Two new species of leaf-toed geckos (*Phyllodactylus*) from Isabela Island, Galápagos Archipelago, Ecuador

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ABSTRACT

In this study, we (1) describe two new species of leaf-toed geckos (*Phyllodactylus*) from Isabela Island in the Galápagos Archipelago, (2) elevate the Mares Leaf-toed Gecko from subspecies (*P. galapagensis maresi*) to species status, (3) present an updated molecular phylogeny of the archipelago's *Phyllodactylus*, and (4) test the limits between gecko species in the archipelago. A unique combination of molecular and morphological characters support the validity of the new species. With these changes, the number of *Phyllodactylus* species reported in Galápagos increases to 12 (eleven endemic and one introduced).

INTRODUCTION

Unlike most reptile groups in the Galápagos Islands, the endemic leaf-toed geckos of the genus *Phyllodactylus* have received little attention from researchers. Only three major scientific works dealing with this group of lizards have been published.

In 1912, John Van Denburgh published the first compendium of the geckos of Galápagos.[1] He recognized six species of *Phyllodactylus* and three subspecies within the Galápagos Leaf-toed Gecko (*P. galapagensis*). In 1973, Benedetto Lanza published a detailed study of the differences in measurements and arrangement of scales among 14 populations of geckos in Galápagos, and described three new subspecies.[2] Recently, in 2014, Omar Torres-Carvajal and collaborators were the first to study the evolutionary relationships among the islands' leaf-toed geckos using DNA sequences.[3] These authors revealed possible colonization and diversification scenarios as well as recognized ten species of endemic *Phyllodactylus* in the archipelago. Of these, two were elevated from subspecies status and two were left as undescribed species.

To this day, the two undescribed gecko species, and possibly many more, remain without formal scientific names. As a result, these endemic Galápagos geckos have not received formal conservation status assessment and, thus, have not been the focus of targeted conservation actions despite facing several threats of extinction such as displacement by introduced geckos, predation by exotic species, and even volcanic activity.

To solve this problem in conservation and taxonomy, here we re-evaluate the validity of these candidate species. First, by generating an updated molecular phylogeny that includes DNA samples from new populations, and second, by using three different species delimitation approaches. Within this evolutionary framework, we assign names (one new, one revalidated) to the two geckos previously recognized as undescribed in Galápagos, and describe a previously unrecognized new species from Wolf Volcano in northern Isabela Island.

MATERIALS AND METHODS

Ethics statement. This study was carried out in strict accordance with the guidelines for the use of live amphibians and reptiles in field research.[4] All procedures with animals (see below) were reviewed by the Galápagos National Park Directorate (DPNG) and the Ministerio de Ambiente del Ecuador (MAE), and specifically approved as part of obtaining the following permits for research and access to genetic resources: PC-31-17, PC-54-18, and DNB-CM-2016-0041-M-0001.

Sampling. We obtained tissue samples, created photo vouchers, and generated DNA sequence data for ten individuals representing four *Phyllodactylus* populations in Isabela Island (Darwin Volcano and Wolf Volcano), Marchena Island, and Gardner Islet.

Laboratory techniques. We extracted genomic DNA from 96% ethanol-preserved tissue samples (tail muscle) following protocols described in Arteaga et al. (2018),[5] and amplified target regions (12S, 16S, and ND4) following procedures described Arteaga et al. (2018),[5] Gamble et al. (2008),[6] and Blair et al. (2009).[7]

DNA sequence analyses. We used a total of 210 DNA sequences (gene fragments 12S, 16S, ND4, RAG1, and c-mos) to build a phylogenetic tree of Ecuadorian *Phyllodactylus* (Fig. 1), of which 25 were generated during this work and 185 were downloaded from GenBank. We edited and assembled new sequences using the program Geneious ProTM 5.4.7[8] and aligned with those downloaded from GenBank (Appendix 1) using MAFFT v.79 under the default parameters in Geneious ProTM 5.4.7. Genes were combined into a single matrix with 11 partitions, one per non-coding gene and three per protein-coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 2[10] under the Bayesian information criterion. We assessed phylogenetic relationships under a Bayesian inference approach in MrBayes 3.2.0[11] following parameters described in Arteaga et al. (2018).[5] GenBank accession numbers are listed in Appendix 1. Genetic distances were calculated using the uncorrected distance matrix in PAUP 4.0.[12]

Species delimitation. We tested the limits within potential species of Ecuadorian leaf-toed geckos using three different species delimitation approaches following parameters described in Koch et al. (2016)[13]: (1) Automatic Barcode Gap Discovery (ABGD)[14]; (2) Bayesian implementation of the Poisson Tree Processes model (bPTP)[15]; and (3) Generalized Mixed Yule Coalescent (GMYC).[16]

Morphological data. We provide maximum total length, from tip of snout to tip of tail, for males and females of each species. These measurements were obtained from Lanza (1973)[2] or by examining comparative alcohol-preserved specimens from the herpetology collections at Fundación Charles Darwin (MVECCD) and the California Academy of Sciences (CAS). Diagnoses and descriptions of the new species of *Phyllodactylus* were based on: (1) the type series; (2) individuals released after tail-tip sampling (labeled JMG under Appendix 1); and (3) images of life individuals that were not collected or sampled.

Distribution maps. We present an overview of the distribution of all leaf-toed geckos in Galápagos as well as detailed distribution maps for the species described or re-described in this work. These maps were created as described in the plan of the book. For some gecko species, we present binary environmental niche models (ENM) to accompany or replace the dot maps. These were created following parameters described in Arteaga et al. (2016).[17]

RESULTS

Molecular phylogeny and taxonomic consequences. The overall topology and support (Fig. 1) of our phylogenetic tree is similar to that obtained by Torres-Carvajal et al. (2014),[3] with minor differences such as the positions of *Phyllodactylus leei* and *P. duncanensis*, as well as those of the newly sampled gecko populations. Our phylogeny, combined with morphological diagnostic traits, supports the existence of two unnamed species from Isabela Island. It also supports the validity of *P. maresi*, a species currently considered a subspecies of *P. galapagensis*.

DNA sequences from Marchena Island are grouped with those from Santiago Island. This clade is distinguishable from geckos on Santa Cruz (*Phyllodactylus galapagensis*) based on DNA sequence data (Fig. 1). Genetic divergence in a 307 bp long fragment of the mitochondrial 12S gene between *P. maresi* and *P. galapagensis* is 3–4%, whereas intraspecific distances are 0–1% in *P. maresi*. However, geckos that inhabit Marchena and Santiago are indistinguishable in coloration from, and overlap largely in number and arrangement of scales with, the geckos that inhabit Mares Islet (see Lanza [1973][2] for comparison between populations of Santiago Island and Mares Islet).

