DATA NOTE

Complete plastome sequences of two Psidium species from the Galápagos Islands [version 1; peer review: 2 approved]

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Open Peer Review

Invited Reviewers

1. Michael O. Dillon, The Field Museum, Chicago, USA
2. Carolyn Proença, University of Brasilia (UnB), Brasilia, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

Abstract
We report the complete plastome sequences of an endemic and an unidentified species from the genus Psidium in the Galápagos Islands (P. galapageium and Psidium sp. respectively).

Keywords
plastome, Psidium, Galapagos, guayabillo

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Competing interests: No competing interests were disclosed.

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Introduction
Over a quarter of all vascular plant species are endemic to islands, making them hotspots of plant diversity and conservation (Kreft et al., 2008). In the Galápagos Islands, there are roughly 560 native species of plants of which approximately 32% are endemic (Lawesson et al., 1987). However, many of these endemic species have remained relatively unstudied since they were originally given scientific descriptions, making the study of the evolutionary histories of these unique taxa difficult. In the present study, we constructed the complete plastome sequences of two species of *Psidium* (guava) from the Galápagos Islands, one endemic and one currently unidentified in hopes of facilitating future work on the evolutionary relationships of these species.

Methods
This research is authorized under the permit: MAE-DNB-CM-2016-004 in compliance with Ecuadorian regulations.

Leaf samples were collected during May of 2017 from the Galápagos endemic *Psidium galapageium* Hook (commonly known as guayabillo) on the island of San Cristobal (0.89094°S, 89.43769°W) and from an unidentified *Psidium* species on the island of Santa Cruz (0.62313°S, 90.38581°W). Based on morphological similarity, the *Psidium sp.* individual is suspected to be *P. acidum* (Landrum, 2016), but no reference or barcode sequence from *P. acidum* is available for confirmation.

Leaf tissue was desiccated immediately after harvesting using silica gel. DNA extractions were performed using a Qiagen DNeasy Plant mini kit (Qiagen, Inc.). Sequence data was generated in the form of paired-end, 150 bp reads using a KAPA library prep kit (Roche Sequencing) and sequenced on an Illumina HiSeq 4000 platform (Illumina, Inc.).

Reads were quality and adapter trimmed using Trim Galore! version 0.4.3 with a minimum phred score value of 20 and minimum read length of 50 bp. Filtered reads were then aligned to the *Psidium guajava* plastome reference available at NCBI (Accession: KX364403) using the mem function within BWA version 0.7.15 (Li & Durbin, 2009). Consensus plastome sequences were generated using the mpileup function within samtools version 1.8 followed by the call and consensus functions within bcftools with a minimum depth of coverage of 10x (Li et al., 2009). Using IRscope (Amiryousefi et al., 2018), the *P. galapageium* and *Psidium sp.* plastomes respectively were confirmed to contain a large single copy of 88,268 bp and 87,747 bp and a small single copy of 18,465 bp and 18,490 bp separated by two inverted repeats of 26,071 bp and 26,360 bp for total lengths of 158,875 bp and 158,957 bp (Figure 1).

Annotations were generated using the program Plann (Huang & Cronk, 2015). Of the 132 gene features annotated previously in the *Psidium guajava* (guava) chloroplast genome on NCBI (Accession: KX364403), all were recovered in the *Psidium sp.* and *P. galapageium* plastome sequences. The non-identity of the two taxa sampled is evidenced by the absolute pairwise sequence divergence of the concatenated sequences of three conserved genes (MatK, psbA, and rbcL) which have been successfully used as barcodes previously in *Psidium* (Kress et al., 2009). Sequences were aligned using MUSCLE within MEGA version 7.0.26 (Tamura et al., 2007), and the number of nucleotide differences were counted between these alignments to estimate divergence. A total of 35 differences were observed among 4011 sites (0.87% uncorrected divergence) between *P. guajava* (Accession: KX364403) and *P. galapageium*, 45 differences (1.1%) between *P. guajava* and *Psidium sp.*, and 40 differences (0.99%) between *P. galapageium* and *Psidium sp.*

![Inverted Repeats](image-url)

**Figure 1.** Genomic structure of junction sites between the long single copy (LSC, light blue), short single copy (SSC, light green), and inverted repeat (iBa and iRb, orange) regions. Proximate genes are shown. The circular genomes have been linearized for illustration.
Data availability
Voucher specimens for *P. galapageium* and *Psidium* sp. are available at the Charles Darwin Research Station herbarium (Index Herbariorum code CDS) with accession numbers 3053515 and 3053562, respectively. The corresponding plastome sequences for *P. galapageium* and *Psidium* sp. are available at NCBI with accession numbers MH491846 and MH491847, respectively.

Grant information
This work was supported by a Louise Coker Fellowship from the University of North Carolina at Chapel Hill (UNC).

All funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Competing interests
No competing interests were disclosed.

References


Carolyn Proença  
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This appears to be a sound paper. Methods are modern, standard and referenced with versions cited when appropriate. Sequences are vouched by herbarium specimens and have been deposited in Genbank. I have checked the accession numbers given and they are correct. Figure 1 is very clear and detailed, and the legend is adequate and complete.

My most important comment is to point out that very recently, Landrum (2017, in Canotia 13:1-101) has treated *Psidium galapageium* Hook.f. as a synonym of widespread *P. oligospermum* DC. He comments in his paper that “As recognized here *Psidium oligospermum* is a widespread and variable species” and that “A geographically broad study with molecular techniques of *Psidium oligospermum*, including related species ... would be valuable.” Reatini’s paper relates directly to this comment by Landrum (2017) and the fact that the independent species status of *P. galapageium* is not accepted by all should be made. Other studies however (Proença *et al.* 2014 Flora de Sergipe: Myrtaceae; and Tuler *et al.* 2017, Flora of Espírito Santo: *Psidium* (Myrtaceae). Rodriguesia 68:1791-1805) do not include *P. galapageium* within *P. oligospermum*.

A minor quibble is with authorities for scientific names in the paper, that are either erroneous or missing. *Psidium galapageium* Hook.f., is the correct form (authority erroneously given in the paper as Hook who is actually a different botanist from Joseph Hooker who described the species). *Psidium acidum* (Mart. ex DC.) Landrum (authorities absent in the paper) is how this species should be cited.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Partly
Are the datasets clearly presented in a useable and accessible format?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Myrtaceae Systematics, Plant Phylogenetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Michael O. Dillon  
Integrative Research Center, The Field Museum, Chicago, IL, USA

This paper reports the results of sequencing efforts of a species native to the Galapagos Islands. The ability to distinguish new species and especially "crypto-species" that are not always obvious due to similar comparative morphologies. The paper should be published and this is the appropriate venue.

**Is the rationale for creating the dataset(s) clearly described?**
Yes

**Are the protocols appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and materials provided to allow replication by others?**
Yes

**Are the datasets clearly presented in a useable and accessible format?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Vascular plant systematist

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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