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Ancient origin of endemic Iberian earth-boring dung beetles (Geotrupidae)

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ABSTRACT

The earth-boring dung beetles belong to the family Geotrupidae that includes more than 350 species classified into three subfamilies Geotrupinae, Lethrinae, and Taurocerastinae, mainly distributed across temperate regions. Phylogenetic relationships within the family are based exclusively on morphology and remain controversial. In the Iberian Peninsula there are 33 species, 20 of them endemic, which suggests that these lineages might have experienced a radiation event. The evolution of morphological adaptations to the Iberian semi-arid environments such as the loss of wings (apterism) or the ability to exploit alternative food resources is thought to have promoted diversification. Here, we present a phylogenetic analysis of 31 species of Geotrupidae, 17 endemic to the Iberian Peninsula, and the remaining from southeastern Europe, Morocco, and Austral South America based on partial mitochondrial and nuclear gene sequence data. The reconstructed maximum likelihood and Bayesian inference phylogenies recovered Geotrupinae and Lethrinae as sister groups to the exclusion of Taurocerastinae. Monophyly of the analyzed geotrupid genera was supported but phylogenetic relationships among genera were poorly resolved. Ancestral character-state reconstruction of wing loss evolution, dating, and diversification tests altogether showed neither evidence of a burst of cladogenesis of the Iberian Peninsula group nor an association between apterism and higher diversification rates. Loss of flight did not accelerate speciation rates but it was likely responsible for the high levels of endemism of Iberian geotrupids by preventing their expansion to central Europe. These Iberian flightless beetle lineages are probably paleoendemics that have survived since the Tertiary in this refuge area during Plio-Pleistocene climatic fluctuations by evolving adaptations to arid and semi-arid environments.

1. Introduction

The earth-boring dung beetles belong to the highly diversified family Geotrupidae Latreille, 1802 that includes around 350 species (Schoolmeesters, 2010), currently classified into three subfamilies (Browne and Scholtz, 1999; Scholtz and Browne, 1996): Geotrupinae mainly distributed across the Holarctic region (≈234 spp.), Lethrinae present in eastern Europe and Asia (≈114 spp.), and Taurocerastinae with only three recognized species distributed in South America (but see Browne and Scholtz, 1995; Howden, 1982; Howden and Peck, 1987; Zunino, 1984, which included Taurocerastinae within Geotrupinae). Former classifications (Howden, 1982; Lawrence and Newton, 1995; Zunino, 1984) included the basically pantropical Bolboceratidae (≈561 spp.) as a subfamily of Geotrupidae, a proposal currently not supported by available phylogenies based on larval and morphological characters (Browne and Scholtz, 1999; Verdú et al., 2004; but see Smith et al., 2006). Geotrupidae are thought to represent an early offshoot of the superfamily Scarabaeoidea that may have diverged from the remaining dung beetles at least during the Lower Cretaceous according to fossil data (Krell, 2007). Although earth-boring dung beetles are fairly represented in the fossil record (Krell, 2007) there is no information regarding lineage-splitting events within the family that lead to current species diversity.

The Iberian Peninsula hosts a remarkably diversified assemblage of earth-boring dung beetles (López-Colón, 2000). A total of 11 genera are present in the Iberian Peninsula representing about 65% and 23% of the total number of European and World Geotrupidae genera, respectively. These 11 Iberian genera include 33 species, which correspond to 62% and 9% of the European and World Geotrupidae species, respectively. Eighteen of the 20 Iberian endemic geotrupid species are apterous (i.e. have lost flight due to atrophy of hindwings and fusion of elytra); thus, their dispersal capacity is limited (Lobo et al., 2006). As in other beetles (e.g. Chown et al., 1998), the origin of apterism in Iberian Geotrupidae may be originally related to adaptation to

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arid or semi-arid conditions since the fusion of elytra diminishes loss of body water.

Despite the wealth of molecular studies centred on Scarabaeoidea (Cabrero-Sañudo and Zardoya, 2004; Forgie et al., 2006; Monaghan et al., 2007; Price, 2009; Smith et al., 2006; Sole and Scholtz, 2010; Villalba et al., 2002), few studies have focused on Geotrupidae, and phylogenetic relationships among earth-boring dung beetles remain poorly understood. Thus far, phylogenetic studies on Geotrupidae have been mostly based on morphological data, and rendered poorly resolved or even contradictory results (Palmer and Cambefort, 1997; Verdú et al., 2004; Zunino, 1984). The only attempt to examine the internal relationships within Geotrupidae based on molecular data focused on the biogeographical patterns of the genus *Trypocopris* (Carisio et al., 2004).

Here, we studied 31 species of earth-boring dung beetles inhabiting the Iberian Peninsula, southeastern Europe, Morocco, Mexico, and Austral South America. We conducted a phylogenetic analysis using both mitochondrial (mt) and nuclear sequence data. In particular, we assessed the phylogenetic placement of the subfamily Taurocerastinae, as well as provided a first phylogenetic hypothesis on the internal relationships within Geotrupinae. As Iberian Geotrupidae species are characterized by a high proportion of endemics we used the recovered phylogenies to test the hypothesis that adaptation to arid and semi-arid environments together with the associated loss of wings could be a major driving force for speciation in this group. First, we performed an ancestral character-state reconstruction to gain insights into the evolution of wing loss within this family. Subsequently, we dated lineage-splitting events within Geotrupidae to finally examine rates of lineage diversification through time in Iberian earth-boring dung beetles.