Mares Islet (see Fig. 3 for location of this small islet) has an area of 4,750 m^2 and is located ~900 m from the northeastern coast of Santiago Island. It is the type locality of *Phyllodactylus galapagensis maresi*.[2] Therefore, we propose that *P. g. maresi* be elevated to full species status (that is, *P. maresi*). This is the most parsimonious scenario given the available data and general biogeographic patterns on the islands; however, although morphologi-

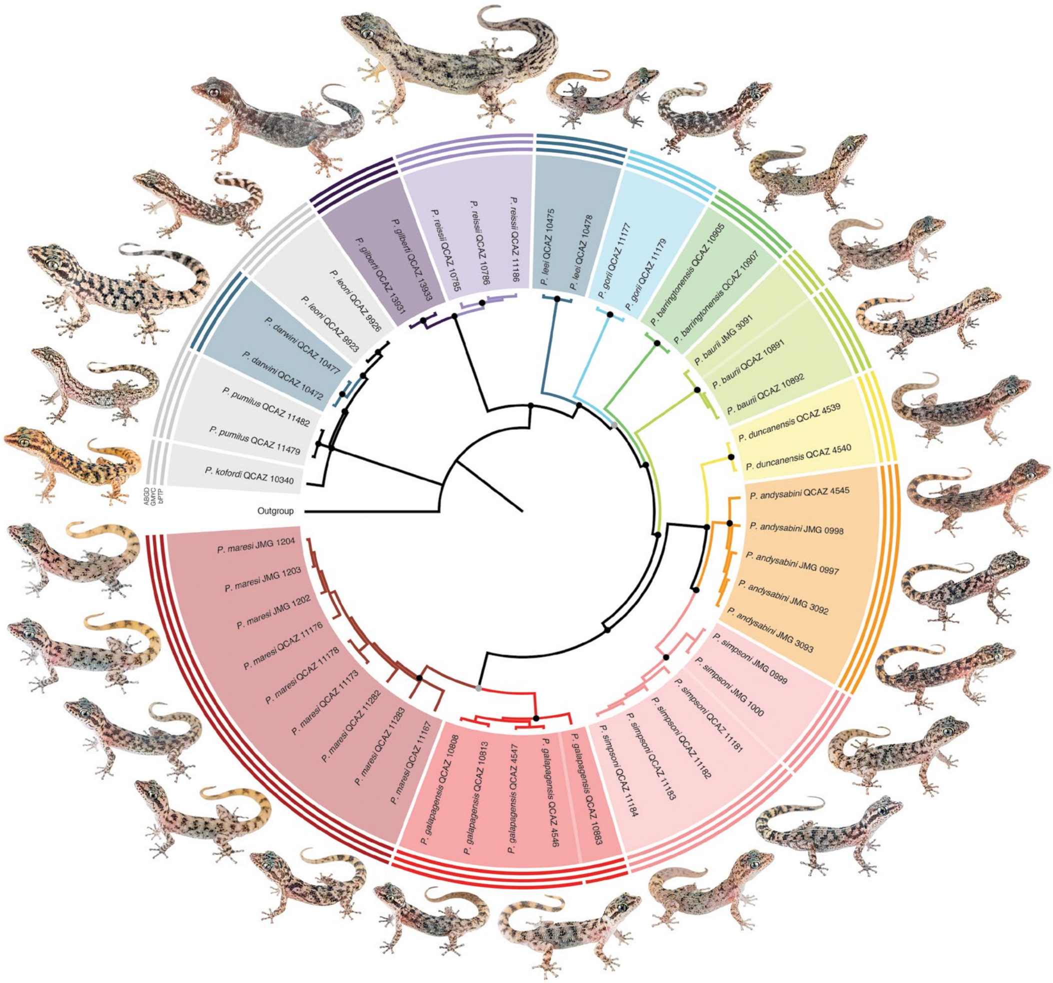

Figure 1. Bayesian consensus phylogeny depicting relationships among Galápagos leaf-toed geckos. The topology is derived from analysis of 2,958 bp of DNA (gene fragments 12S, 16S, ND4, RAG1, and c-mos). Voucher numbers for sequences are indicated for each terminal. Black dots indicate clades with 95–100% posterior probability values. Grey dots indicate values from 45–94%. Support values on some intraspecific branches are not shown for clarity. Colored branches represent lineages occurring in Galápagos, and each color corresponds to each species' distribution on the archipelago (see Fig. 2). Images of geckos adjacent to the labels of the tree correspond with the species indicated on the label, but not necessarily with the same voucher. Results of the species delimitations are illustrated by continuous semicircular bars. Each bar represents a species detected by one of three approaches (from the outside in, these are ABGD, GMYC, and bPTP).

cal data are unambiguous, the genetic assignment of the Mares Islet population to gecko populations that inhabit Bartolomé, Marchena, Santiago, and Rábida islands is still pending.

Species delimitation. Two of the approaches (GMYC and bPTP) we used to delimit species within Ecuadorian *Phyllodactylus* recognized the same number of confirmed species of geckos in Galápagos as Torres-Carvajal et al. (2014),[3] but they also recognized geckos from Darwin Volcano, Wolf Volcano, and Gardner Islet (off the coast of Floreana Island) as three distinct species. A third approach (ABGD) rec-

