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Alectryon vitiensis: A New Species of Sapindaceae Endemic to Fiji

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Abstract. A new species of Alectryon Gaertn. (Sapindaceae) endemic to the Fijian archipelago is described as A. vitiensis Buerki, Lowry, Munzinger & Callm. based on morphological and molecular evidence. It can easily be distinguished from the two congeners currently known from Fiji by its smaller leaves, subsessile leaflets, apetalous flowers, and crested fruits. A phylogenetic analysis using ITS sequence data shows that the new species is closely related to two Australian endemics, A. diversifolius (F. Muell.) S. T. Reynolds and A. oleifolius (Desf.) S. T. Reynolds, but differs in having compound leaves covered with a golden indument. Moreover, the Australian taxa are associated with dry habitats, whereas the new species from Fiji is confined to evergreen humid forests. Among apetalous species (all of which belong to a well-supported clade), A. vitiensis morphologically most closely resembles the generic type, A. excelsus Gaertn., endemic to New Zealand, but they differ from one another in the type of indument covering their branches and leaves and the arrangement, shape, and nature of the indument on their leaflets; and they belong to different clades. The new species is provisionally assigned a conservation status of "Endangered" according to the IUCN Red List criteria.

Key words: Alectryon, conservation, Cupania group, IUCN Red List, molecular systematics, Pacific islands, Sapindaceae.

As part of a collaborative research program being conducted on the systematics, evolution, and biogeography of Sapindaceae in Southeast Asia (Buerki et al. 2013a, 2013b) and the Pacific islands (Buerki et al., 2012; Munzinger et al., 2013, 2016; Pierre et al., 2014), two field expeditions were conducted to the Fijian archipelago in 2011 and 2015 in order to collect material of the indigenous species and gain insights into their morphology and ecology as well as their relationships with other members of the family. During each of these trips, material was collected of a previously undescribed member of the genus Alectryon Gaertn. growing in humid evergreen forest on the island of Vanua Levu, initially in fruit and then, during the second expedition, in flower. Careful examination of the material deposited at SUVA enabled us to match these collections with an earlier flowering specimen (Dept. of Agriculture 19647) gathered in 1986 on the island of Viti Levu. In the most recent treatment of Fijian Sapindaceae (Smith, 1985), two species of Alectryon were recognized, A. grandifolius A. C. Sm.

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Alectryon comprises ca. 25 to 30 species distributed from eastern Malesia. Australia. New Zealand, and New Caledonia across the Pacific to Hawaii (Buerki et al., 2009). Australia is the center of diversity, with 15 species (13 endemic; Edwards & Gadek, 2001), while 10 species are reported in the area covered by the Flora Malesiana project (Leenhouts, 1988; Adema et al., 1994), and the remainder of the diversity is spread across islands in the Pacific (see Table 1 in Edwards & Gadek, 2001, for a summary of the number of species per region). Edwards and Gadek (2001) published a phylogenetic study of *Alectryon* based on molecular sequence data, but their limited sampling from outside the genus precluded inferences regarding its position within Sapindaceae, Buerki et al. (2012), in a broad study with representation from throughout the family that included samples of 12 species of Alectryon, showed that the genus belongs to a clade they recognized as the "Cupania group," within which it was placed as sister to the endemic New Caledonian genus Podonephelium Baill. (see Munzinger et al., 2013, for more details on this genus). Their sampling did not, however, include any material from Fiji. To confirm that our new entity indeed belongs to Alectryon and to enable a preliminary assessment of its phylogenetic relationships within the genus, we have generated a nuclear ITS sequence for one of the collections (Callmander et al. 941) and added it to the dataset used by Buerki et al. (2012). We limited our sequencing to ITS because in previous studies this region yielded a majority of the nucleotide polymorphisms that differed among species of Alectryon (Edwards & Gadek, 2001; Buerki et al., 2009, 2012, 2013b), suggesting that this marker would provide ample information to confirm the generic placement and evaluate the relationships of our new material.

