgiella
10. Fruits without wings, dehiscent (if rarely indehiscent then ovary and fruit distinctly stalked); carinal anther resembles short anthers (anthers 4+6) ………………… Wiborgia
11. Style straight or rarely down-curved, anthers all similar in size and shape ………………… 12
11. Style curved upwards, anthers dimorphic ………………… 14
12. Stamens nine (five fertile and four lacking anthers) ………………… 16
12. Stamens 10 (all fertile) ………………… 13
13. Anthers monomorphic, prostrate annuals ……………… Rothia
13. Anthers slightly dimorphic, four basifixed, six attached slightly higher up, all elongate, perennial herbs or shrubs ………………… Pearsonia
14. Bracteoles absent ……………………………………… 15
14. Bracteoles present ……………………………………… 19
15. Geophyte with woody tuber; leaves (at least the basal ones) flat, simple and sessile ………………… Euchlora
15. Annuals, suffrutices, shrubs; if leaves flat and simple then not sessile ………………… 16
16. Stipules paired or absent. ……………………………… 17
16. Stipules dimorphic or single ……………………………… 18
17. Keel obtuse, hairy ……………………………………… Leobordea
17. Keel beaked, glabrous ……………………………………… Lotononis
18. Keel obtuse, hairy ……………………………………… Leobordea
18. Keel beaked, glabrous ……………………………………… Lotononis
19. Keel obtuse ………………………………………………… 20
19. Keel beaked or helically coiled ……………………………… 22
20. Stamipes single at each node; keel (and standard petal) densely hairy over most of the surface of the lamina …………………………… Leobordea
20. Stamipes paired at each node (sometimes dimorphic); keel and standard petal glabrous or with a few hairs only ……………… 21
21. Calyx subequally lobed; fruit more than 20 mm long; stems without adventitious roots; seeds ± 4 mm long; anthers 5+5 ………………… Ezoloba
21. Calyx zygomorphic (upper and lateral lobes on either side fused higher up in pairs); fruit less than 20 mm long; stems often with adventitious roots; seeds ± 1 mm long; anthers 4+1+5 ………………… Lista
22. Keel and style helically coiled through several turns ……………………………… Bolusia
22. Keel and style not helically coiled ……………………………… Crotalaria

Taxonomy


Listiae E. Mey. similis, sed stipulis aequimagnis, calyce subpariter lobato, 5+5 dispositione antherarum, leguminibus magnis (longioribus 20 mm) et seminibus (longioribus 4 mm) et habitu non stiliferi differt.

The generic concept proposed here is based on a unique combination of characters, namely the stipules that are equal in size, the sub-equally lobed calyx, presence of bracteoles, 5+5 anther arrangement, the very large fruit with a warty upper suture and the large seeds (Fig. 4). The bracts are often minutely toothed along the margins (Fig. 4C1, C2), a character not yet found in any other genera of the tribe. The molecular and morphological evidence presented here indicates that this species is more closely related to the ‘Cape’ group of Crotalariaeae than to Lotononis s.l. The single species is known from only a few localities in the Western Cape Province of South Africa. The generic name commemorates Ecklon and Zeyher who first described this species.


The genus is easily recognisable by the large underground tuber, simple, sessile leaves (in some forms) and the large, inflated pods (illustrations are provided by Dahlgren, 1964). The results presented by Boatwright & al. (2008a) and in this study indicate that this genus forms part of the early diverging elements of Crotalariaeae and that it is closely related to Bolusia and Crotalaria. The single species occurs in the Northern and Western Cape Provinces of South Africa.


The concept of *Listia* proposed here conforms to that of *Lotononis* sect. *Listia* subsect. *Listia* as described by Van Wyk (1991a), which includes seven species. The genus can be distinguished from especially *Ezoloba*, *Lotononis* s.str. and *Leobordea* by a unique combination of characters: stoloniferous habit, paired, dimorphic stipules, presence of bracteoles, 4+5+1 anther arrangement, largely glabrous petals and pods, the latter often folded like a concertina. The root nodules differ from all other members of Crotalarieae in being lupinoid (sleeve-like). The species mainly occur in the interior of southern Africa, but *L. angolensis* and *L. heterophylla* extend into central Africa.


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**Fig. 4.** *Ezoloba macrocarpa* [J. Burrows 5 (NBG), Voëlvlei Dam, Gouda, South Africa – A1, A2, D2, E2, F2, G2, I2; Ecklon & Zeyher 1271 (SAM), Clanwilliam, South Africa – B1, B2, D1, E1, F1, G1, H1, I1, J1. A, Leaf with stipules; B, flowers in side view; C, bracts and bracteoles; D, calyces (opened out with the upper lobes to the left); E, standard petals; F, wing petals; G, keel petals; H, anthers (H1 = long basifixed anther, H2 = carinal anther, H3 = short dorsifixed anther); I, gynoecium; J, fruit. Scale bars: 1 = 5 mm; 2 = 3 mm (bracts and bracteoles only); 3 = 1 mm (anthers only).
Van Wyk, 1991a; BM!, K!, herb. Hook., S!, TCD!, isolec-
totypes); Cape Province, ‘prope Wildschutshoek’, Drège
s.n. b (BM!, K!, S!).

    nov. = Lotononis marlothii Engl. in Bot. Jahrb. Syst. 10:
    26. 1888 – Type: South Africa, Cape Province, Griqualand
    West, Kimberley, Marloth 765 (K!, herb. Engl., lectotype,
designated by Van Wyk, 1991a; BOL!, K!, PRE!, isolec-
totypes).