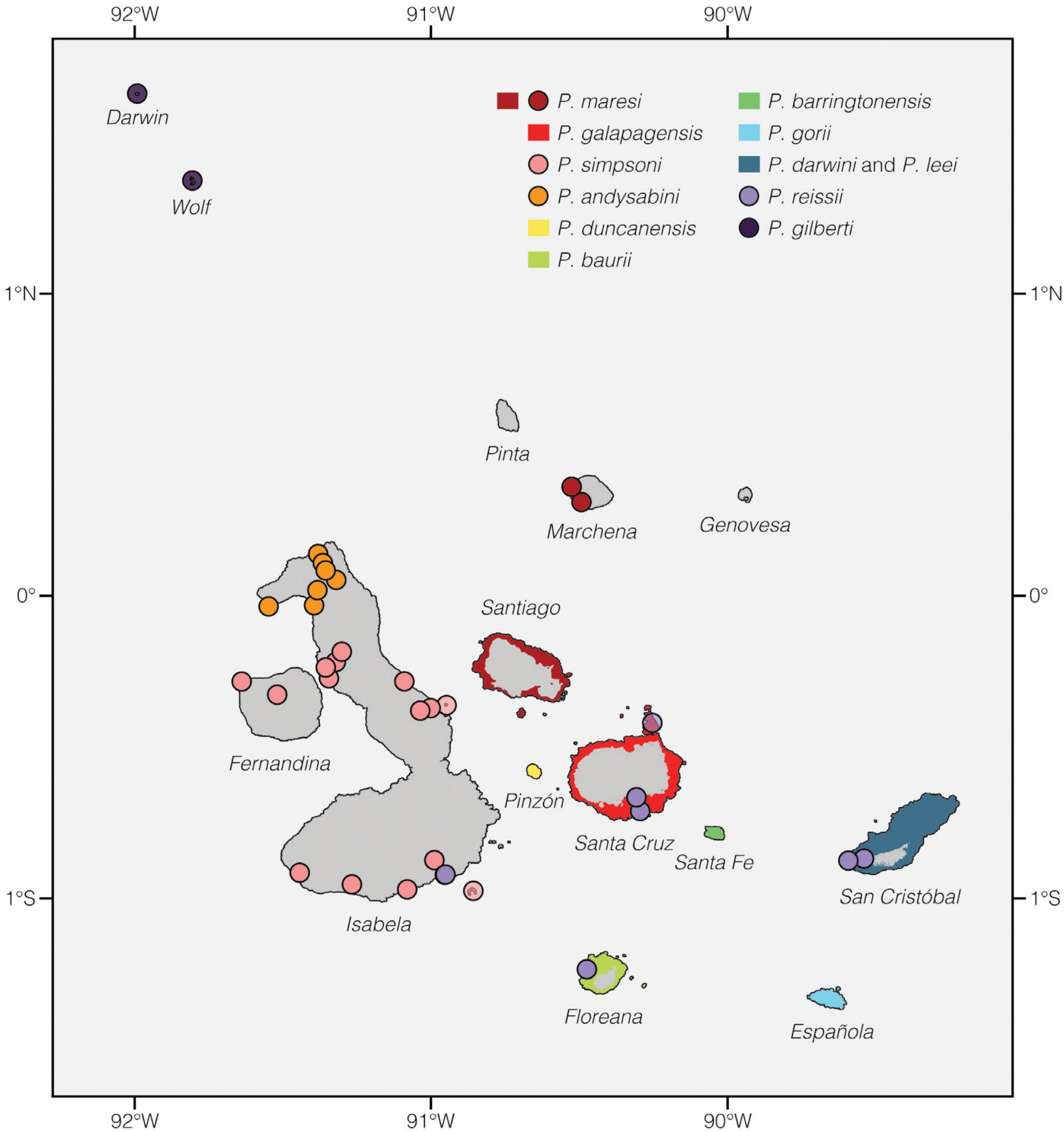

Figure 2. Distribution of leaf-toed geckos (*Phyllodactylus*) in Galápagos.

ognized the same limits, in addition to finding limits within *P. galapagensis* from Santa Cruz Island and recognizing *P. darwini*, *P. kofordi*, and *P. pumilus* as a single species.

Systematic accounts. We name species that are: (1) monophyletic, (2) identified as distinct by the three different species delimitation approaches implemented (Fig. 1), and (3) share diagnostic features such as coloration, shape of digits, and arrangements of scales. Based on these species delimitation criteria, which follow the general species concept of de Queiroz (2007),[18] we describe two new *Phyllodactylus* from Isabela Island and recognize the populations on Bartolomé, Marchena, Santiago, and Rábida islands, and Mares Islet as a valid species: *Phyllodactylus maresi*.

Phyllodactylus maresi **Lanza 1973**

Proposed English common name. Mares Leaf-toed Gecko.

Proposed Spanish common names. Geco de Mares, salamanquesa de Mares.

Holotype. MF (Museo Zoologico dell'Università di Firenze) 15325, an adult female collected by Benedetto Lanza on Mares Islet (-0.2356, -90.57467; 0 m), Galápagos, Ecuador.

Diagnosis. ♂♂ 7.6 cm ♀♀ 9.1 cm. We compare *Phyllodactylus maresi* to other geckos traditionally assigned to *P. galapagensis*. From *P. andysabini* sp. n., *P. simpsoni* sp. n., and *P. duncanensis*, it differs in having pointed tubercles on the top of the head and having asymmetrical pine-cone-shaped fingertips as opposed to blunt and symmetrical fingertips (Fig. 6). From *P. galapagensis*, it differs in having a higher (39–53 vs 27–45) number of tubercles in a paravertebral row from base of tail to head. A description of *P. maresi* and a more detailed comparison with *P. galapagensis* is given in Lanza (1973).[2] Genetic divergence in a 307 bp long fragment of the mitochondrial 12S gene between *P. maresi* and *P. galapagensis* is 3–4%, whereas intraspecific distances are 0–1% in eight individuals of *P. maresi*.

Natural history. Individuals of *Phyllodactylus maresi* have been found active by night on soil, rocks, leaf litter, and trunks of trees up to 2 m above the ground in areas of deciduous forest, dry shrubland, and dry grassland. During the daytime, individuals have been found under the bark of trees,[1] under rocks, and inside rotten logs. Members of this species are preyed upon by Thomas' Racers (*Pseudalsophis thomasi*).[19] Eggs of *P. maresi* have been found in crevices of an abandoned house in Santiago Island.

Etymology. The specific epithet *maresi* honors Lodovico Mares, an Italian businessman and Maecenas who funded the expedition that led to the discovery of the species on the islet now known as Mares.

Distribution. *Phyllodactylus maresi* is endemic to an estimated 226 km^2 area on four islands (Bartolomé, Marchena, Santiago, and Rábida) and Mares Islet in Galápagos, Ecuador, at elevations between 0 and 83 m above sea level (Figs 3 and 4).

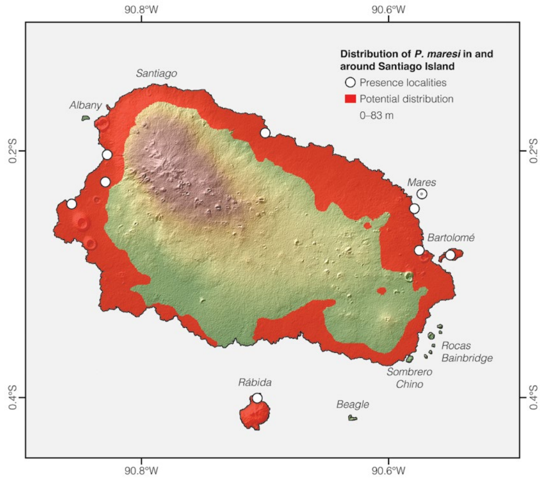

Figure 3. Distribution of *Phyllodactylus maresi* on and around Santiago Island, Galápagos, Ecuador.

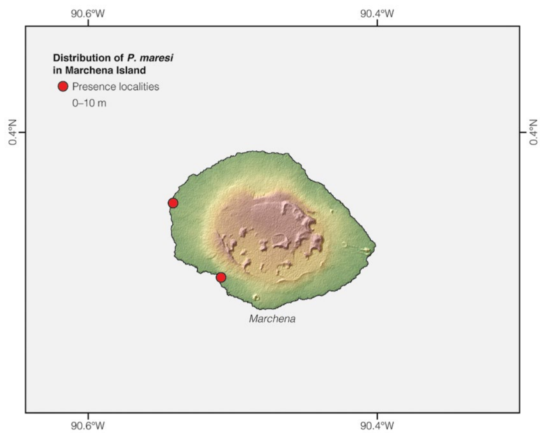

Figure 4. Distribution of *Phyllodactylus maresi* on Marchena Island, Galápagos, Ecuador.

