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## **Are the Mascarene frog (*Ptychadena mascareniensis*) and Brahminy blind snake (*Indotyphlops braminus*) really alien species in the Seychelles?**

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### **INTRODUCTION**

Through resource competition, predation and the introduction of diseases, invasive alien species (IAS) pose a major threat to native biota (Kraus, 2009; Rocamora & Henriette, 2015; Young et al., 2017), particularly on remote islands with small and naïve populations. Alien (introduced) species, are those introduced by man, accidentally or intentionally, outside of their natural geographic range into an area where they would not naturally occur. Where alien species cause economic or environmental harm they are referred to as IAS. It can be difficult to determine whether species are alien or native within given habitats. Molecular genetic methods offer one approach to testing hypotheses of alien status, and determining putative source and introduction pathways. Alien populations are predicted to vary little genetically from populations from their native range (Tsutsui et al., 2000), and relatively recently introduced populations are also predicted to have low within-population genetic variation if founded by a small number of founding individuals (*i.e.* the founder effect) (e.g. Sakai et al., 2014).

The biota of the granitic Seychelles islands have high levels of endemism, largely due to geographic isolation from other landmasses for approximately ca. 65 million years (MY) (Davies, 1968; Mart, 1988; Collier et al., 2008; Chatterjee et al., 2013; Ali, 2018). In addition, the Seychelles are considered to be home to alien (introduced) species and IAS (Rocamora & Henriette, 2015), including plants (Fleischmann, 1997), mammals (Merton et al., 2002), reptiles (Nussbaum, 1980, 1984; Vences et al., 2004b), amphibians (Vences et al., 2004a), birds (Canning, 2011) and insects (Gerlach, 2004).

We examined mitochondrial (mt) DNA sequence data to test the hypotheses that the Mascarene frog, *Ptychadena mascareniensis* (Duméril & Bibron, 1841), and Brahminy blind snake, *Indotyphlops braminus* (Daudin, 1803), are introduced species in the Seychelles (Nussbaum, 1980; Vences et al., 2004a). The Mascarene frog has been reported from nine of the Seychelles islands (Labisko et al., 2015) and, based on genetic data for the single Seychelles individual thus far sampled, is hypothesised to have been introduced by humans from Madagascar in the recent past (Vences et al., 2004a). The Brahminy blind snake, sometimes considered native to India (Hedges et al., 2014), has a global distribution including Asia, Europe, the Middle East, Africa, Australia and the Americas (Uetz et al., 2019). It is well equipped to invade new regions because of its small size, tolerance of dry and human-modified habitats, and because it reproduces by parthenogenesis (McDowell, 1974; Nussbaum, 1984; Wynn et al., 1987). It has been reported from seven Seychelles islands (Nussbaum, 1980; Rocha et al., 2009) where it is hypothesised to be non-native (Nussbaum, 1980). Genetic data for the single *I. braminus* from the Seychelles sampled in previously published studies are identical to those from localities in Europe, Africa, the Comoro islands, Asia and Central America (Rato et al., 2015).

To date no studies have assessed intraspecific molecular genetic variation within Seychelles *P. mascareniensis* or *I. braminus*. We address this data gap and provide further tests of the hypotheses that these taxa are alien species in the Seychelles.

## **METHODS**

We generated DNA sequence data for a region of the mt 16s rRNA gene (*I6s*), for multiple individuals of each species from the Seychelles and additional samples of *I. braminus* from Sri Lanka (Tables 1, 2). Tissue samples (frog liver or toe tips; snake liver or muscle) were obtained from 10 Seychelles (from the islands of Curieuse, La Digue, Mahé, Praslin and Felicité – the latter a new record for the species taking the known Seychelles range to eight islands) and five Sri Lankan *I. braminus*, and 19 Seychelles *P. mascareniensis*

(La Digue, Mahé, North, Praslin, Silhouette). All available published *16s* sequence data (16) for *I. braminus* and the single previously published sequence of *P. mascareniensis* from the Seychelles were obtained from Genbank and added to our datasets (Tables 1, 2). The mitochondrial marker *16s* was targeted because this is the locus that most published sequence data exists for *I. braminus* and is what was targeted in previous Seychelles studies incorporating both *I. braminus* and *P. mascareniensis* (Vences et al., 2004a; Rato et al., 2015).

Genomic DNA was extracted from tissue using a Qiagen DNeasy™ Blood and Tissue Kit following manufacturer's guidelines. Partial fragments of *16s* were amplified using the polymerase chain reaction (PCR) and sequenced following protocols reported by Maddock et al. (2014, 2017). Sequences were manually trimmed using Geneious Prime (Biomatters) and aligned using default parameters in Muscle (Edgar 2004).