5. Listia minima (B.-E. van Wyk) B.-E. van Wyk & Boatwr.,
    comb. nov. = Lotononis minima B.-E. van Wyk in S. Af-
    rican J. Bot. 54(6): 628. 1988 – Type: South Africa, Cape
    Province, Kenhardt Div., Jagbult, floor of Uilpan, Acocoks
    12664 (PRE!, holotype; K!, isotype).

6. Listia solitudinis (Dümm.) B.-E. van Wyk & Boatwr.,
    comb. nov. = Lotononis solitudinis Dümm. in Trans. Roy.
    Soc. South Africa 3(2): 297. 1913 – Type: South Africa,
    Vaal River, Wilms 400 (BM!, holotype).

7. Listia subulata (B.-E. van Wyk) B.-E. van Wyk & Boatwr.,
    comb. nov. = Lotononis subulata B.-E. van Wyk in Bothalia
    bridge over Vaal River on Potchefstroom Road, B.-E. van
    Wyk 2884 (PRE!, holotype; JRAU!, K!, MO!, S!, isotypes).

IV. Leobordea Del. in Laborde, Voy. Arabie Pétrée (“Delile,
    Fl. Arabie Pétrée”): 82, 86. 1830 emend. B.-E. van Wyk &
    Boatwr., emend nov. – Type: Leobordea platycarpa (Viv.)
    B.-E. van Wyk & Boatwr. (= Leobordea platycarpa (Viv.)
    B.-E. van Wyk & Boatwr.).

Note. – Leobordea is here reinstated and its concept
    greatly broadened to include Lotononis sects. Digitata,
    Lep-
    tis, Leobordea, Lipozygis and Synclistus. We were unable to
determine which of the two species names were published
first in 1830 and provisionally follow previous authors in ac-
    cepting Lotus platycarpos Viv. as the oldest available name.

The genus Leobordea now includes 51 species that occur
mostly in the eastern parts of South Africa and extend into
    tropical Africa and the Mediterranean region. They can be
distinguished from especially Listia by the habit that is never
    stoloniferous, the single stipules that are sometimes similar
to the leaflets, pubescent vegetative and reproductive parts,
abscence of bracteoles (except in four species) and the 4+6 an-
other arrangement (very rarely 4+5+1). After Lotononis s.str.
    which now comprises 91 species, this is the largest of the genera
proposed here. The species have been classified into distinct
sections by Van Wyk (1991a) based on cladistic analyses of
    morphological, chemical and cytological data. The number of
species sampled for the molecular studies does not allow for
a re-evaluation of the infrageneric classification system and
the sectional classification of Van Wyk (1991a) is followed
here. See Van Wyk (1991a) for complete species synonymies
as well as keys to the sections and species. Fortunately, the
nomenclature of the 91 species remaining in Lotononis s.str.
are not affected (see Van Wyk, 1991a).

    & Boatwr., comb. nov. = Lotononis sect. Digitata B.-E. van
digitata (Harv.) B.-E. van Wyk & Boatwr. (= Lotononis
digitata Harv.)

This section can be distinguished by the following combi-
nation of characters: prostrate shrubs or shrublets with the basal
parts of the branches woody, leaves mostly 5-foliolate with long,
sleender petioles, stipules single at each node, bracteoles absent,
calyx zygomorphic, keel petals obtuse and glabrous pods that
are stipitate to long-stipitate and flat, linear or falcate and some-
times plicate (Van Wyk, 1991a). The section includes six species.

1.1. Leobordea digitata (Harv.) B.-E. van Wyk & Boatwr.,
    comb. nov. = Lotononis digitata Harv. in Harvey &
    Sonder, Fl. Cap. 2: 52. 1862 – Type: South Africa, in some
    part of the eastern provinces, Capt. Carmichael s.n. (TCD!,
holotype).

1.2. Leobordea benthamiana (Dümm.) B.-E. van Wyk &
    Boatwr., comb. nov. = Lotononis benthamiana Dümm. in
    Africa, Cape Province, Little Namaqualand; Near Ookiep,
    Scully s.n. sub Herb. Norm. Austr. Afr. 1127 (K!, lectotype,
designated by Van Wyk, 1991a; BOL!, K!, isolec-
totypes), Scully 150 (BM!, isotypy), Morris s.n. sub BOL 5622
    (BOL!, K!, isotypes); Teinkopf, Schlechter 39 (BM!,
    BOL!, GRA!, MO!, isotypes).

