First records of the Brahminy blindsnake, *Indotyphlops braminus* (Daudin, 1803), in Italy (Squamata: Typhlopidae)

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Invasive alien species are among the main threats to global biodiversity (Butchart et al., 2010). Their growing distribution and invasiveness are linked to various factors, including the sharp increase in international trade (Westphal et al., 2008) and climate change (Dukes and Money, 1999; Sutherst, 2000). Some snake species have a high dispersal capacity and can easily acclimatise to new ecological conditions, which are typical traits of successful invasive species. Snake dispersal patterns are mainly driven by the pet trade (Meshaka, 2011; Willson et al., 2011; Cabrera-Peréz et al., 2012) and accidental translocation (Fritts, 1987; Silva-Rocha et al., 2015).

The Brahminy blindsnake, *Indotyphlops braminus* (Daudin, 1803), is a very small fossorial snake, its length usually not exceeding 17 cm in total (Mateo, 2013). A probable native of the Indo-Malayan region (Mateo et al., 2011), *I. braminus* has been introduced in at least 54 countries, mostly located in tropical and subtropical areas of Africa, the Americas, Asia, and Australia (including many islands of the Atlantic, Indian, and Pacific Oceans) (Global Invasive Species Database, 2018). Its remarkable dispersal success is attributed to two reasons: it is able to live inside pots (it is also known as the 'flowerpot snake') and therefore is easily dispersed through the international plant trade (Kraus, 2003), and it is a triploid, unisexual, obligate parthenogenetic species (Cagle, 1946; Kamosawa

and Ota, 1996), which means that a single female can potentially give rise to an entire population.

Few records of this species exist for the Mediterranean Basin. *Indotyphlops braminus* populations have been reported from Libya (Joger et al., 2008), Egypt (Baha El Din, 1996), and Spain (Mateo et al., 2011; Mateo, 2013; Zamora-Camacho, 2017). In the Mediterranean Region of Spain, the species has been reported in Almeria (Mateo, 2013) and on the Balearic Islands (Mateo et al., 2011); observations of several individuals have been made in the Granada and Girona regions (Zamora-Camacho, 2017). Here, we report the first record of this species in Italy.

On 6 May 2017, in a suburban area near Paceco (Trapani Province, western Sicily, Italy), a living blindsnake (Fig.1) was found at 1900 h on a private garden patio (37.9889°N, 12.5602°E) adjacent to a plant nursery. It measured 71.4 mm in length and presented 20 scales around the body as well as the typical head scalation, thus leading to its identification as *I. braminus* (Ota et al., 1991).

From May–December 2017, we placed three pitfall traps in the private garden where the first specimen was found, and we actively searched for snakes every two weeks. These efforts did not produce any additional individual. However, on 12 November 2018, around noon, a second living Brahminy blindsnake was found in the same area. This second individual had a total length of 73.2 mm and also 20 scales around the body.

Both blindsnakes died shortly after capture, were preserved in 96% alcohol and deposited at the Zoology Museum "Pietro Doderlein," University of Palermo (accession numbers MZPA R-975 and R-976). During 2019, two additional specimens were found in the same garden. The third (81 mm total length) was found dead on 14 July 2019 and the fourth (58 mm total length) was found alive and entangled in a spider web on 14 August 2019. Based on their small sizes, we consider all discovered blindsnakes as immature individuals (see