Based on detailed examination of the morphological features of the material of Alectroon collected in Fiji and a careful comparison with other members of the genus, we have concluded that this entity is sufficiently distinct to warrant description as a new species, which we describe below as A. vitiensis Buerki, Lowry, Munzinger & Callm. Using information from the expanded molecular phylogenetic analyses, we also discuss the affinities of this new species within the genus and explore how phylogenetic relationships align with selected morphological characters. The new species is provided with a line drawing, color photos, and a preliminary risk of extinc-tion assessment using the IUCN Red List Categories and Criteria (IUCN, 2012).

MATERIALS AND METHODS

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Genomic DNA of Callmander et al. 941 was extracted from silica-gel-dried leaf material following the same

Table 1. Comparison of Alectron vitiensis Buerki, Lowry, Munzinger & Callm, with its phylogenetically most closely related species (A. diversifolius (F. Muell.) S. T. Reynolds and A. oleifolius (Desf.) S. T. Reynolds), species occurring in Fiji (A. grandifolius (A. C. Sm. and A. samoensis Christoph.), and the most morphologically similar species (A. excelsus Gaertn.). See text for more details.

	A. vitiensis	A. diversifolius	A. oleifolius	A. grandifolius	A. samoensis	A. excelsus
Corolla	absent	absent	absent	present	present	absent
Leaf type	compound	simple	simple	compound	compound	compound
Phyllotaxy of leaflets	opposite	N/A	N/A	opposite	opposite to subopposite	alternate (sometimes subopposite when young)
Leaf surface	with golden indument	glabrous	with white indument	rapidly glabrous	rapidly glabrous	with ferruginous indument
Fruit crests	present	absent	weakly developed	absent	absent	present
Geographic distribution	Fiji	Australia	Australia	Fiji	Fiji and Samoa	New Zealand
Phylogenetic position	clade BI	clade BI	clade BI	not evaluated	not evaluated	clade BIII

modified 2× CTAB method used by Buerki et al. (2009). A nuclear ITS sequence was then produced following the same primers and polymerase chain reaction (PCR) and sequencing protocols described in Buerki et al. (2009).

DNA SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

The program Geneious 8.1.3 (Biomatters, Auckland, New Zealand) was used to assemble complementary strands and verify software base-calling. The sequence of Callmander et al. 941 (GenBank accession number KY454869) was aligned using MUSCLE (implemented in Geneious; Edgar, 2004) into the ITS dataset of Buerki et al. (2012), which includes all the available sequences of Alectryon (representing a total of 12 species). Identifications of species of Podonephelium sampled in Buerki et al. (2012) have been updated based on taxonomic results of Munzinger et al. (2013). Phylogenetic inferences were carried out employing both max-imum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) analyses, performed using the facilities offered by the CIPRES portal in San Diego California, U.S.A. (http://www.phylo.org/>). The ML analysis was done using RAxML 8.1.11 (Stamatakis, 2006; Stamatakis et al., 2008) with a 1000 rapid bootstrap analysis followed by the search of the bestscoring ML tree in a single run. The default model, GTRCAT, was used to perform the ML analysis. The Bayesian MCMC analysis was performed in MrBayes 3.2 (Ronquist et al., 2012) and the best-fit model (GTR+G) was estimated using MrModeltest 2.3 (Nylander, 2004) and the Akaike information criterion (Akaike, 1974). Three Metropolis-coupled Markov chains with an incremental heating temperature of 0.2 were run for 10 hours on the CIPRES portal yielding 7,802,000 generations and sampled every 1000th generation. Each analysis was repeated twice starting with random trees. The MCMC sampling was considered sufficient when the effective sampling size was higher than 200, as verified in Tracer 1.4 (Rambaut & Drummond, 2007). After a burn-in period of 25% per run, the remaining trees were used to construct a majorityrule consensus tree and its associated Bayesian posterior probabilities (BPP).

