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Multiple introductions and environmental factors affecting the establishment of invasive species on a volcanic island

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ABSTRACT

Invasive species pose significant challenges to local biodiversity and ecosystem function, especially on islands. Understanding the factors affecting the establishment of invasive species and how these relate to their genetic background is crucial to improve our ability to manage biological invasions. Here, we performed a phylogeographic study of two cosmopolitan megascolecid earthworms of Asian origin: *Amynthas gracilis* and *Amynthas corticis* at 38 localities on São Miguel Island in the Azores archipelago (Portugal). Samples from putative source populations in China, Taiwan, Malaysia, as well as 'outlier' populations in USA, Mexico, Brazil and Spain were also included, resulting in a total of 565 earthworms genotyped at the mitochondrial cytochrome oxidase I (COI) and 16S ribosomal RNA genes. Soils were characterised for elemental composition, water holding capacity, organic matter content, texture and pH, and some habitat features were recorded. Both species showed a wide distribution across São Miguel and their abundances were negatively associated, suggesting spatial segregation/competition, with the parthenogenetic *A. corticis* being relatively more successful. The presence of multiple mitochondrial lineages within each species, one of them found exclusively in the Azores, suggests a complex invasion history. Environmental factors affected the establishment of the different lineages, with metal concentrations, topographical elevation and the degree of human influence being differently linked to their abundances. Lineage diversity was negatively correlated with metal concentrations. These results emphasise the importance of genetically characterising invasive species to better understand their invasion patterns.

1. Introduction

Invasive species can adversely affect local biodiversity due to alterations of recipient ecosystems, impacts on native species, such as competition, predation or hybridisation, or as carriers of disease (Pejchar and Mooney, 2009). Global environmental change and globalisation of trade networks mean that introductions are more likely to occur and their proper prediction, prevention and management is crucial. Therefore understanding the influence of the environment, geography and genetic features on colonisation and invasiveness of species should be a priority. Islands are particularly vulnerable to invasions, which are the main cause of population declines and species extinctions as well as having substantial socioeconomic impacts within these areas (Reaser et al., 2007). Invasive species have shown rapid adaptations to new biotic and abiotic environments and much of this evidence has been observed on islands, highlighting them as evolutionary hotspots (Mooney and Cleland, 2001).

Terrestrial invertebrates have been identified as one of the groups with the most profound ecological and economic impact through their roles in environmental processes that give rise to ecosystem services (Vila et al., 2010). However, attention has been focused on invasions above ground while soil organisms have been largely unexplored, even though their invasions may profoundly affect soil ecosystems (Hendrix et al., 2008). Alterations of the soil

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structure by earthworms can cause a cascade of ecological effects (Frelich et al., 2006). In the last decade several authors have studied the effects of introduced earthworms on temperate and tropical regions, showing their capacity to greatly modify nutrient and organic matter dynamics, above- and below-ground community structures, and soil structural properties; the role of humans in earthworm dispersal has also been shown to be significant (Hendrix et al., 2008 and references therein). A well-documented case concerns the invasion of European earthworms (Lumbricidae) in North America, which has resulted in a change in forest floor litter dynamics with resulting effects on ecosystem processes (e.g., Bohlen et al., 2004; Hale et al., 2005; Frelich et al., 2006). Less understood are the effects of megascolecid species, although Burtelow et al. (1998) showed that *Amynthas gracilis* in particular has impacted C and N fluxes in soils of the northeastern United States.

The Azores archipelago is located in the North Atlantic Ocean and comprises nine islands. Of these, São Miguel is the largest and is made up of active volcanic areas including fumarolic fields and cold and thermal springs with soil-diffuse degassing (Viveiros et al., 2009). Humans first populated the island in the late 1430's (Santos et al., 2005) and dramatic changes in land-use started after colonization. Native vegetation at low and middle altitudes became extinct or was modified and exotic plants and animals were introduced (Borges et al., 2006 and references therein). Among the Macaronesian archipelagos, the Azores contain fewer single-island endemic species (Amorim et al., 2012), which has been referred to as the 'Azores diversity enigma' (Carine and Schaefer, 2010). This relatively depauperate native species diversity could make the Azores archipelago more susceptible to successful invasions because more niches are unoccupied and, for example, 70% of the current flora and 58% of the arthropod fauna are exotic (Borges et al., 2006).

Most phylogeographic studies of earthworms to date have been carried out within the natural ranges of the species, including megascolecids (e.g., Chang and Chen, 2005; Chang et al., 2008; Buckley et al., 2011; Fernandez et al., 2011; Novo et al., 2011). Cameron et al. (2008) and Cunha et al. (2014) investigated the population genetic structure of invasive earthworms in the US and the Azores respectively, in relation to human-mediated dispersal and landscape features. Porco et al. (2013) and Shekhovtsov et al. (2014) have also applied DNA barcoding to study invasions of earthworms in the US and Kamchatka. More studies are needed to better understand the dynamic forces affecting earthworm invasions given the potential consequences for native species and ecosystems.