Conservation. Least Concern. We provisionally consider *Phyllodactylus maresi* to be in this category following IUCN criteria because, given available data, the species does not seem to be facing major immediate threats of extinction. The islands where *P. maresi* occurs are not populated by humans nor major introduced lizard predators (such as cats) and all of these islands are protected within the Galápagos National Park. However, in Santiago Island, *P. maresi* is facing the threat of predation by introduced black rats.

Plate 64. Mares Leaf-toed Geckos from Santiago (▲) and Marchena (■) islands, Galápagos.

Phyllodactylus andysabini sp. n.

http://zoobank.org/BCC57F57-A51D-422D-AE9C-775F5A933A8A

Proposed English common names. Andy Sabin's Leaf-toed Gecko, Wolf Volcano Leaf-toed Gecko.

Proposed Spanish common names. Geco de Andy Sabin, salamanquesa de Andy Sabin, geco del Volcán Wolf, salamanquesa del Volcán Wolf.

Holotype. MVECCD 1924, an adult of undetermined sex collected by Alizon Llerena on the western slopes of Wolf Volcano (-0.00225, -91.39602; 576 m), Isabela Island, Galápagos, Ecuador, July 20, 2006.

Description of holotype. See Lanza (1973)[2] for an explanation of the following characters and scale counts: 11 scales between eye and nostril; 8/8 supralabials; 7/7 infralabials; mental bell shaped, bordered posteriorly by 3 postmentals; dorsal surfaces of head covered by granules of variable size, none of which are clearly enlarged; dorsum with 12 longitudinal rows of enlarged barely keeled tubercles; 49 tubercles in a paravertebral row between rear of head and base of tail; paravertebral rows separated by 3 rows of granules at middle of body; 6 longitudinal rows of tubercles at base of tail; 73 rows of ventral scales from gular region to vent; dorsal surface of forearm lacking enlarged tubercles; digits having barely enlarged, blunt, and symmetrical distal pads; snout-vent length 39 mm; tail length 32 mm. In ethanol, dorsal surfaces reddish brown with faint dark irregular cross-bars; dark brown postocular streak extends to 5–6 scales behind the tympanum; ground color of ventral surfaces cream with each scale having dark brown speckles.

Diagnosis. ♂♂ 7.8 cm ♀♀ 8 cm. This species is placed in the genus *Phyllodactylus* based on phylogenetic evidence (Fig. 1). The species is compared to other geckos traditionally assigned to *P. galapagensis*. From *P. galapagensis*, it differs in lacking pointed tubercles on the top of the head. From *P. maresi* and *P. duncanensis*, it differs in having blunt and symmetrical fingertips, as opposed to asymmetrical pinecone-shaped fingertips (Fig. 6). From *P. simpsoni*, it differs in having supranasals not in contact in 9 out of 9 individuals examined (versus in contact in 8 out of 14 individuals of *P. simpsoni*; Fig. 7) and having the throat densely stippled with dark brown pigment (versus immaculate throat in 9 out of 14 individuals of *P. simpsoni*; Fig. 8). Genetic divergence in a 181 bp long fragment of the mitochondrial 12S gene between the sister species *P. andysabini* and *P. simpsoni* is 3–5%, whereas intraspecific distances are 0% in five individuals of *P. andysabini* from two localities.

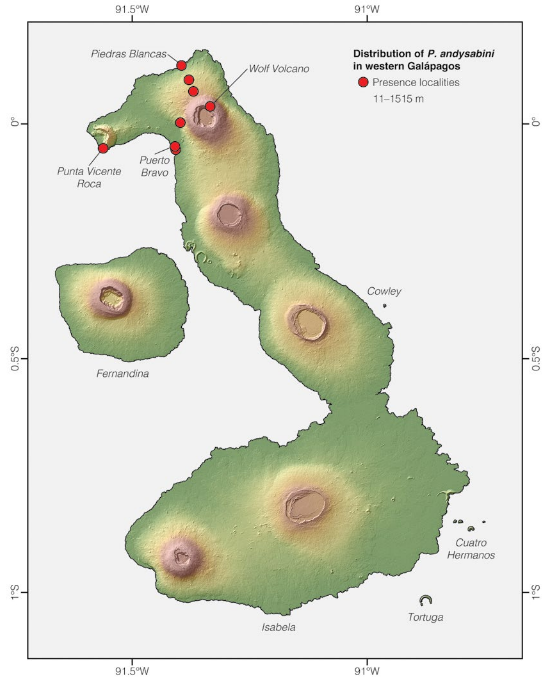

Figure 5. Distribution of *Phyllodactylus andysabini* in western Galápagos, Ecuador. DNA samples included in the genetic analyses come from Piedras Blancas and along the slopes of Wolf Volcano.

Natural history. Individuals of *Phyllodactylus andysabini* have been found active at night on soil, rocks, and tree trunks up to 40 cm above the ground in deciduous forests and evergreen foothill forests. Eggs have been found in holes up to 3 m above the ground in mangrove trees growing on the beach.[1]

Etymology. The specific name *andysabini* honors American philanthropist and con-