MORPHOLOGY

In order to identify potentially informative morphological characters of the new entity of Alectryon and to assess its affinities with other members of the genus, material deposited in the following herbaria was examined: BM, G, K, MO, MPU, NOU, P, and SUVA. This was complemented by accessing digital images of type specimens through the Global Plants website (http:// plants.jstor.org/>), especially for Pacific island species with types at BISH and US. To encompass the full range of morphological variation of species across their distributional ranges, we also consulted the descriptions provided in several floras (Cheeseman, 1925; Reynolds, 1985; Smith, 1985; Adema et al., 1994). Data were compiled on key morphological characters for each taxon. The terminology used here follows Beentje (2016).

PECIES DISTRIBUTION MAP AND CONSERVATION ASSESSMENT

GPS coordinates were available for all collections except Dept. of Agriculture 19647, which was g localized a posteriori using Google Earth (freely available at http://www.google.com/earth/). A species distribution map was prepared using ArcGIS 10.3.1 software by ESRI, using the Fiji_Coast_BND shape file provided by ArcGIS REST Services Directory (http://services3. arcgis.com>). The preliminary risk of extinction asse ment of our new taxon was assessed using the IUCN Red List Categories and Criteria (IUCN, 2012). The extent of occurrence (EOO) and area of occupancy (AOO) were calculated by using the GeoCAT tool (Bachman et al., 2011) with a grid cell size of 2×2 km used to calculate

PHYLOGENETIC ANALYSES

The ML and Bayesian analyses based on ITS data rielded congruent overall tree topologies and inferred the same phylogenetic position for the sample of our new entity. Only the Bayesian majority-rule consensus tree is presented here (Fig. 1), but we have also indicated the bootstrap support (BS) values obtained from the RAxML analysis. The monophyly of Alectryon is confirmed with strong support (BPP: 1.00. BS: 99%), although its sister relationship with the New Caledonian endemic Podonephelium is only moderately supported by the ITS data alone (BPP: 0.79; BS: 78%). Within Alectryon, two moderately to well-supported clades are recovered (Fig. 1), clade A (BPP: 0.84, BS: 63%), comprising species whose flowers have petals, and clade B (BPP: 1.00, BS: 96%), all of whose members are apetalous. Three subclades are retrieved within clade B with varying levels of support (subclades BI, BII, and BIII), although the relationships among them are not resolved in the tree based on ITS data. The inclusion of the sample of the new taxon from Fiji within Alectryon is strongly supported by the molecular data, and it is placed in subclade BI, sister to two other apetalous species, A. diversifolius (F. Muell.) S. T. Reynolds and A. oleifolius (Desf.) S. T. Reynolds, both endemic to Australia (Fig. 1).

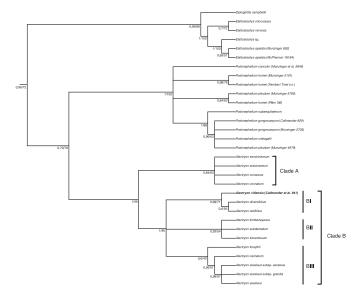


Figure 1. Bayesian half-compatible consensus tree of Sapindaceae inferred from the nuclear ITS region focusing on the phylogenetic position of Alectryon vitiensis Buerki, Lowry, Munzinger & Callm. (Callmander et al. 941). Bayesian posterior probabilities (BPP) and bootstrap support (BS) values are displayed at nodes. See main text for the discussion of the clades within Alectryon Gaertn.

MORPHOLOGY

The phylogenetic inferences presented above indicate that, among the taxa included in our sampling, our material is most closely related to two Australian species of Alectryon (A. diversifolius and A. oleifolius). While this result should be regarded as preliminary because we do not yet have comprehensive sampling from within the genus, including the two currently recognized species from Fiji (A. grandifolius and A. samoensis), it nevertheless provides a useful framework for comparing morphological features. Drawing on our observations of herbarium material (including scans of type specimens) and information gathered from the published literature, we compiled an extensive list of morphological characters in order to compare our new entity with an appropriate set of currently recognized species (see Table 1).