## RESULTS

The *I. braminus* and *P. mascareniensis* *16s* datasets comprised a total of 502 and 588 aligned base pairs (bp), respectively. For the *I. braminus* alignment all overlapping sequences from across the globe (Seychelles, Sri Lanka, Thailand, Mexico, Equatorial Guinea, Spain (+Tenerife), Comoro Islands and China) were identical, with the exception of one individual from southern India (JN172940), which differs by 9 bp, including one deletion. All *P. mascareniensis* sampled were identical. Newly generated sequences have been deposited in GenBank (Table 1 & 2).

## DISCUSSION

Our multi-specimen mtDNA sequence data are consistent with (and provide additional support for) the hypotheses that both the Mascarene frog and the Brahminy blind snake are alien species in Seychelles. Our much expanded sampling found no intraspecific molecular variation for either taxa within the Seychelles and is in agreement with results from previous studies of both *P. mascareniensis* (Vences et al., 2004a) and *I. braminus* (Rato et al., 2015).

In the Seychelles, the Mascarene frog occurs in lowland, often human modified landscapes, including habitats much more degraded than those that support Seychelles native frogs (sooglossids and the Seychelles treefrog, *Tachycnemis seychellensis*) (pers. obs.). For sooglossids and Seychelles treefrogs, *16s* sequence data vary substantially among the four islands on which they occur (Maddock et al., 2014; Labisko et al., 2019), contrasting strongly

with the single *16s* haplotype found within *P. mascareniensis* across the five sampled islands. Vences et al. (2004a) found this same *16s* haplotype in *P. mascareniensis* from Mauritius, with this differing from a Reunion and Madagascar haplotype by only a single substitution, consistent with a recent introduction of one (or very few) individual lineages of this species in the Seychelles and Mascarenes. In addition, Zimkus et al. (2017) indicated intra-specific variation of *P. mascareniensis* within Madagascar, supporting a single introduction source to Seychelles.

Published studies of native Seychelles reptile species occurring on multiple islands display a substantial amount of intraspecific mtDNA variation among populations from at least some islands (e.g. Rocha et al., 2013; Valente et al., 2014; Harris et al., 2015), with the only exceptions discovered thus far being the Brahminy blind snake and the terrapins *Pelusios castanoides* and *P. subniger* (Silva et al., 2010). The two terrapin species are considered to be recent (likely human mediated) arrivals in the Seychelles based on haplotype sharing with conspecifics from Madagascar and Africa (Fritz et al., 2013). Interpretation of the biogeographic history of Seychelles Brahminy blind snakes is complicated by scant sampling across this species' global range. Although our data are consistent with an introduction to the Seychelles, we cannot rule out that the islands occupy part of its natural range because of the apparent global universal genetic homogeneity of the sampled mtDNA marker. The genetic homogeneity may be, in part, due to a slowly evolving *16s* mtDNA marker and due to the species' ability to reproduce via parthenogenesis. Additionally, the high dispersal ability of the species may reduce genetic diversification. The discovery of *I. braminus* on the island of Felicité takes the known Seychelles range of the species to eight islands.

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Table 1. Sample information for the Mascarene frog *Ptychadena mascareniensis*. Individuals with a “Sample ID” had *16s* sequence data generated for them in this study. GenBank accession numbers are provided (<https://www.ncbi.nlm.nih.gov/genbank/>).

<b>Species</b>	<b>Sample ID</b>	<b>Locality</b>	<b>Latitude (S)</b>	<b>Longitude (E)</b>	<b>GenBank #</b>
<i>P. mascareniensis</i>	SM086	Mahé, Seychelles	04°36'21.4"	055°26'20.8"	MT509738
<i>P. mascareniensis</i>	SM124	Praslin, Seychelles	04°19'33.24"	055°45'29.10"	MT509739
<i>P. mascareniensis</i>	SM126	Praslin, Seychelles	04°19'33.24"	055°45'29.10"	MT509740
<i>P. mascareniensis</i>	SM127	Praslin, Seychelles	04°19'33.24"	055°45'29.10"	MT509737
<i>P. mascareniensis</i>	SM244	Silhouette, Seychelles	04°29'01.2"	055°14'56.9"	MT509741
<i>P. mascareniensis</i>	SM253	La Digue, Seychelles	04°20'57.4"	055°49'48.6"	MT509742
<i>P. mascareniensis</i>	SM255	La Digue, Seychelles	04°20'25.38"	055°50'11.88"	MT509743
<i>P. mascareniensis</i>	SM256	La Digue, Seychelles	04°20'25.38"	055°50'11.88"	MT509744
<i>P. mascareniensis</i>	SM258	La Digue, Seychelles	04°21'43.20"	055°49'57.84"	MT509745
<i>P. mascareniensis</i>	SM434	North, Seychelles			MT509755
<i>P. mascareniensis</i>	SM435	North, Seychelles			MT509754
<i>P. mascareniensis</i>	SM436	North, Seychelles			MT509753
<i>P. mascareniensis</i>	SM437	North, Seychelles			MT509752
<i>P. mascareniensis</i>	SM438	North, Seychelles			MT509751
<i>P. mascareniensis</i>	SM439	North, Seychelles			MT509750
<i>P. mascareniensis</i>	SM440	North, Seychelles			MT509749
<i>P. mascareniensis</i>	SM441	North, Seychelles			MT509748
<i>P. mascareniensis</i>	SM442	North, Seychelles			MT509746
<i>P. mascareniensis</i>	SM443	North, Seychelles			MT509747
<i>P. mascareniensis</i>		Praslin, Seychelles			AF517589 <sup>1</sup>

