A molecular assessment of European populations of *Indotyphlops braminus* (DAUDIN, 1803)

The alarming rates at which species are introduced, directly or indirectly from human intervention, as well as their potentially deleterious impact on native biota, make biological invasions a major threat to biodiversity at a global scale (SIMBERLOFF et al. 2013). Among vertebrates, reptiles are largely involved in invasion processes, both as alien invaders and as native sufferers (LEVER 2003; KRAUS 2009). Because of that, early detection of allocthonous populations and inference of invasion pathways are crucial to develop successful management strategies.

Phylogeographic analysis and population genetics are now widely used to reveal invasion pathways in reptiles. Namely, by analyzing selected genetic markers of a number of introduced specimens and fitting these sequence data into the known phylogeographic and population structure for a given species in its native range, it is possible to determine their allocthonous status, the source area and even the number of invasion events (KOLBE et al. 2007; GRACIÁ et al. 2011; SILVA-ROCHA et al. 2012; MICHAELIDES et al. 2013).

The Flowerpot Snake *Indotyphlops braminus* (DAUDIN, 1803) (formerly *Ramphotyphlops braminus* (see HEDGES et al. 2014; for a recent generic rearrangement of

Table 1: Codes of the specimens of *Indotyphlops braminus* used in this study, with the corresponding locality and GenBank accession number for the 16S rRNA fragment.

Code	Locality	GenBank Accession No.	Reference
DB14631	Araya, Candelaria, Tenerife, Spain	KJ783469	This study
DB13781	Aguadulce, Almería, Spain	KJ783468	This study
671An	Annobón, Equatorial Guinea	KJ783466	This study
673An	Annobón, Equatorial Guinea	KJ783467	This study
STA14	Annobón, Equatorial Guinea	KJ783475	This study
MH27	Fomboni, Moheli, Comoro islands	KJ783471	This study
MH30	Fomboni, Moheli, Comoro islands	KJ783472	This study
MY47	Ouagani, Mayotte, Comoro islands	KJ783473	This study
MY48	Ouagani, Mayotte, Comoro islands	KJ783474	This study
DB6923	Trail to Anse Intendance, Mahé, Seychelles	KJ783470	This study
	Phang-Nga City, Muang District, Thailand	AF544823	(Vidal & Hedges 2002)
	Chipancingo-Acapulco, Mexico	GQ469240	(Adalsteinsson et al. 2009)
	South India	JN172940	(Shekhar & Sandip 2011)

the family) represents a relevant case of a successful invasive species. This fossorial typhlopid is restricted to warm areas with high humidity, usually in the tropics (Ota et al. 1991). However, its parthenogenic reproduction (Kamosawa & Ota 1996), small size (up 175 mm SVL with a diameter of only 5 mm) and association with garden plants have allowed the species to spread from India, where it is said to be native (Kraus 2009), to numerous tropical and subtropical regions from Asia, Oceania, Africa and the Americas (Global Invasive Species Database 2013). Closer to Europe, it has been reported from the Canary Islands (López-Jurado et al. 2006; Urioste & Mateo 2011), Madeira (Jesus et al. 2013), Northern Africa (Baha El Din 2001; Joger et al. 2012) and, more recently, from the Balearic Islands and Southern Iberian Peninsula (Mateo et al. 2013) apparently in gardens and greenhouses with tropical plants.

In the present study, mitochondrial markers were used to assess the putative origin(s) of some of these European populations, namely those from the Canary Islands and Iberia. By combining newly generated sequences from these and other regions as well as those deposited in genetic databases, the authors aimed at elucidating the invasion pathways of this alien snake species.

Ten specimens of *I. braminus* were collected from Tenerife (Canary Islands) and Almería (Southern Spain), which were added to samples previously collected from Equatorial Guinea, Seychelles and Comoro Islands. Precise information on the geographic locality and specimen codes of all the samples are given in Table 1.

From all of the above specimens, genomic DNA was extracted using the DNeasy Blood & Tissue Kit from QIAGEN GmbH (Hilden), following the manufacturer's instructions. The ribosomal 16S rRNA gene was amplified for all samples using the primers from Palumbi (1996). The PCR reaction was carried out in a total volume of 25 µl, containing 2.5 µl of 10X Reaction Buffer, 3.0 mM of $MgCl_2$, 0.5 mM of each dNTP, 0.2 mM of each primer, 1 U of Taq DNA polymerase (Invitrogen™ with W-1), 1.25 µl of detergent W-1 and approximately 100 ng of template DNA. The PCR cycle program was the same as the one recommended by Invitrogen™ with an annealing temperature of 50 °C.

All amplicons were sequenced directly from the PCR products on an ABI Prism® 377 DNA automated sequencer and all sequences were deposited in GenBank. Further, three 16S rRNA sequences of *I. braminus* were added from GenBank (Table 1). The obtained sequences were aligned using MAFFT v.7.017 (Katoh & Standley 2013) with default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2).

The 16S rRNA fragments obtained in this study consisted of 361 bp and were all identical among each other and with two entries in GenBank. The only exception was the sequence downloaded from GenBank with accession number JN172940 from South India, which showed a total of eight polymorphic positions compared to the remaining sequences.

It is obvious that the present results do not completely allow ascertaining the population sources for Canarian and Iberian *I. braminus*. Even if markers with faster mutation rates could provide better detail, it has already been reported that in scenarios of repetitive introductions, inferring the exact colonization patterns may prove difficult if not impossible (ROCHA et al. 2005, 2010). In fact, European populations shared the same haplotype not only between them, but also with other unrelated populations from three continents (see localities in Table 1), suggesting repeated invasions. Parthenogenic reproduction, enhancing the colonizing potential of the species, may also account for this genetic uniformity [see FREITAS (2011) for a similar case in the parthenogenic lacertid *Darevskia unisexualis* (DAREVSKY, 1966)]. Significantly, the only divergent sequence came from India, already pointed to (without any genetic evidence) as the native range of the species (KRAUS 2009).

At present, no impacts of this snake on the native biota have been described either at a global level (GLOBAL INVASIVE SPECIES DATABASE 2013) or for Spain (MATEO et al. 2013). Although effects on native reptiles may be unlikely, the analysis of the impact on the arthropod communities deserves deeper investigation (MATEO et al. 2011). Also, the species seems to be spreading across the Canarian Archipelago associated with plant gardening (URIOSTE & MATEO 2011). Hence, a simple principle of caution recommends preventing new introductions and monitoring the populations already naturalized in Europe using both censuses and genetic markers.

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