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Molecular phylogenetics unveils the ancient evolutionary origins of the enigmatic fairy armadillos

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ABSTRACT

Fairy armadillos or pichiciegos (Xenarthra, Dasypodidae) are among the most elusive mammals. Due to their subterranean and nocturnal lifestyle, their basic biology and evolutionary history remain virtually unknown. Two distinct species with allopatric distributions are recognized: *Chlamyphorus truncatus* is restricted to central Argentina, while *Calyptophractus retusus* occurs in the Gran Chaco of Argentina, Paraguay, and Bolivia. To test their monophyly and resolve their phylogenetic affinities within armadillos, we obtained sequence data from modern and museum specimens for two mitochondrial genes (12S RNA [MT-RNR1] and NADH dehydrogenase 1 [MT-ND1]) and two nuclear exons (breast cancer 1 early onset exon 11 [BRCA1] and von Willebrand factor exon 28 [VWF]). Phylogenetic analyses provided a reference phylogeny and timescale for living xenarthran genera. Our results reveal monophyletic pichiciegos as members of a major armadillo subfamily (Chlamyphorinae). Their strictly fossorial lifestyle probably evolved as a response to the Oligocene aridification that occurred in South America after their divergence from Tolypeutinae around 32 million years ago (Mya). The ancient divergence date (~17 Mya) for separation between the two species supports their taxonomic classification into distinct genera. The synchronicity with Middle Miocene marine incursions along the Paraná river basin suggests a vicariant origin for pichiciegos by the disruption of their ancestral range. Their phylogenetic distinctiveness and rarity in the wild argue in favor of high conservation priority.

1. Introduction

The two living species of fairy armadillos or pichiciegos, *Chlamyphorus truncatus* (pink fairy armadillo) and *Calyptophractus retusus* (Chacoan fairy armadillo), are probably among the most elusive mammals. Encounters with these small and enigmatic creatures are extremely rare and incidental. The two species have allopatric distributions (Fig. 1A). *Ch. truncatus* is endemic to the provinces of central Argentina, where it occurs in dry grasslands and on sandy plains, whereas *Ca. retusus* is distributed in the Gran Chaco region spanning central and south-eastern Bolivia, western Paraguay, and extreme northern Argentina (Abba and Superina, 2010). These cryptic animals are subterranean and strictly nocturnal, but the details of their ecology and population biology remain unknown (Cuéllar, 2001; Meritt, 1985; Superina, 2006). As a consequence, both species are classified as Data Deficient by the IUCN Red List of Threatened Species (Abba and Superina, 2010), which means that insufficient scientific information is available to realistically assess their conservation status. Field observations suggest, however, worrying population declines for the pink fairy armadillo (Superina, 2006), while the Chacoan fairy armadillo is affected by habitat loss and persecuted by indigenous people who believe it to be an omen of bad luck, foretelling an impending death in the family (Abba and Superina, 2010; Cuéllar, 2001).

The two fairy armadillo species are the smallest armadillos (both weigh around 100 g) and share similar morphological adaptations to the subterranean lifestyle, such as enlarged digging claws, reduced eyes, a fusiform body shape, and a vertical, rounded plate that caps the rump (Fig. 1B and C). Marked differences exist nonetheless, notably in the carapace structure, the shape of the cephalic shield and ears, and the shape of the tail. Chacoan fairy armadillos bear a dorsal carapace that is fully attached to the skin

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Abbreviations: MT-RNR1, mitochondrially encoded 12S RNA; MT-ND1, mitochondrially encoded NADH dehydrogenase 1; BRCA1, breast cancer 1 early onset; VWF, von Willebrand factor; Mya, million years ago; Myr, million years; bp, base pairs; ML, Maximum likelihood; MCMCMC, Metropolis-coupled Markov Chain Monte Carlo.

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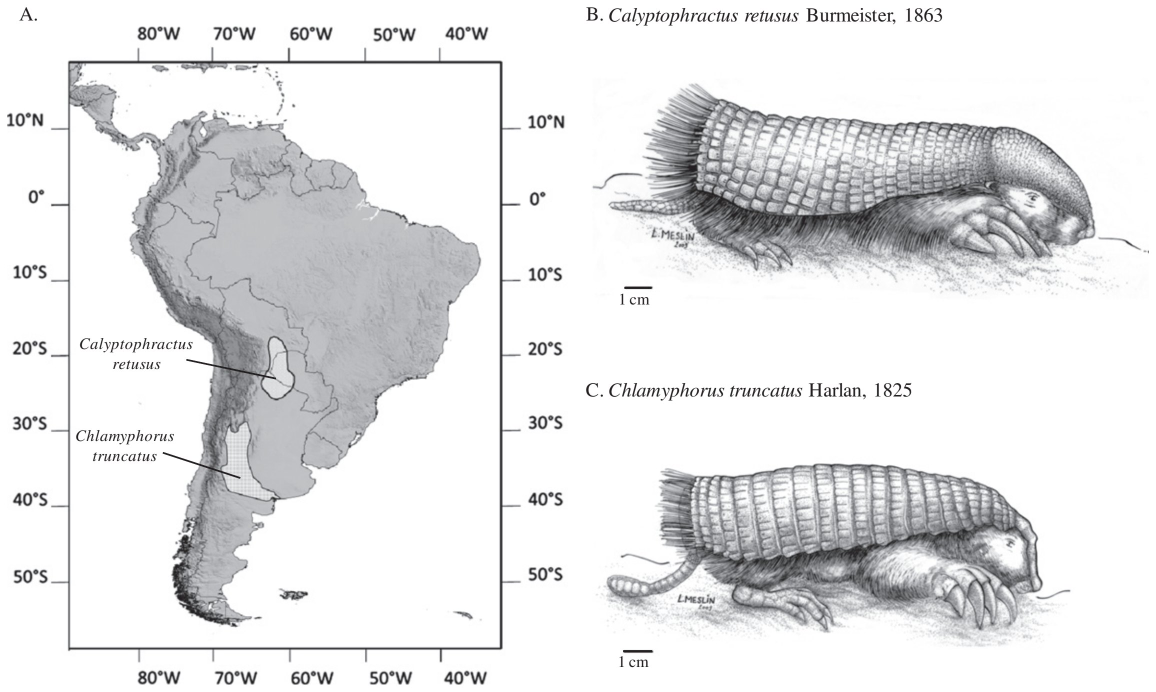

