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### A new record for Carpodetus (Rousseaceae) in Vanuatu

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#### Abstract

A molecular phylogenetic analysis of a plant specimen collected in Vanuatu (southwest Pacific) that was initially identified as *Alphitonia* (Rhamnaceae) because of its fruit morphology was found through molecular phylogenetic analysis to in fact belong to *Carpodetus* (Rousseaceae), a genus previously reported only from New Zealand, New Guinea and the Solomon Islands. The plant appears relatively distinct genetically from both species currently accepted in this genus, *C. serratus* and *C. arboreus*. However we cannot exclude the possibility that it belongs to one of the taxa currently placed in the synonymy of the polymorphic *C. arboreus*. Beyond the range extension of the genus, this observation also stresses the need for a revision of *Carpodetus*, particularly in New Guinea.

Keywords: Carpodetus, New Guinea, Rousseaceae, Vanuatu

Introduction

Rousseaceae sensu APG (2009) is an assemblage of four poorly known genera: the Mauritian endemic *Roussea* Sm. (monotypic), two Australian endemics *Abrophyllum* Hook f. (two species) and *Cuttsia* F. Muell. (monotypic), and *Carpodetus* J.R. Forst. & G. Forst. (two or more species). The latter genus has been reported from New Guinea, the Solomon Islands and New Zealand (Gustafsson 2007; Gustafsson & Bremer 1997) and has also been collected from the islands of Bougainville and Halmahera (Moluccas). The affinities of these four genera have mostly been uncovered using molecular phylogenetics (Gustafsson & Bremer 1997; Lundberg 2001). Alternative placements for *Carpodetus* in the past included Grossulariaceae, Escalloniaceae and Carpodetaceae.

The systematics of *Carpodetus* (syn. *Argyrocalymma* K. Schum. & Lauterb.) is somewhat obscure. IPNI (2013) lists a total of 13 specific epithets for the genus *Carpodetus*. Reeder (1946) recognized at least six species in New Guinea besides C. *arboreus* (K. Schum. & Lauterb.) Schltr. and also described *C. amplus* Reeder, apparently known only from the type, collected on Guadalcanal, Solomon Islands.. In contrast, van Royen (1983) recognized only two species, *Carpodetus serratus* J.R. Forst. & G. Forst. from New Zealand and C. *arboreus*. In the latter, he included all described species based on material from New Guinea, as well as *C. amplus*. Van Royen did not discuss the taxonomy of *C. arboreus* except for noting that it is a "very variable species in almost all details." *Carpodetus arboreus* sensu van Royen is particularly variable in the size and shape of its leaves and in its indumentum.

During 'Santo 2006', a multidisciplinary expedition to Vanuatu lead by IRD (Institut de Recherche pour le Développement), MNHN (Muséum National d'Histoire Naturelle, Paris) and Pro-Natura (a non-governmental organisation), new plant material was collected from Santo,

also known as Espiritu Santo, the largest and highest island of this archipelago. Several specimens had preliminary identifications as *Alphitonia* sp. (Rhamnaceae), because they had simple leaves and dark, globular, leathery fruits, c. 6-6.5 mm diameter, with an annular scar near their widest point and the lower part of the fruit suggesting a leathery cupule. The molecular evidence presented here, however, suggests instead an affinity with *Carpodetus*.

#### Materials and Methods

For one specimen, *Pillon et al.* 571, we sequenced three loci: the chloroplast coding *rbcL*, the chloroplast *trnL* region (*trnL* intron and *trnL-trnF* intergenic spacer) and the nuclear ribosomal transcribed spacer ITS (Genbank accession numbers: KF780482, KF780483 & KF780481), using lab protocols from Richardson et al. (2000) and Pillon et al. (2007). Alignment of these DNA sequences with other accessions of *Alphitonia* was problematic, and subsequent BLAST searches in Genbank indicated affinities with Carpodetus. We aligned the sequences from this plant with sequences available for Rousseaceae in Genbank (see Appendix) and one species of the sister-family Campanulaceae as an outgroup. The best-fit evolution models for *rbcL*, *trnL* and ITS were, respectively, GTR+I, GTR+ $\gamma$  and GTR+ $\gamma$ , according to jModeltest analyses (Posada 2008). We conducted two Bayesian phylogenetic analyses using MrBayes (Ronquist & Huelsenbeck 2003), one combining *rbcL* and *trnL* and one using the single ITS locus, and ran 1,000,000 generations of Markov Chain Monte Carlo (MCMC), sampling one tree every 1,000 generations, using the gene-specific substitution model and default settings otherwise. Loci were unlinked in the combined *rbcL+trnL* analyses. Convergence of runs and adequate MCMC sampling were checked using Tracer (Rambaut & Drummond 2007). The burn-in period per run was then set to 250,000 generations for both

datasets. The remaining trees were used to construct a half-compatible consensus tree and its associated estimated Bayesian posterior probabilities (figure 1).