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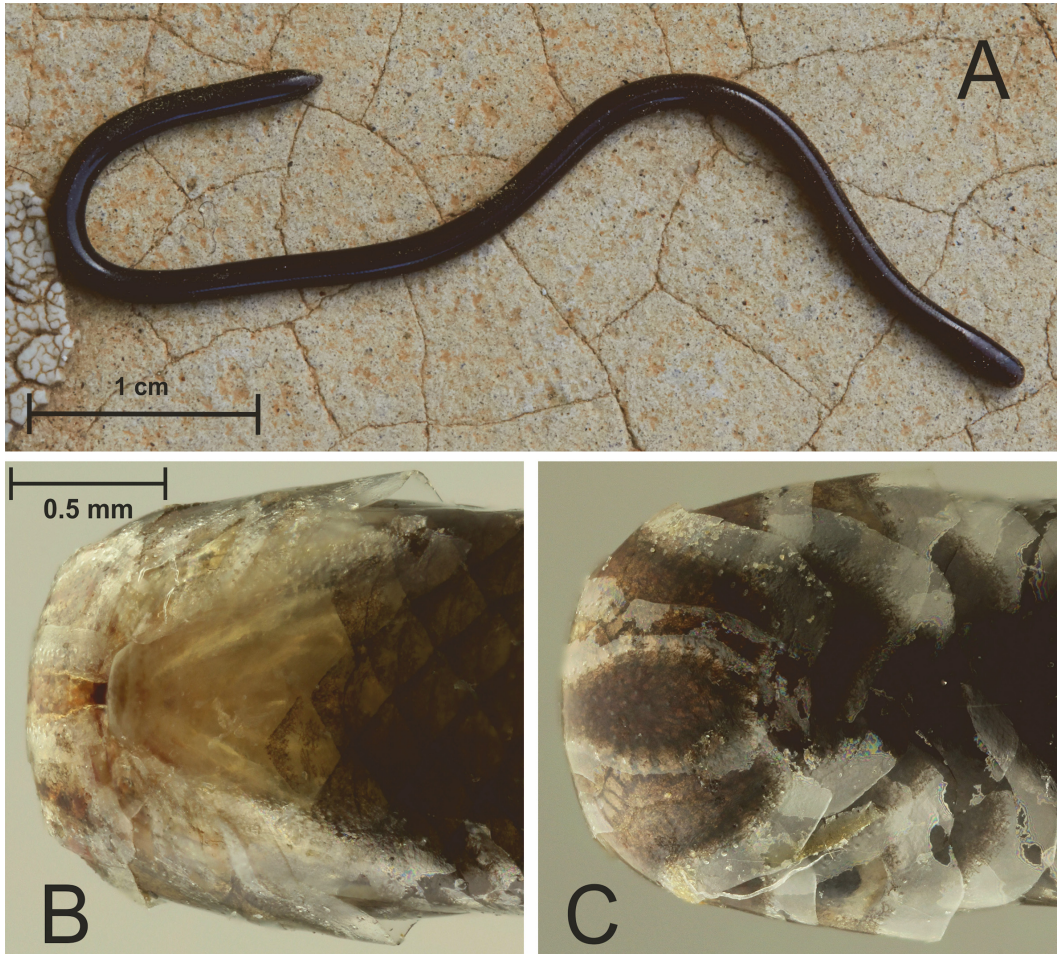[*] Corresponding author. E-mail: paolofaraone@libero.it

Figure 1. The first Brahminy blindsnake (*Indotyphlops braminus*) recorded for Sicily, Italy, photographed *in situ* at the time of its discovery (A). Ventral (B) and dorsal (C) views of the second specimen. Photos by Luigi Barraco (A) and Enrico Schifani (B,C).

Kamosawa and Ota, 1996), most likely yearlings (see Mateo, 2013).

To confirm the morphology-based identification and to assess the putative origin of the collected blindsnakes, we obtained molecular data of the specimens. DNA extraction was performed on a small tissue sample taken from each of the first two collected specimens. Prior to DNA extraction, the tissue was carefully cleaned and soaked in double-distilled water for 10 min, and then processed for DNA extraction using the BIORON GmbH "Ron's Tissue DNA Mini Kit" following manufacturer instructions. In addition, in order to compare the new sequences obtained with those already available from other *I. braminus* specimens, all 13 available 16S rRNA sequences were downloaded from GenBank and

included in the analyses (see Fig. 2 for their GenBank Accession numbers, AN). In addition, three 16S rRNA sequences of *Typhlops* spp. were also downloaded from GenBank and included in the analyses to be used as outgroups (GenBank AN: KF993227;KF993229–30).