These include the two currently recognized species present in Fiji as well as the two taxa included in the phylogenetic study to which our novelly appears most closely related (A. diversifolius and A. oleifolius). Moreover, because the overall morphology of our new taxon most closely resembles that of the generic type, A. excelsus Gaertin., which is endemic to New Zealand, we also included it in the comparison, despite the fact that our phylogenetic analyses placed it in a different subclade within the apsetalous clade B (Fiz. 1).

Our new taxon can easily be distinguished from its apparent close relatives, Alectryon diversifolius and A. oleijolius, by its compound (vs. simple) leaves covered with a golden indument (vs. glabrous in A. diversifolius and with a white indument in A. oleijolius). The new entity differs from the two other species present in Fiji, A. grandifolius and A. samoensis, not only by being apetalous, but also by having leaflets that are shorter (11–19 cm vs. 45–90 cm in A. grandifolius and 30-40 cm in A. samoensis) and nearly subsessile (vs. petiolules 15 mm in A. grandifolius and > 10 mm in A. samoensis), along with crested fruits (vs. smooth in the other two taxa). Finally, while our new taxon shares apetalous flowers and crested fruits with A. excelsus, these two entities can easily be distinguished by the color of the indument covering their branches and leaves (golden in the novelty from Fiji vs. ferruginous in A. excelsus) as well as several aspects of their leaflets, including their arrangement (opposite in the new taxon vs. alternate in A. excelsus) and shape (elliptic vs. obliquely ovate-lanceolate), and the distribution of the golden indument (covered throughout vs. restricted to the venation below and glabrous on the upper surface).

Discussion

The results of our phylogenetic analyses agree with those of Edwards and Gadek (2001) in supporting the subdivision of Alectryon into two main clades characterized by flower morphology, one (clade A) in which the species have flowers with an evident corolla and another (clade B) that exclusively comprises apetalous species (Fig. 1). Unfortunately, we have not been able to sequence either of the two currently recognized species of Alectryon occurring in Fiji. We can, however, hypothesize that, based on the presence of a fully developed corolla in both $A.\ grandifolius$ and $A.\$ samoensis, they would belong to clade A. This hypothesis will have to be tested by including these species in a further expanded phylogenetic analysis, but the morphological evidence presented here strongly supports the conclusion that the newly collected material of Alectryon from Vanua Levu and the older collection of the same entity from Viti Levu do not belong to either of the previously described species in Fiii.

Our sample of the new taxon from Fiji is placed within a subclade of clade B that also includes two Australian species (Fig. 1), both of which were originally described in the genus Heterodendrum Desf. based on the presence of simple leaves and apetalous flowers (Reynolds, 1985) but were subsequently transferred to Alectryon by Reynolds (1987), a decision later confirmed by molecular phylogenetic evidence (Edwards & Gadek, 2001; Buerki et al., 2012). While our collections of the new Alectryon from Fiji share apetalous flowers with these two Australian species, as indicated above, they can easily be distinguished on the basis of several morphological characters (Table 1). Furthermore, these two Australian taxa occur in dry habitats, viz. in brigalow scrub

in the case of A. diversifolius and mixed woodland for A. oleifolius, according to Reynolds (1985), whereas the new Fijian species is known only from lowland evergreen humid forest.

Finally, while our new species resembles the New Zealand endemic Alectryon excelsus (Table 1), the phylogenetic inferences resulting from our analyses of ITS sequence data show that these two taxa are not closely related (Fig. 1). Our taxonomic novelty belongs to subclade BI, whereas A. excelsus is placed in subclade BIIII, where it is sister to the New Caledonian endemic A. carinatum Radlk. (Fig. 1).

The evidence provided above clearly supports the recognition of the material from Fiji as a new species, which we describe below as Alectron vitiensis. We accompany the formal description of this novelty with additional information on its habitat and conservation status.