#### Results

The phylogenetic analysis based on rbcL+trnL DNA sequences (figure 1) strongly supported the placement of the Vanuatu plant within Rousseaceae as sister to *Carpodetus serratus* (posterior probability of 1). In the ITS analysis (figure 1), where sequences were available for both the currently recognised species of *Carpodetus*, the plant was recovered as sister to *C. arboreus* (New Guinea) + *C. serratus* (New Zealand). This group of three terminals is strongly supported (posterior probability of 1), although their exact relationships are not confidently resolved.

#### **Discussion and Conclusions**

The phylogenetic analysis of the plant from Vanuatu places this specimen unambiguously in the genus *Carpodetus*, and this represents an important range extension for the genus and the family Rousseaceae (figure 2). Re-examination of the herbarium specimen, *Pillon et al.* 571, agreed with its placement in *Carpodetus*. Externally the fruits of *Alphitonia* and *Carpodetus* are quite similar; both are globose with a ring-scar at or just below the widest point. In most species of *Alphitonia*, however, the black pericarp contains a reddish spongy to powdery mesocarp surrounding three woody endocarpids, each of which dehisces to reveal a single, erect, often persistent, arillate seed (Bean 2010). The fruits of *Carpodetus*, in contrast, are indehiscent berries with numerous small angular seeds (van Royen 1983, Gustafsson 2007). Two other specimens collected during the same expedition, *Munzinger* 3720 & *Munzinger* 3896, also initially identified as *Alphitonia*, are conspecific with *Pillon et al.* 571. A further specimen, from the Royal Society New Hebrides expedition in 1971, *Gillison & Beveridge* RSNH 3524 (K and P), is also from Santo and belongs to the same taxon. This specimen has "*?Carpodetus*" typed on the collectors' label, although P.S. Green later annotated the sheet as "unknown." A scanned-image of the duplicate at P is available online

(http://sonneratphoto.mnhn.fr/2011/09/29/10/P03616858.jpg).

The morphogical variation within these four specimens is within the accepted range of the variable *Carpodetus arboreus*. It is not possible to ascertain the taxonomic status of *Carpodetus* sp. Vanuatu any further because of the taxonomic uncertainty within *C. arboreus*. There is over 40 base differences between the ITS sequence from the plant from Vanuatu and the sequences available in Genbank for the two species currently recognized, *C. arboreus* and *C. serratus*, suggesting that the former may represent a third species. However, we cannot exclude the possibility that it could be identical to one of the taxa described from New Guinea or the Solomon Islands currently placed in synonymy with *C. arboreus*. The genus *Carpodetus* therefore seems in need of a taxonomic revision that better reflects the morphological diversity present in New Guinea and the Pacific Islands.

Material seen: Vanuatu, Santo:

Gillison & Beveridge RSNH 3524 (K!, P[image]!

[http://sonneratphoto.mnhn.fr/2011/09/29/10/P03616858.jpg, accessed 1 December 2013]) Mt Tabwemasana, 5800 ft (~1770 m), wind damaged and slightly stunted forest. 27 August 1971 (fr.).

Munzinger 3720 (MO, NOU!, PVNH, SUVA) Penaorou Valley, 166°39'21.69''E 14°58'0.17''S,
900m, montane forest, volcanic rocks. Tree 15m. 7 November 2006 (fr.).
Munzinger 3896 (CANB, MO, NOU!, PVNH, SUVA) Penaorou Valley, 166°39'21.69''E
14°58'0.17''S, 900m, montane forest. Tree 5m. 12 November 2006 (fr.)
Pillon, Sam, Plunkett & Rouhan 571 (NOU!, P, PVNH) Mt Tabwemasana 166°43'40''E
15°20'10''S, 1000m, grassy ridge ("white grass"). Tree 15m. 6 November 2006 (fr.)

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Appendix. Genbank accession numbers for previously published DNA sequences of *rbcL*, *trnL* and ITS used in this study.

*Abrophyllum ornans*: AF299090, GQ984068, -; *Carpodetus arboreus*: -, -, DQ499091; *Carpodetus serratus*: Y08461, AJ430971, DQ499090; *Clermontia fauriei*: -,-, JX500338; *Cuttsia viburnea*: Y10676, GQ984071, DQ499092; *Hippobroma longiflora*: DQ356140, DQ356206, -; *Roussea simplex*: AF084477, AJ430977, -. Figure 1. Half-compatible Bayesian phylogenetic trees of Rousseaceae based on combined plastid *rbcL-trnL* and nuclear ribosomal ITS data. Trees were rooted with *Hippobroma* or *Clermontia* (Campanulaceae). Numbers at nodes are posterior probabilities.



Figure 2. Distribution of *Carpodetus* (Rousseaceae). The arrow indicates the novel locality on the island of Santo, Vanuatu.