The selective amplification of a 366-bp-long fragment from a portion of the large ribosomal subunit 16S rRNA (16S) was carried out using the primer pair 16Sar-L-myt and 16Sbr-H-myt (Lydeard et al., 1996). Composition of the polymerase chain reaction (PCR) mix, thermal cycles, and phylogenetic analyses were performed according to Hedges et al. (2014). Only the *I. braminus* specimen collected in 2018 was successfully amplified. Otherwise, no results were obtained from the DNA template extracted from the degraded
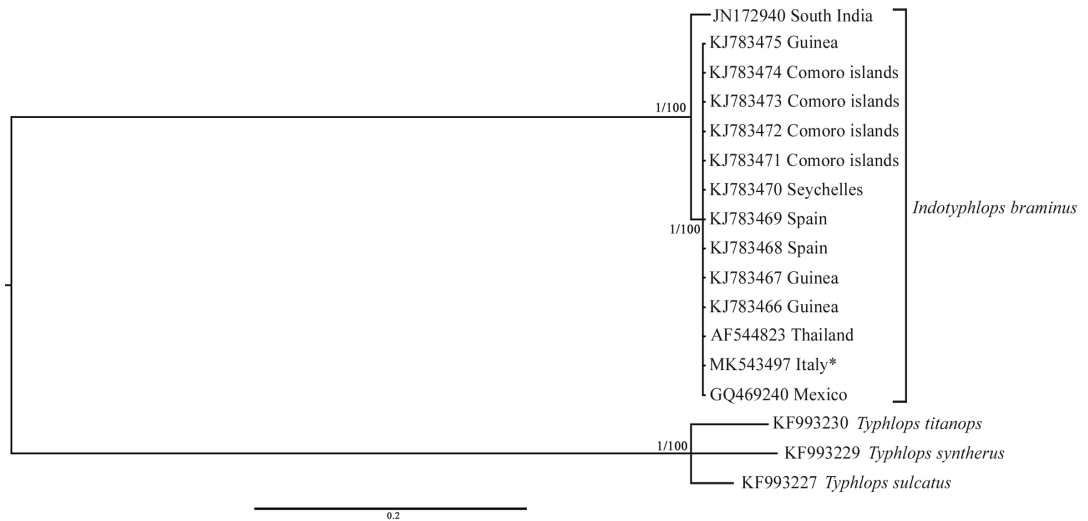
Figure 2. Bayesian phylogram (95% majority rule consensus tree) of *Indotyphlops braminus* based on the 366 bp fragment of the 16S rRNA. Samples of *Typhlops* spp. were used as outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI) / bootstrap values (Maximum Likelihood, ML). A novel sequence is indicated by an asterisk (*).

specimen collected in 2017, thus suggesting that DNA degradation was already substantial in this specimen. The single obtained chromatogram showed no peak ambiguities, and the obtained 16S rRNA sequence was uploaded to GenBank (AN: MK543497). The sequence was analysed and manually proofread with the DNA sequencing software Chromas v. 2.6.2 (Technelysium Pty. Ltd. 1998, Queensland, Australia) and then aligned with MEGA7 (Kumar et al., 2016). The alignment of our novel sequence with those downloaded from GenBank led to a 16S rRNA aligned fragment of 366 base pairs.

The molecular identification of the studied specimens and the reconstruction of the phylogenetic relationships among the taxa was performed with Bayesian Inference (BI) and Maximum Likelihood (ML) methods as implemented in MrBayes v. 3.2.6 (Ronquist et al., 2012) and PhyMl v. 3 (Guindon and Gascuel, 2003), respectively. As a measure of branch support, bootstrap values (Felsenstein, 1985) were calculated with 1000 replicates in the ML tree, and posterior probability values were reported on the BI tree. The choice of the best evolutionary model was made using Partition Finder v. 1.0.1 (Lanfear et al., 2012) according to the Akaike Information Criterion (AIC; Akaike, 1974). The BI and ML analyses were performed using a Hasegawa-Kishino-Yano model of evolution with gamma-distributed rate variation among sites (HKY

$+ \Gamma$; nst=2). In the BI analyses, two independent Markov Chain Monte Carlo analyses were run with one million generations (temp.: 0.2; default priors). Trees and parameter values were sampled every 100 generations, resulting in 10,000 saved trees per analysis; in the analysis, convergence was reached (Effective Sample Size (ESS) above 2034.54); 2500 trees were conservatively discarded as "burn-in".