The Megascolecidae is the largest earthworm family and has a suggested Pangean origin (Jamieson et al., 2002). The genus *Amynthas* is thought to be native to the eastern Palearctic, with species being described as cosmopolitan, peregrine and invasive. *Amynthas corticis* and *A. gracilis* are amongst the most invasive earthworms on earth, mainly due to their inherent environmental plasticity, rapid growth, high reproductive performance and relatively large adult body size (Burtelow et al., 1998; Garcia and Fragoso, 2002). Additionally, some lineages of *A. corticis* including those from the Azores (unpublished observations by the authors) are parthenogenetic. This mode of reproduction can facilitate invasiveness (Terhivuo and Saura, 2006). For example, parthenogenetic species can found stable populations initiated by a single individual in novel environments. The benefit of uniparental reproduction is highest after long-distance dispersal (Baker, 1965, 1967), which would be the case following human introductions.

It is not presently known whether the introduction of megascolecid worms to the Azores was a single or multiple event(s); neither is it known whether the invaders originated from one or more populations in the Eastern Palearctic (Hendrix et al., 2008). Given that the establishment on São Miguel Island by both *A. corticis* and *A. gracilis* is probably a relatively recent human-mediated event, they constitute an ideal model to test how the genetic background of an invader responds to local geography and the chemical characteristics of a new environment, in this case an island characterised by a volcanic-driven topography and geology. Physical features are known to affect earthworm distributions even on a very small scale (Rossi et al., 1997; Nuutinen et al., 1998; Hernandez et al., 2007; Jiménez et al., 2014).

Our aim was to study the phylogeography of megascolecids in São Miguel Island in the Azores (Portugal), placing their human introduction into a global context and exploring the likely factors affecting their establishment on this island. Specifically we aimed to: i) compare the haplotypes of Azorean *A. gracilis* and *A. corticis* to those of both species from other global sources (including Asian) in an attempt to detect different introduction events; ii) study the extent of genetic variability at the landscape scale in São Miguel by identifying genetic lineages within both species and their spatial distributions; iii) explore the relationships between the abundances of different lineages and environmental characteristics. Addressing these issues has allowed us to ascertain whether the invasion success of *Amynthas* on São Miguel is attributed to just one highly adapted genetic lineage per species, or to multiple lineages with potentially different adaptative capacities to spatially heterogeneous environmental conditions.

2. Materials and methods

2.1. Sampling

Samples were collected from throughout São Miguel Island (Azores, Portugal). Its area is 757 km^2 and includes three active volcanoes: Furnas (eastern end, ca. 1 million years old), Sete Cidades (western end, 550–750 thousand years old) and Fogo (center, ca. 350 thousand years old) (Gomes et al., 2006; Cunha et al., 2014). The oldest area of the island, to the east is ca. 4 million years old (Harris et al., 2013). The climate in São Miguel is oceanic and temperate being strongly influenced by altitude (Ricardo et al., 1977). The summits are higher in the eastern part (Pico da Vara, 1080 m) than in the western part (Eguas, 873 m), and the central part is the lowest (maximum of 400 m) (Louvat and Allègre, 1998). Mean annual temperatures range between 11.5 °C at the summit of Agua de Pau volcano (800–900 m) and 17.3 °C in Ponta Delgada (sea level). Annual rainfall and average relative humidity increase with elevation: rainfall ranges from 1000–1500 mm/yr in the driest part of the island (the south coast) to 3000 mm/yr at Lagoa do Fogo; relative humidity ranges from 77–78% on the coast to 87–88% at the highest elevation (Malucelli et al., 2006). Around 500 megascolecid earthworms were collected from 37 localities (Supplementary Table 1, Fig. 1B, C). The sampling effort was standardized in all the locations in order to record relative (but not absolute) abundances and to compare these between sites. These relative numbers are named abundances hereafter within the text. We sampled at least 8 *quadrats* (50 cm × 50 cm) randomly distributed in the sampling area that were separated by at least 3 m. Sampling effort was limited by time and people making the combination uniform among sites (generally 4 people digging for 60 min) with the search targeted to *Amynthas* specimens. All individuals were hand collected, washed in distilled water, weighted *in vivo* and subsequently fixed and preserved in ca. 96% EtOH. A portion of the integument (ca. 25 mg) was cleansed and preserved at −20 °C for DNA extraction. Samples from other countries in the species' global distribution, including from the presumed native range (Malaysia: MAL, China: YN, HB; Taiwan: TW), were donated