B. *Calyptophractus retusus* Burmeister, 1863

C. *Chlamyphorus truncatus* Harlan, 1825

Fig. 1. Geographic ranges and comparative morphologies of the two living species of fairy armadillos. (a) Updated distribution maps of the Chacoan fairy armadillo *Calyptophractus retusus* (light gray) and the pink fairy armadillo *Chlamyphorus truncatus* (hashed lines) modified from Abba and Superina (2010); (b) *Calyptophractus retusus* Burmeister, 1863; (c) *Chlamyphorus truncatus* Harlan, 1825. A major distinction between the two species is the dorsal carapace, which is only attached to the body through a thin membrane along the spine in *Chlamyphorus*, whereas it is fully attached to the body in *Calyptophractus*, as in other armadillos. Note the spatula-shaped tail and the well-defined head shield in *Chlamyphorus*, and the rounded tail and visible ears in *Calyptophractus*. Drawings by Laurence Meslin. ©Meslin-CNRS2009.

of the back and head. Their rounded head shield is wider than in the other species and extends laterally and ventrally to the level of the eye. Their ears are visible, and the tip of their tail is rounded. In contrast to this, the carapace of pink fairy armadillos is only united to the body by a thin membrane on the dorsal mid-line. A row of large osseous plates (osteoderms or scutes) at the posterior margin of their well-defined head shield gives the appearance of a distinct "step" in the dorsal surface. Their ears are not visible, and the extremity of their tail is characteristically flattened and diamond-shaped (Wetzel, 1985a,b).

These similarities and differences have been the cause of taxonomic debate for decades. The pink fairy armadillo has been named *Ch. truncatus* since its first description by Harlan in 1825 (Harlan, 1825). The Chacoan fairy armadillo, initially described as a second species of the genus *Chlamyphorus* by Burmeister in 1863 (Burmeister, 1863), was later re-assigned to the genera *Burmeisteria* by Gray in 1865 (Gray, 1865) and *Calyptophractus* by Fitzinger in 1871 (Fitzinger, 1871). Moeller (1968) proposed retaining *Burmeisteria* in order to reflect the degree of morphological differentiation between the two species. However, *Burmeisteria* was previously attributed to a trilobite (Salter, 1865), and the appropriate name should thus be *Calyptophractus* if a separate genus name were deemed advisable (Wetzel, 1985a). Wetzel, the longstanding taxonomic authority for xenarthrans, did not advocate such a generic distinction, arguing that the two species represented different evolutionary points on one generic gradient of fossorial adaptation (Wetzel, 1985a). The generic distinction has, nevertheless, been retained in recent classifications (Gardner, 2005; IUCN, 2011).

The morphological distinctiveness of pichiciegos within armadillos also prompted Wetzel (1985a) to assign them to a separate subfamily, Chlamyphorinae. Cladistic analyses of morphological characters including either *Ch. truncatus* (Billet et al., 2011; Engelmann, 1985; Patterson et al., 1989) or *Ca. retusus* (Gaudin and Wible, 2006) specimens suggest that fairy armadillos are closely related to the Euphractinae. The first molecular phylogenetic studies using both mitochondrial and nuclear genes divided armadillos into three major lineages corresponding to the currently accepted subfamilies Dasypodinae (*Dasypus*), Tolypeutinae (*Tolypeutes*, *Priodontes*, and *Cabassous*), and Euphractinae (*Euphractus*, *Chaetophractus*, and *Zaedyus*), with a close relationship between Tolypeutinae and Euphractinae (Delsuc et al., 2002, 2003). However, these early studies did not include fairy armadillo representatives. The only molecular study that included a Chlamyphorinae species (*Ch. truncatus*) found strong support for a sister-group relationship between the pink fairy armadillo and Tolypeutinae based on phylogenetic analyses of non-coding regions flanking retroposed elements (Möller-Krull et al., 2007).

Because the two species of fairy armadillo have never been included in morphological or molecular phylogenies, their monophyly remains untested and their relationships among armadillos are unsettled. Two alternative hypotheses may explain the morphological similarities between fairy armadillos: either they reflect common ancestry – and illustrate the monophyly of both species – or they are the result of adaptive convergence due to the extreme selective pressures induced by their subterranean lifestyle, which would suggest a diphyletic origin of the two species. To examine the possibility of convergent evolution in fairy armadillos, we used ancient DNA techniques on a *Ca. retusus* museum specimen to obtain the first molecular data for this species. We sequenced candidate markers already used to unveil xenarthran evolutionary relationships, i.e., two mitochondrial genes (12S RNA [MT-RNR1] and NADH dehydrogenase 1 [MT-ND1]) and two nuclear exons (breast cancer 1 early onset exon 11 [BRCA1] and von Willebrand factor exon 28 [VWF]) (Delsuc et al., 2003). Phylogenetic and molecular dating analyses of these data, including new sequences from the other fairy armadillo (*Ch. truncatus*), resolves their evolutionary origins and provides a phylogenetic framework and time scale including all extant xenarthran genera. The data presented here will further inform conservation decisions for these enigmatic species.