1.3. Leobordea longiflora (H. Bolus) B.-E. van Wyk & Boatwr.,
    comb. nov. = Lotononis longiflora H. Bolus in J. Linn.
    Soc. 25: 159. 1889 – Type: South Africa, Cape Province,
    Namaqualand, Dowdle s.n. sub BOL 6568 (BOL!, holotype;
    K!, isotype).

1.4. Leobordea magnifica (B.-E. van Wyk) B.-E. van Wyk
    & Boatwr., comb. nov. = Lotononis magnifica B.-E. van


The most useful character to distinguish species of sect. *Leobordea* from other sections are the leaves that are opposite on the flowering nodes and not alternate as in all the other sections (Van Wyk, 1991a). The section includes six species.


*Note.* – As noted under the generic citation, the relative priority of *Leobordea newtonii* Del. in relation to *Lotus platycarpus* Viv. is in need of further investigation.


The species of this section are mostly distributed in central and southern Africa and extend into the Mediterranean region. They are perennial suffrutescent herbs, shrublets or annuals with the stipules single at each node, leaves predominantly 3-foliolate, the inflorescences few-flowered, bracteoles absent and the keel petals obtuse and hairy. The section includes 20 species.


Two subspecies are recognised:

3.2.1. **Leobordea adpressa** (N.E. Br.) B.-E. van Wyk & Boatwr. subsp. **adpressa** = *Lotononis adpressa* N.E. Br. subsp. **adpressa**.


3.6.1. **Leobordea decumbens** (Thunb.) B.-E. van Wyk & Boatw. subsp. **decumbens**.


3.12. **Leobordea maroccana** (Ball) B.-E. van Wyk & Boatw., **comb. nov.** = *Lotononis maroccana* Ball in J. Bot. 11: 302. 1873 – Type: ‘Oourika’, Ball *s.n.* (K!, upper specimen on sheet with illustration, lectotype, designated by Van Wyk, 1991a; BM!, K!, bottom of second sheet, isolectotypes), *Hooker* *s.n.* (K!, isosyntype); Morocco, ‘in regione inferiori Atlantis Majoris: prope Tasseremout’ *Ball* *s.n.* (K!, isosyntype); ‘Ait Mesan’, *Ball* *s.n.* (K!, 2 sheets, isosyntypes); ‘Amsmiz’, *Ball* *s.n.* (K!, 2 sheets, isosyntypes), *Hooker* *s.n.* (K!, isosyntype).


The bracts of this section are suffrutescent, pyrophytic herbs. The bracts are inserted near the middle of the pedicel rather than at the base as in the other sections (Van Wyk, 1991a). The section includes two subsections and ten species.


In this subsection the bracts are linear to narrowly lanceolate and bracteoles absent or vestigial (Van Wyk, 1991a). This subsection includes eight species.


*Leobordea* sect. *Synclistis* differs from the other sections of the genus in the dense heads of sessile flowers and the small, few-seeded and indehiscent pods that remain enclosed in the persistent calyx. The section includes nine species.


*Note.* – Thunberg described the broad bracts and flower colour as red, so that the identity of his plant as that later described as *L. rosea* is beyond doubt. The only Thunberg specimen in UPS is chosen as lectotype.


*Note.* – Thunberg described the flowers of this species as white which confirms the identity of his plant as the species generally known as *Lotononis bolusii* that has cream-coloured and not yellow flowers. The only Thunberg specimen in UPS is chosen as lectotype.


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**LITERATURE CITED**


Appendix 1. List of characters and character states for eight morphological, cytological and chemical characters scored for the accessions included in the combined molecular and molecular/morphological analysis. Characters were assessed from personal observations or the following literature sources (Polhill, 1974, 1976, 1982; Dahlgren, 1975, 1988; Schutte & Van Wyk, 1988; Van Wyk 1991a; Campbell & Van Wyk, 2001; Boatwright & al., 2008b, 2009, 2010; Boatwright & Van Wyk, 2009; Van Wyk & al., 2010).

(1) Stipule symmetry: symmetrical or absent = 0, asymmetrical or single = 1. (2) Bracteoles: present = 0, vestigial or absent = 1. (3) Calyx symmetry: lateral lobes not fused higher up = 0, lateral lobes fused higher up = 1. (4) Anther arrangement: 5+5 = 0, 4+1+5 = 1, 4+6 = 2. (5) Verrucose upper suture of fruit: absent = 0, present = 1. (6) Funicle length: normal = 0, exceptionally long = 1. (7) Chromosome base number: $x = 7 = 0$, $x = 8 = 1$, $x = 9 = 2$. (8) Cyanogenic glucosides: absent = 0, present = 1.