Both BI and ML trees showed a congruent topology highlighting the presence of a well-supported clade which includes all the available sequences of *I. braminus* (Fig. 2). The novel sequences shared the same haplotype of all the other populations from other continents, with the only exception of a single sample from southern India (Rato et al., 2015; AN: JN172940). The topologies of the BI and ML trees based on the 16S rRNA marker stress the molecular homogeneity of all *I. braminus* populations worldwide.

This is the first evidence for the occurrence of this alien taxon in Italy. The present *I. braminus* records are most likely related to the presence of a plant nursery close to the site of the findings, since it is known that these structures play a fundamental role in the inadvertent dispersal of this species (Zamora-Camacho, 2017). Brahminy blindsnakes have an enormous capacity for passive dispersion through propagules (pots and roots) but this is combined with a low tendency to actively

spread from the release point due to limited motility and because a blindsnake's survival requires specific environmental characteristics (moist and soft soil) (Mateo, 2013). For this reason, in the subtropical and Mediterranean area, this species is mainly present in 'managed' soils, like those of plant nurseries (Zamora-Camacho, 2017).

Unfortunately, the nursery close to the Sicilian occurrence site of the species has not yet been properly investigated due to its inaccessibility and therefore the actual presence of the species and the consistency of the population could not be verified. However, imports of soil or plants into the nursery were very limited between 2008 and 2018 and involved only Sicilian material, and it is possible that the reported blindsnakes are part of a population that is able to survive inside the study area and/or in Sicily. On the other hand, the nursery owner has reported that soil exchanges with other local plant nurseries are frequent, suggesting that future research efforts should include these areas.

Despite its enormous range as alien species, it is perhaps surprising that the impact of introduced *I. braminus* populations on the ecosystems where they have been introduced is almost completely unknown (Global Invasive Species Database, 2018). Blindsnakes are considered 'binge feeders', which prey on large numbers of tiny arthropods at the same time, whereas snakes usually consume a small number of large preys (Kley, 2001; Webb et al., 2001). Ants and termites are often generically reported as the main preys of *I. braminus* (e.g., Fields and Horrocks, 2011; Ineich et al., 2017). We attempted an approximate, preliminary, assessment of soil-nesting ants and termite species in the plant nursery through direct sampling. No soil-nesting termites were found. Six likely native ant species commonly found in anthropogenic habitats were collected *Hypoponera eduardi* (Forel, 1894), *Messor capitatus* (Latreille, 1798), *M. ibericus* Santschi, 1931, *Pheidole pallidula* (Nylander, 1849), *Plagiolepis pygmaea* (Latreille, 1798), *Tapinoma magnum* (Mayr, 1861), and one very abundant exotic species, *Nylanderia* cf. *jaegerskioeldi* (Mayr, 1904), which is part of a group of exotic taxa quickly spreading in Sicilian gardens (Schifani and Alicata, 2018).

Indotyphlops braminus can easily remain long unnoticed both due to its resemblance to earthworms and its elusive habits and its presence may be thus underestimated. The finding of the four individuals in three different years in the same site stresses the need of further investigations in the area. In the light of its establishment under similar climatic conditions, as those in Spain (Mateo et al., 2011; Mateo, 2013; Zamora-Camacho, 2017), *I. braminus* appears to be a species fully capable of becoming established in Italy. Its possible ecological impacts are hard to predict due to the general lack of knowledge on this topic. The Brahminy blindsnake may affect not only the invertebrate communities by direct predation, but also vertebrates through transmission of pathogens and parasites or through competition for food (Field and Horrocks, 2011; Ineich et al., 2017). It will be our future goal to deepen the research in the area of the present findings as well as to investigate among other plant nurseries through surveys, questionnaires (see Zamora-Camacho, 2017) and citizen science projects on social networks (see Faraone et al., 2017), with the aim of gathering further data on the species presence in the Sicilian territory.

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