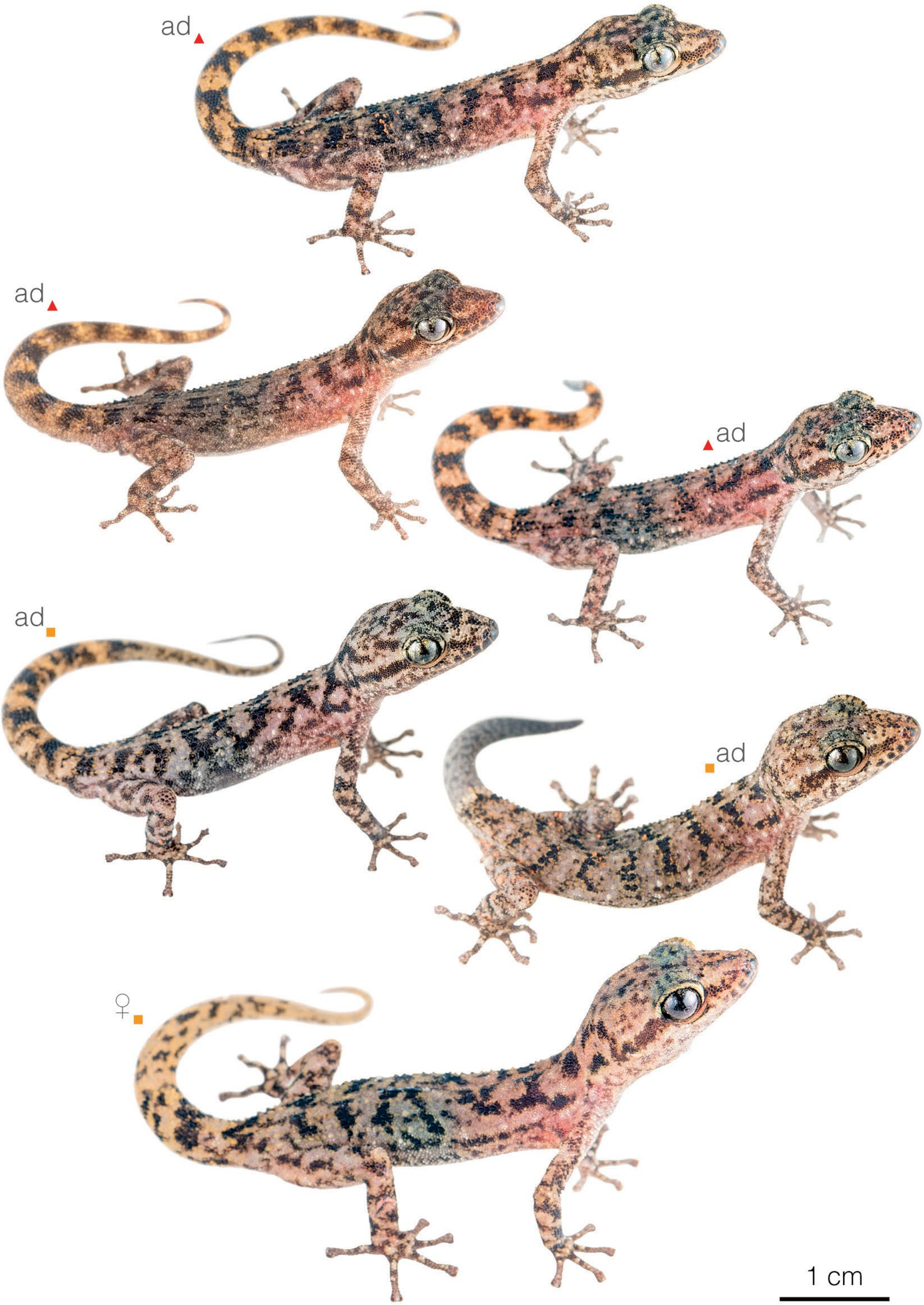

Plate 65. Andy Sabin's Leaf-toed Geckos from Piedras Blancas (▲) and slopes of Wolf Volcano (■), Galápagos.

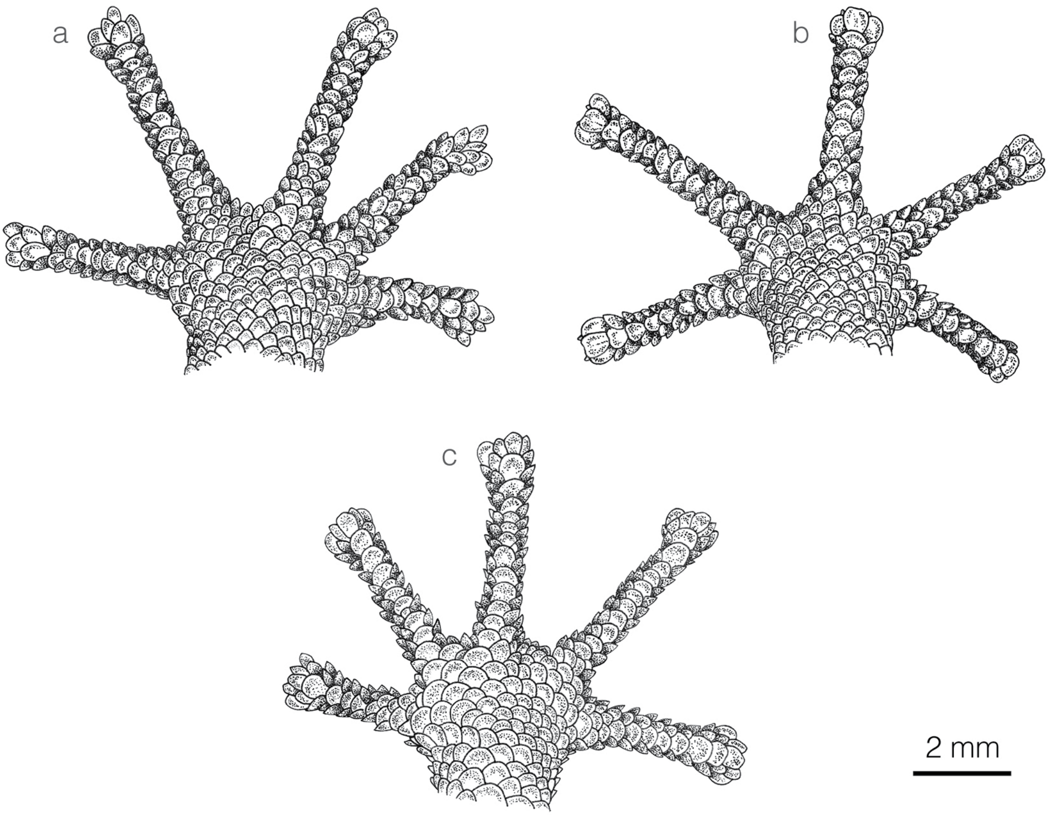

Figure 6. Shape of the fingertips in three leaf-toed geckos of Galápagos. (**a**) Pine-cone-shaped and asymmetrical, *Phyllodactylus maresi*. (**b**) Blunt and symmetrical, *P. andysabini*. (**c**) Pine-cone-shaped and symmetrical, *P. simpsoni*. Illustration by Valentina Nieto Fernández.

servationist Andrew "Andy" Sabin, known also as "Mr. Salamander," in recognition of his life-long support of environmental programs around the world and for his passion for the preservation of amphibians and reptiles. In addition to providing financial support, both personally and through the Andrew Sabin Family Foundation to hundreds of organizations, Andy is directly involved in conservation and field research. He has effectively protected over 264,365 acres of habitat and also participates in expeditions to remote places across the globe in search of new species.

Distribution. *Phyllodactylus andysabini* is endemic to an estimated 250 km^2 area on northern Isabela Island in Galápagos, Ecuador, at elevations between 11 and 1,515 m above sea level (Fig. 5). There are records of this species from the northern slopes of Wolf Volcano, from Piedras Blancas (0.12398, -91.39362; 11 m) to the rim of the volcano (0.03678, -91.33259; 1,515 m). Geckos from Puerto Bravo (-0.04843, -91.40742; 11 m) and Punta Vicente Roca (-0.05250; -91.56040, 15 m) are only tentatively identified as this species pending genetic assignment and upcoming field work.