TAXONOMY

Alectryon vitiensis Buerki, Lowry, Munzinger & Callm., sp. nov. TYPE: Fiji. Vanua Levu: Macuata Prov., Tikina Wailevu Region, Yavusa Sauniduna, Lambasa, near Nasaqa village, 16°33'24"S, 179°18'53"E, 500 m, 10 Feb. 2011, fr., Callmander et al. 941 (holotype, G [barcode] G00341663!; isotypes, BM!, M0-6703556!, MPU [bc] MPU310596!, SUVA).

Diagnosis. Alectryon vitiensis Buerki, Lowry, Munzinger & Callm, differs from other members of the genus by a combination of (11–1)2–19–cm-long paripinnately compound leaves with opposite, subsessile leaflets bearing golden indument on the veins; lateral racemose inflorescences densely covered with golden indument; apetalous flowers with a villous cally outside, and 1– to 2-lobed, crested fruits.

Tree, 3-8 m tall, trunk 8 cm diam., monoecious (or possibly andromonoecious); bark smooth, gray; branches ca. 3.5-6 mm diam. just below insertion of mature leaves, blackish, covered with golden indument. Leaves alternate, paripinnate, (11-)12-19 cm; petiole (2-)5.5 cm, ca. 1.5-2(-3) mm diam. at base, hirsute to sericeous; leaflets (4 to)5 to 7 pairs, opposite, blades medium to dark green on both surfaces (upper surface slightly darker), papyraceous, elliptic, 4–7 \times 1.2-3.5 cm, venation hirsute to sericeous with golden indument, base subcordate to cordate, margins entire, rarely slightly dentate distally when young, apex acute; petiolules very short, < 1 mm, slightly enlarged at the base. Inflorescence a lateral compound raceme, with solitary flowers or spikelets, or secondarily producing solitary flowers or spikelets by reinitiation in the axils of bractlets, 5-9 cm, hirsute with golden indument. Male flowers ca. 1.5-3 mm, borne singly or in pairs;

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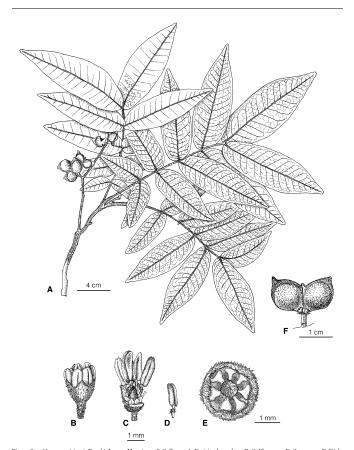


Figure 2. Alectryon vitiensis Buerki, Lowry, Munzinger & Callm.—A. Fruiting branch.—B, C. Flower.—D. Stamen.—E. Disk. —F. Pistillate spikelet. A, F based on Callmander et al. 941 (MPU); B–E based on Munzinger et al. 7376 (MPU). Drawn by L. Ramon.

bracts subtending the inflorescence axes linear or narrowly triangular, 2–3 mm, caducous, leaving an evident scar; pedicels 1–2.5 mm, shortly sericeous. Calyx cupuliform, toothed, lobes (4 to)5 to 6, triangular, 0.5 mm, greenish in vivo, brown in sicco, villous outside, puberulent inside. Petals absent. Disk present, ca. 1 mm diam., forming separate T-shaped portions equal in number to stamens. Stamens 3 to 6; filaments 0.5 mm at anthesis, shortly villous in upper half; anthers cream-colored, ca. 1.5 mm. Ovary vestigial, conical, ca. 0.5 mm high, densely sericeous. Female (or possibly hermaphroditic) flowers not seen.



Figure 3. Photographs of Alectryon vitiensis Buerki, Lowry, Munzinger & Callm. —A. Flowering branch. —B. Detail of flowers. —C, D. Fruiting branch. A, B from Munzinger et al. 7376; C from Callmander et al. 941; D from Callmander et al. 945. A, B photos by P. Lowry; C, D photos by M. W. Callmander.