2. Materials and methods

2.1. DNA sources and extraction

A total of 31 specimens representing the same number of species, 13 genera and the three subfamilies of Geotrupidae were collected from the localities indicated in Fig. 1. All biological material used in this study is deposited in the DNA and tissues collection of the Museo Nacional de Ciencias Naturales of Madrid. Based on previous work (Cabrero-Sañudo and Zardoya, 2004; Villalba et al., 2002) species belonging to the other two lineages of dung beetles, Aphodiidae (*Aphodius conjugatus*) and Scarabaeidae (*Bubas bison*) were selected as outgroups. Total DNA was extracted from frozen tissue with a DNA Easy extraction kit (Qiagen).

2.2. PCR amplification and sequencing

Primers COI-Sca-F, COI-Sca-R, COIIam-Sca, and COIIB-605-Sca (Villalba et al., 2002), were used to amplify by PCR two overlapping fragments that comprised the 3′ end of the mt *cox1*, the adjacent complete *trnL (uur)*, and the 5′ end of the *cox2* genes of 31 specimens of earth-boring dung beetles, as well as of two outgroup species. Additionally, primers 8029fin-4F and 8029fin-7R (Regier et al., 2008) were used to amplify by PCR a fragment of 718 bp of the nuclear gene neurofibromin. Initially, only a few samples were amplified successfully. Therefore, two internal primers Sca nuc-F (CATATGATGTGGGGTGAYATCGC) and Sca-nuc-R (AAACGCTCGCGATCTGGCGAAGC) were designed to amplify by PCR a fragment of 420 bp in a total of 23 specimens, including the outgroup *Bubas*

Table 1
List of subfamilies and species used in this study, geographic range, endemic species from the Iberian Peninsula (IP), and GenBank acession numbers for nuclear and mitochondrial gene fragments.

Subfamily	Species	Geographic range	Iberian Peninsul endemics	GeneBank accession number	
				Nuclear	*coxl + LEU + cox2*
Geotrupinae	*Ceratophyus hoffmannseggi*	Southwestern Europe, North Africa	No		GU984631
	Ceratophyus martinezi	North-Central IP	**Yes**		GU984624
	Geotrupes stercorarius	Western Europe	No	GU984581	GU984634
	Geotrupes ibericus	IP	**Yes**	GU984590	GU984608
	Geotrupes mutator	Western Europe	No	GU984579	GU984606
	Geotrupes spiniger	Europe-Western Asia	No		GU984614
	Jekelius albarracinus	Central-southeasthern IP	**Yes**	GU984592	GU984604
	Jekelius catalonicus	Northeastern IP	**Yes**	GU984578	GU984629
	Jekelius castillanus	Central Iberian Peninsula	**Yes**	GU984597	GU984603
	Jekelius hispanus	Southwestern IP	**Yes**	GU984599	GU984628
	Jekelius hernandezi	Southeastern IP	**Yes**	GU984593	GU984627
	Jekelius nitidus	Northwestern IP	**Yes**	GU984583	GU984626
	Jekelius punctatolineatus	Southeastern IP	**Yes**	GU984582	GU984625
	Jekelius balearicus	Balearic islands	**Yes**	GU984580	HM625867
	Silphotrupes orocantabricus	Northwestern IP	**Yes**	GU984591	GU984623
	Silphotrupes punctatissimus	North-Central IP	**Yes**	GU984588	GU984622
	Thorectes baraudi	South-Central IP	**Yes**	GU984598	GU984636
	Thorectes ferreri	South IP	**Yes**	GU984585	GU984635
	Thorectes lusitanicus	South IP	**Yes**		GU984615
	Thorectes valencianus	Southeastern IP	**Yes**	GU984584	GU984632
	Thorectes armifrons	North Africa	No		GU984605
	Trypocopris pyrenaeus	Europe	**No**	GU984596	GU984621
	Typhaeus momus	South IP	**Yes**	GU984600	GU984620
	Typhaeus typhoeus	Europe-North Africa	No		GU984619
	Haplogeotrupes guatemalensis	Central America	No	GU984601	GU984617
	Sericotrupes niger	Southwestern Europe, North Africa	No	GU984589	GU984609
	Onthotrupes nebularum	Mexico	No	GU984594	GU984618
	Onthotrupes herbeus	Mexico	No		GU984616
Taurocerastinae	*Taurocerastes patagonicus*	Austral South America			GU984611
	Frickius variolosus	Austral South America		GU984595	GU984610
Lethrinae	*Lethrus raymondi*	Southeastern Europe, West Asia			GU984607
Aphodiinae	*Aphodius conjugatus*	–			AY223690
Scarabaeinae	*Bubas bison*	–	–	GU984602	AY039339