Conservation. Endangered. We consider *Phyllodactylus andysabini* to be in this category following IUCN criteria because the species' area of occupancy is estimated to be no larger than 250 km^2, its habitat is fragmented by lava flows, and, although there is no information on population trends, there is an ongoing threat of decline in the number of mature individuals due to predation by introduced species

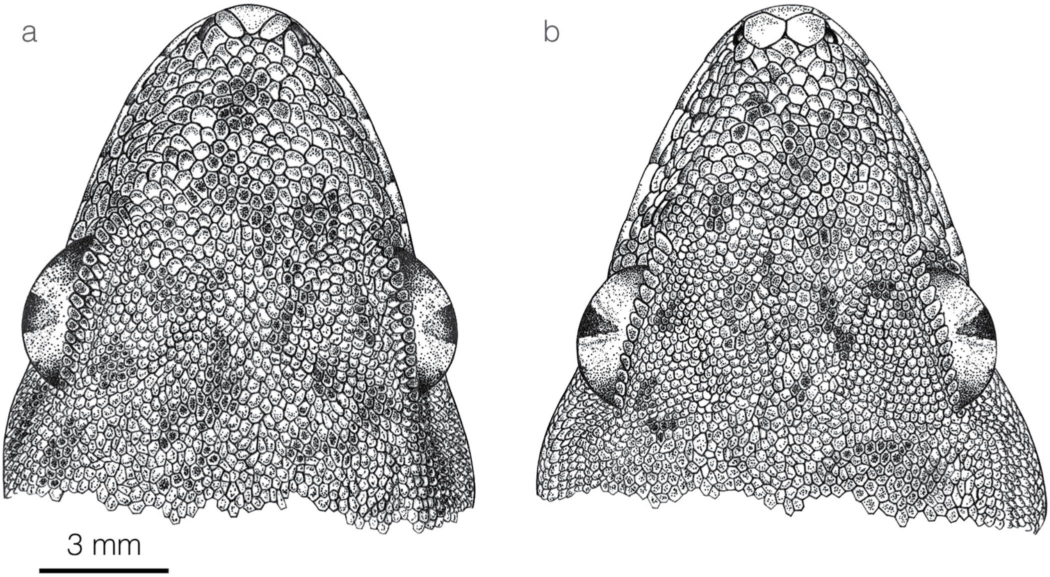

Figure 7. Condition of the supranasal scales (the large scales between the nostrils) in leaf-toed geckos of Isabela Island. (**a**) Not in contact, *Phyllodactylus andysabini*. (**b**) In contact, *P. simpsoni*. Illustration by Valentina Nieto Fernández.

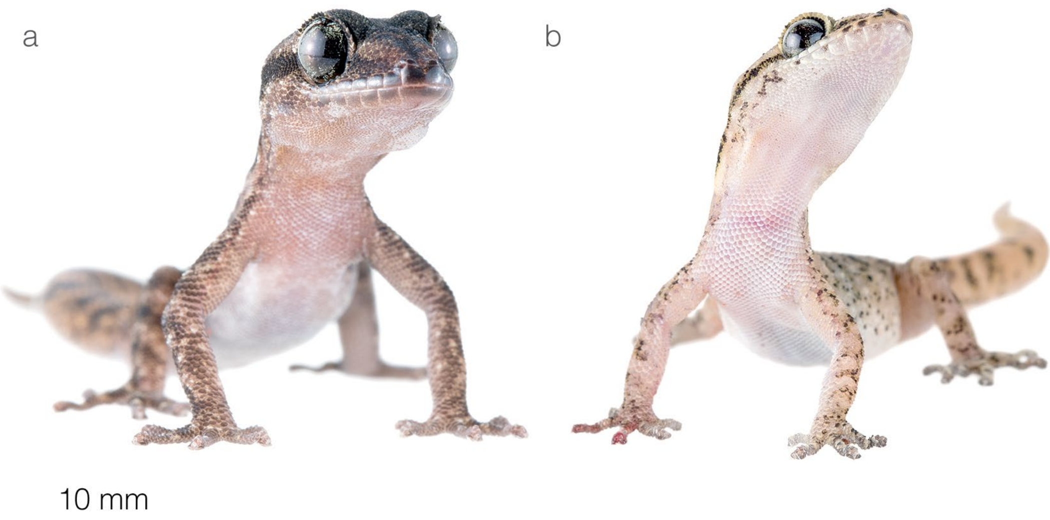

Figure 8. Throat pigmentation of two leaf-toed geckos of Isabela Island. (**a**) Densely stippled with brown pigment, *Phyllodactylus andysabini*. (**b**) Immaculate, *P. simpsoni*.

(cats and black rats). Alien predators are believed to have decimated the populations of other geckos on the Galápagos (see conservation section in the account of *P. duncanensis*). Volcanic eruptions are also a serious threat to *P. andysabini*. Wolf Volcano is in constant activity, with its last eruption recorded in May 2015.

***Phyllodactylus simpsoni* sp. n.**
http://zoobank.org/912E2C62-C91C-4BD1-ABE3-7FD5583C7FF8

Proposed English common names. Simpson's Leaf-toed Gecko, Western Galápagos Leaf-toed Gecko.

Proposed Spanish common names. Geco de Simpson, salamanquesa de Simpson, geco occidental de Galápagos.

Holotype. CAS 10339, an adult of undetermined sex collected by Joseph Slevin at Puerto Villamil (-0.95622, -90.97023; 3 m), Isabela Island, Galápagos, Ecuador, on November 1, 1905.

Paratypes. CAS 11245, an adult of undetermined sex collected by Joseph Slevin at the type locality on March 9, 1906. CAS 10351, an adult of undetermined sex collected by Francis Williams at the type locality on November 3, 1905.

Description of holotype. See Lanza (1973)[2] for an explanation of the following characters and scale counts: 12 scales between eye and nostril; 8/8 supralabials; 6/6 infralabials; mental bell shaped, bordered posteriorly by 3 postmentals; dorsal surfaces of head covered by granules of variable sizes, some of which are slightly enlarged but not pointed; dorsum with 12 longitudinal rows of enlarged keeled tubercles; 48 tubercles in a paravertebral row between rear of head and base of tail; paravertebral rows separated by 3 rows of granules at middle of body; 5 longitudinal rows of tubercles at base of tail; 70 rows of ventral scales from gular region to vent; dorsal surface of forearm without enlarged tubercles; digits with enlarged, pine-cone-shaped, and symmetrical distal pads; snout-vent length 37 mm; tail length 34 mm. In ethanol, dorsal surfaces grayish brown with faint dark irregular cross-bars; dark brown postocular streak extends to above the forelimbs; ground color of ventral surfaces cream with each scale having dark brown speckles.