Fruit 1- to 2-lobed, ca. $0.8-1.5 \times 1-1.8$ cm, narrowed to the base, crested, puberulent to hirsutulous; exocarp 0.2 mm thick, inner surface of locules glabrous; seed with a translucent arillode when young. (See Figs. 2 and 3 for more details on the morphology of $Alectryon\ vitiensis$.)

Etymology. The epithet refers to the distribution of the species, which is only known from the islands of Vanua Levu and Viti Levu in the Fijian archipelago.

Habitat, distribution, and phenology. Alectryon vitiensis is only known from Vanua Levu and Viti Levu, the two largest islands in the Fiji archipelago (Fig. 4). It

occurs in evergreen humid forest ranging between 110 and 550 m in elevation. Flowering material has been collected in March and November, whereas the fruiting collections were made in February (see Figs. 2, 3).

IUCN Red List category. With an EOO of 431.6 km², an AOO of 16 km², and three subpopulations representing three locations with respect to the main threat (logging), none of which are situated within Fiji's protected areas network and all of which are projected to face continuing decline in quality of habitat, Alectryon vitiensis is assigned a preliminary status of "Endangered" [EN B1ab](iii)+2ab(iii)] based on the IUCN Red

Figure 4. Species distribution map of Alectryon vitiensis Buerki, Lowry, Munzinger & Callm. in the Fiji archipelago.

List Categories and Criteria (IUCN, 2012). The new species appears to be rare and endemic to the lowland humid forests in the Fijian archipelago, which are

Paratypes. FIJI. Vanna Levu: Cakaudrove Prov., Wailevu Region, W of Savusavu, Waisali, 16'33'75.7'S. 179'12'52'TE, 510 m, 3 Feb. 2011, fr., Callmander et al. 945 (G. K. P. SUVA); Cakaudrove Prov., Wailevu-West distr., along Wailevu West coast rd., betw. Naviavia village & Keka village jet., 16'45'54''S, 179'05'0'0' E, 114 m, 6 Nov. 2015, fl., Manninger et al. 7376 (BM, G. K. I., MO, MPU [barcode] MPU310598, P. SUVA). Vii Levu: Serua Prov., Naboutini, 11.3 km on Nabukelevu rd., (interpreted as 18*09/31.41°S, 177:51*15.43°E), 300 m, 13 Mar. 1986, fl., Dept. of Agriculture 19647 (G. MPU [be] MPU310779, NOU [be] NOU054618, SUVA).

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R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com.

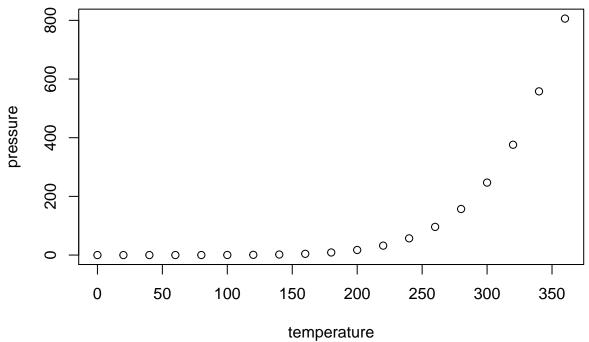
When you click the \mathbf{Knit} button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

summary(cars)

```
##
        speed
                          dist
           : 4.0
##
    Min.
                    Min.
                            :
                               2.00
    1st Qu.:12.0
                    1st Qu.: 26.00
##
##
    Median:15.0
                    Median : 36.00
##
    Mean
            :15.4
                    Mean
                            : 42.98
    3rd Qu.:19.0
                    3rd Qu.: 56.00
##
                            :120.00
##
    Max.
            :25.0
                    Max.
```

Including Plots

You can also embed plots, for example:



Note that the echo = FALSE parameter was added to the code chunk to prevent printing of the R code that generated the plot.



Alectryon vitiensis: A New Species of Sapindaceae Endemic to Fiji

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Figure 1: Alternate Name