<sup>1</sup> Vences et al. (2004a)

Table 2. Sample information for the Brahminy blind snake *Indotyphlops braminus*.

Individuals with a “Sample ID” had *I6s* sequence data generated for them in this study.

MW numbers from Sri Lanka are field tags of uncatalogued National Museum of Sri Lanka, Colombo specimens. New island locality records are denoted with a \*. GenBank accession numbers are provided (<https://www.ncbi.nlm.nih.gov/genbank/>).

Species	Sample ID	Locality	Latitude (S)	Longitude (E)	GenBank #
<i>I. braminus</i>	RAN25158	Curieuse, Seychelles			MT509731
<i>I. braminus</i>	SM313	La Digue, Seychelles	4°21'43.20"S	55°49'57.84"E	MT509730
<i>I. braminus</i>	MW10438	La Digue, Seychelles	4°21'43.20"S	55°49'57.84"E	MT509729
<i>I. braminus</i>	MW10439	La Digue, Seychelles	4°21'43.20"S	55°49'57.84"E	MT509728
<i>I. braminus</i>	MW10443	La Digue, Seychelles	4°21'43.20"S	55°49'57.84"E	MT509727
<i>I. braminus</i>	MW10240	Mahé, Seychelles	4°36'24.05"S	55°26'25.05"E	MT509725
<i>I. braminus</i>	SM455	Mahé, Seychelles	4°36'24.05"S	55°26'25.05"E	MT509725
<i>I. braminus</i>	SM481	Praslin, Seychelles	4°19'54.88"S	55°44'23.81"E	MT509724
* <i>I. braminus</i>	SM482	Félicité, Seychelles	4°19'36.9"S	55°52'26.5"E	MT509723
* <i>I. braminus</i>	SM483	Félicité, Seychelles	4°19'36.9"S	55°52'26.5"E	MT509722
<i>I. braminus</i>	MW1777	Sri Lanka	6°7'22.20"N	80°33'43.80"E	MT509736
<i>I. braminus</i>	MW1778	Sri Lanka	6°23'26.40"N	80°14'29.40"E	MT509735
<i>I. braminus</i>	MW1779	Sri Lanka	6°23'26.40"N	80°14'29.40"E	MT509734
<i>I. braminus</i>	MW1780	Sri Lanka	6°24'51.00"N	80°19'22.20"E	MT509733
<i>I. braminus</i>	MW1781	Sri Lanka	6°24'51.00"N	80°19'22.20"E	MT509732
<i>I. braminus</i>		Mahé, Seychelles			KJ783470 <sup>1</sup>
<i>I. braminus</i>		Thailand			AF544823 <sup>2</sup>
<i>I. braminus</i>		Unknown			DQ343649 <sup>3</sup>
<i>I. braminus</i>		Unknown			NC_010196 <sup>3</sup>
<i>I. braminus</i>		Mexico			GQ469240 <sup>4</sup>
<i>I. braminus</i>		Southern India			JN172940
<i>I. braminus</i>		Equatorial Guinea			KJ783466 <sup>1</sup>
<i>I. braminus</i>		Equatorial Guinea			KJ783467 <sup>1</sup>
<i>I. braminus</i>		Spain			KJ783468 <sup>1</sup>
<i>I. braminus</i>		Tenerife, Spain			KJ783469 <sup>1</sup>
<i>I. braminus</i>		Comoro Islands			KJ783471 <sup>1</sup>
<i>I. braminus</i>		Comoro Islands			KJ783472 <sup>1</sup>
<i>I. braminus</i>		Comoro Islands			KJ783473 <sup>1</sup>
<i>I. braminus</i>		Comoro Islands			KJ783474 <sup>1</sup>
<i>I. braminus</i>		Equatorial Guinea			KJ783475 <sup>1</sup>
<i>I. braminus</i>		China			MK194179

<sup>1</sup> Rato et al. (2015), <sup>2</sup> Vidal & Hedges (2002), <sup>3</sup> Yan et al. (2008), <sup>4</sup> Adalsteinsson et al. (2009)