Diagnosis. ♂♂ 10 cm ♀♀ 9.6 cm. *Phyllodactylus simpsoni* is placed in the genus *Phyllodactylus* based on phylogenetic evidence (Fig. 1). The species is compared to other geckos traditionally assigned to *P. galapagensis*. From *P. galapagensis*, it differs in lacking pointed tubercles on the top of the head and having fewer (71–80 vs 83–103) midbody scales (see Lanza [1973][2] for an explanation of this measurement). From *P. maresi* and *P. duncanensis*, it differs in having pine-cone-shaped and symmetrical fingertips, as opposed to asymmetrical pine-cone-shaped fingertips (Fig. 6). From *P. andysabini*, it differs in having supranasals occasionally (in 8 out of 14 individuals examined by us) in contact (versus in not in contact in 9 out of 9 *P. andysabini*; Fig. 7), and having the throat usually

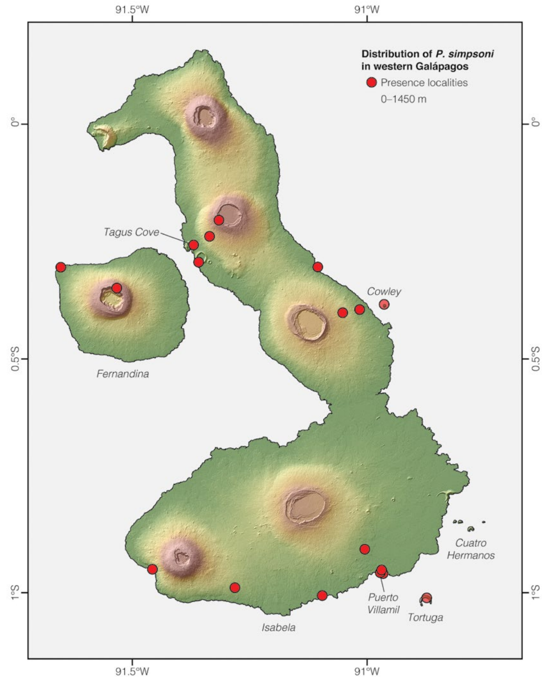

Figure 9. Distribution of *Phyllodactylus simpsoni* in western Galápagos. DNA samples included in the genetic analyses come from Tagus Cove and Puerto Villamil.

(in 9 out of 14 individuals) immaculate as opposed to densely stippled with dark brown pigment (Fig. 8). Genetic divergence in a 181 bp long fragment of the mitochondrial 12S gene between the sister species *P. simpsoni* and *P. andysabini* is 3–5%, whereas intraspecific distances are less than 2.3% in six individuals of *P. simpsoni*.

Natural history. Individuals of *Phyllodactylus simpsoni* have been found active at night on soil, rocks, tree trunks, fence posts, and walls of buildings up to 5 m above the ground. During the daytime, they have been found beneath rocks, old tortoise shells, the bark of trees, and old dead stumps. Eggs of *P. simpsoni* have been found beneath rocks. Simpson's Leaf-toed Geckos inhabit deciduous forests, dry grasslands, and dry shrublands. They are preyed upon by native predators such as mockingbirds and Western Galápagos Racers (*Pseudalsophis occidentalis*),[19] as well as by introduced species such as cats and black rats.

Etymology. The specific name *simpsoni* honors Dr. Nigel Simpson for his long-standing and visionary leadership in conservation. Nigel is a founding board member of the Ecuadorian conservation organizations Fundación Jocotoco and Fundación Ecominga. His passion and strong support for protecting the whole range of biodiversity, from birds to orchids, frogs, and moths,

Plate 66. Simpson's Leaf-toed Geckos from Puerto Villamil (▲) and Tagus Cove (■), Galápagos.

has been pivotal for establishing the network of private reserves owned by both organizations. We greatly appreciate his dedication to the conservation of some of the most threatened, yet most diverse places on Earth.

Distribution. *Phyllodactylus simpsoni* is endemic to an estimated 1,219 km^2 area on central and southern Isabela Island, as well as on Fernandina Island, Cowley Islet, and Tortuga Islet in Galápagos, Ecuador. The species occurs at elevations between 0 and 1,450 m above sea level (Fig. 9). Geckos from Fernandina Island, volcanoes Alcedo and Cerro Azul, and islets Cowley and Tortuga are only tentatively identified as this species pending genetic assignment and upcoming field work.

Conservation. Near Threatened. We consider *Phyllodactylus simpsoni* to be in this category following IUCN criteria because the species is facing the threat of displacement by introduced geckos (*Hemidactylus frenatus* and *P. reissii*) in areas where the latter have become invasive (currently only in urban areas), as well as predation by housecats, and, therefore, may qualify for a threatened category in the near future if these threats are not addressed. However, there is no current information on the population trend of *P. simpsoni* to determine whether its numbers are declining.

DISCUSSION

In this study, we expand the knowledge about the diversity and biogeography of Galápagos leaf-toed geckos.

We provide evidence for the existence of at least 12 species (eleven native; one introduced) of leaf-toed geckos in the Galápagos Islands; three more species than are traditionally recognized. We corroborate the validity of the four species that Torres-Carvajal et al. (2014)[3] identified within *Phyllodactylus galapagensis* as traditionally defined, and provide evidence for the existence of a fifth one that inhabits Wolf Volcano.

We show that geckos that inhabit Marchena Island are genetically most similar to, and their genetic variation included within the variation of, geckos from Santiago Island. This suggests that geckos from Santiago colonized Marchena recently. Our data suggests that geckos from southern (Puerto Villamil) and northern (Tagus Cove at Darwin Volcano) Isabela Island are closely related. Although they are identified as different species by the three delimitation approaches used in this work (Fig. 1), the samples analyzed come from localities separated by an airline distance of 85 km (Fig. 12), and may actually represent geographic genetic structure within a single species rather than two distinct species. Including samples from intermediate localities will certainly clarify the identity of geckos of Isabela Island.

Despite these advances, our understanding of the diversity and biogeography of Galápagos leaf-toed geckos is still far from complete. For example, genetic samples from geckos that inhabit Bartolomé and Rábida islands and Mares Islet have never been included in genetic analysis, and, therefore, the populations on these islands are regarded as the same as those on Santiago and Marchena Island based only on similar morphology. Geckos that inhabit Fernandina Island as well as those on Alcedo Volcano and Darwin Volcano have also not been studied at the genetic level, and their assignment to *Phyllodactylus simpsoni* is preliminary. Finally, there are two subspecies of *P. galapagensis* whose validity has not been tested using genetic information: *P. galapagensis daphnensis* of Daphne Island and *P. galapagensis olschkii* of Plaza Sur Island.

We suspect that there are numerous additional species of *Phyllodactylus* to be discovered in Galápagos, and we suggest that a more exhaustive sampling of all islands and islets in the archipelago be authorized by the Galápagos National Park in order to uncover, catalogue, and protect this hidden diversity.

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[1]Van Denburgh (1912b). [2]Lanza (1973). [3]Torres-Carvajal et al. (2014). [4]Beaupre et al. (2004). [5]Arteaga et al. (2018). [6]Gamble et al. (2008). [7]Blair et al. (2009). [8]Drummond et al. (2010). [9]Katoh & Standley (2013). [10]Lanfear et al. (2016). [11]Ronquist & Huelsenbeck (2013). [12]Swofford (2002). [13]Koch et al. (2016). [14]Puillandre et al. (2012). [15]Zhang et al. (2013). [16]Fujisawa & Barraclough (2013). [17]Arteaga et al. (2016). [18]de Queiroz (2007). [19]Ortiz-Catedral et al. (2019).

Appendix 1. GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*). Locality acronyms: EC = Continental Ecuador, ES = Española Island, FL = Floreana Island, GI = Gardner Islet, IS = Isabela Island, MR = Marchena Island, PB = Piedras Blancas, PV = Puerto Villamil, PZ = Pinzón Island, SC = Santa Cruz Island, SCY = San Cristóbal Island, SF = Santa Fe Island, SN = Santiago Island, TC = Tagus Cove, WI = Wolf Island, WF = Wolf Volcano.

Species	Voucher	Locality	12S	16S	ND4	RAG1	c-mos
P. andysabini	JMG 0997	PB	MN057713*	MN057703*	-	-	-
P. andysabini	JMG 0998	PB	MN057714*	MN057704*	MN117903*	-	-
P. andysabini	JMG 3092	WV	MN057715*	MN057705*	MN117904*	-	-
P. andysabini	JMG 3093	WV	MN057716*	MN057706*	MN117905*	-	-
P. andysabini	QCAZ 4545	PB	KJ914432	KJ914432	KJ914246	KJ913985	KJ914164
P. barringtonensis	QCAZ 10905	SF	KJ914388	KJ914388	KJ914212	KJ913941	KJ914124
P. barringtonensis	QCAZ 10907	SF	KJ914389	KJ914389	KJ914213	KJ913942	KJ914125
P. baurii	JMG 3091	GI	MN057717*	MN057707*	MN117906*	-	-
P. baurii	QCAZ 10891	FL	KJ914386	KJ914386	KJ914210	KJ913939	KJ914122
P. baurii	QCAZ 10892	FL	KJ914387	KJ914387	KJ914211	KJ913940	KJ914123
P. darwini	QCAZ 10472	SCY	KJ914369	KJ914369	KJ914194	KJ913922	KJ914105
P. darwini	QCAZ 10477	SCY	KJ914372	KJ914372	KJ914197	KJ913925	KJ914108
P. duncanensis	QCAZ 4539	PZ	KJ914429	KJ914429	KJ914243	KJ913982	KJ914161
P. duncanensis	QCAZ 4540	PZ	KJ914430	KJ914430	KJ914244	KJ913983	KJ914162
P. gilberti	QCAZ 13931	WI	KX268814	KX268798	KX268806	KX268810	KX268804
P. gilberti	QCAZ 13933	WI	KX268815	KX268799	KX268807	KX268811	KX268805
P. gorii	QCAZ 11177	ES	KJ914395	KJ914395	KJ914217	KJ913948	KJ914131
P. gorii	QCAZ 11179	ES	KJ914397	KJ914397	KJ914218	KJ913950	KJ914133
P. kofordi	QCAZ 10340	EC	KJ914363	KJ914363	KJ914188	KJ913916	KJ914099
P. leei	QCAZ 10475	SCY	KJ914371	KJ914371	KJ914196	KJ913924	KJ914107
P. leei	QCAZ 10478	SCY	KJ914373	KJ914373	KJ914198	KJ913926	KJ914109
P. leoni	QCAZ 9923	EC	JQ821780	JQ821780	JQ821769	JQ821791	JQ821737
P. leoni	QCAZ 9926	EC	JQ82178	JQ82178	JQ821770	JQ821792	JQ821739
P. maresi	JMG 1202	MR	MN057718*	MN057708*	MN117907*	-	-
P. maresi	JMG 1203	MR	MN057719*	MN057709*	-	-	-
P. maresi	JMG 1204	MR	MN057720*	MN057710*	-	-	-
P. maresi	QCAZ 11167	MR	-	KJ914391	-	KJ913944	KJ914127
P. maresi	QCAZ 11173	MR	KJ914393	KJ914393	-	KJ913946	KJ914129
P. maresi	QCAZ 11176	MR	KJ914394	KJ914394	KJ914216	KJ913947	KJ914130
P. maresi	QCAZ 11178	MR	KJ914396	KJ914396	-	KJ913949	KJ914132
P. maresi	QCAZ 11282	SN	KJ914407	KJ914407	-	KJ913960	KJ914143
P. maresi	QCAZ 11283	SN	KJ914408	KJ914408	KJ914224	KJ913961	KJ914144
P. pumilus	QCAZ 11479	EC	KJ914409	KJ914409	KJ914225	KJ913962	KJ914145
P. pumilus	QCAZ 11482	EC	KJ914410	KJ914410	KJ914226	KJ913963	KJ914146
P. reissii	QCAZ 10785	SC	KJ914378	KJ914378	KJ914203	KJ913931	KJ914114
P. reissii	QCAZ 10786	SC	KJ914379	KJ914379	KJ914204	KJ913932	KJ914115
P. reissii	QCAZ 11186	IS	KJ914402	KJ914402	KJ914221	KJ913955	KJ914138
P. simpsoni	JMG 0999	TC	MN057721*	MN057711*	-	-	-
P. simpsoni	JMG 1000	TC	MN057722*	MN057712*	-	-	-
P. simpsoni	QCAZ 11181	PV	KJ914398	KJ914398	-	KJ913951	KJ914134
P. simpsoni	QCAZ 11182	PV	KJ914399	KJ914399	KJ914219	KJ913952	KJ914135
P. simpsoni	QCAZ 11183	PV	KJ914400	KJ914400	KJ914220	KJ913953	KJ914136
P. simpsoni	QCAZ 11184	PV	KJ914401	KJ914401	-	KJ913954	KJ914137
P. galapagensis	QCAZ 4546	SC	KJ914433	KJ914433	KJ914247	KJ913986	KJ914165
P. galapagensis	QCAZ 4547	SC	KJ914434	KJ914434	KJ914248	KJ913987	KJ914166
P. galapagensis	QCAZ 10813	SC	KJ914381	KJ914381	KJ914206	KJ913934	KJ914117
P. galapagensis	QCAZ 10808	SC	KJ914380	KJ914380	KJ914205	KJ913933	KJ914116
P. galapagensis	QCAZ 10883	SC	KJ914382	KJ914382	KJ914207	KJ913935	KJ914118