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Phylogeography of Cuban *Rivulus*: Evidence for allopatric speciation and secondary dispersal across a marine barrier

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ABSTRACT

The genus *Rivulus* is currently comprised of two species, *R. cylindraceus* and *R. insulaepinorum*, which are endemic to Cuba. However, the taxonomic status of the latter species remains dubious because of the poor quality of the original description. In addition, a recent barcoding survey suggests that the two species may be conspecific. The aim of this study was to test the hypothesis that the two species represent a single evolutionary clade. To delimit the species and their evolutionary history, we used a combination of molecular phylogenetic analyses, with both mitochondrial and nuclear sequences, tests of phylogeographic hypotheses, combined with morphological measurements and information on known dispersal barriers and species distribution. None of the data sets support *R. insulaepinorum* and *R. cylindraceus* as separate taxa. However, a new species, restricted to the northwestern part of the main island, was identified by phylogenetic analyses, body colour pattern and geographical distribution. The evolutionary distance between the two lineages (*cyt*b, $d = 15\%$; *CAM*-4, $d = 2.5\%$) indicates a long period of divergence. Phylogeographic analyses shed light on the dispersal history of *R. cylindraceus*, which probably originated on the Isla de la Juventud. They also suggest that each lineage had contrasting histories; *Rivulus* sp. is restricted to a relatively small geographic area whereas *R. cylindraceus* has dispersed considerably and more than once from its centre of origin, probably facilitated by sea level fluctuations. These results strengthen previous findings, i.e. that the diversity of Cuban freshwater fishes is far from well-known and deserves more in-depth studies, and that vicariance and dispersal events have resulted in a complex biogeographical landscape which has had a significant impact on the freshwater fishes of the Caribbean islands.

1. Introduction

The genus *Rivulus* Poey (1860) belongs to the family Rivulidae, represented by over 350 species occurring between Florida and northeastern Argentina (Costa, 2003, 2006, 2011; Valdesalici et al., 2011). *Rivulus* is endemic to Cuba and is one of the least diverse and among the more geographically restricted of the 35 genera of freshwater fishes in the archipelago (de la Cruz and Dubitsky, 1976). At present, two species are recognized (Costa, 2011): *R. cylindraceus* Poey, 1860 (Green rivulus) endemic to western Cuba, and *R. insulaepinorum* de la Cruz and Dubitsky, 1976 (Isle of *Pines rivulus*), endemic to Isla de la Juventud. Both species are restricted to freshwater mountain streams, lowland rivers, and lagoons (Fig. 1).

The delimitation of *R. cylindraceus* and *R. insulaepinorum* was based on relatively few morphological characters: *i.e.* the number of lateral scales and the relative position of the dorsal fin (Poey, 1860; de la Cruz and Dubitsky, 1976). The taxonomic status of the latter is particularly controversial because of the poor quality of the original description (Huber, 1992) and remains unresolved. Moreover, a recent DNA barcoding study suggests that *R. insulaepinorum* may be conspecific with *R. cylindraceus* (Lara et al., 2010). The estimated COI sequence divergence (1.8 ± 0.4%) is

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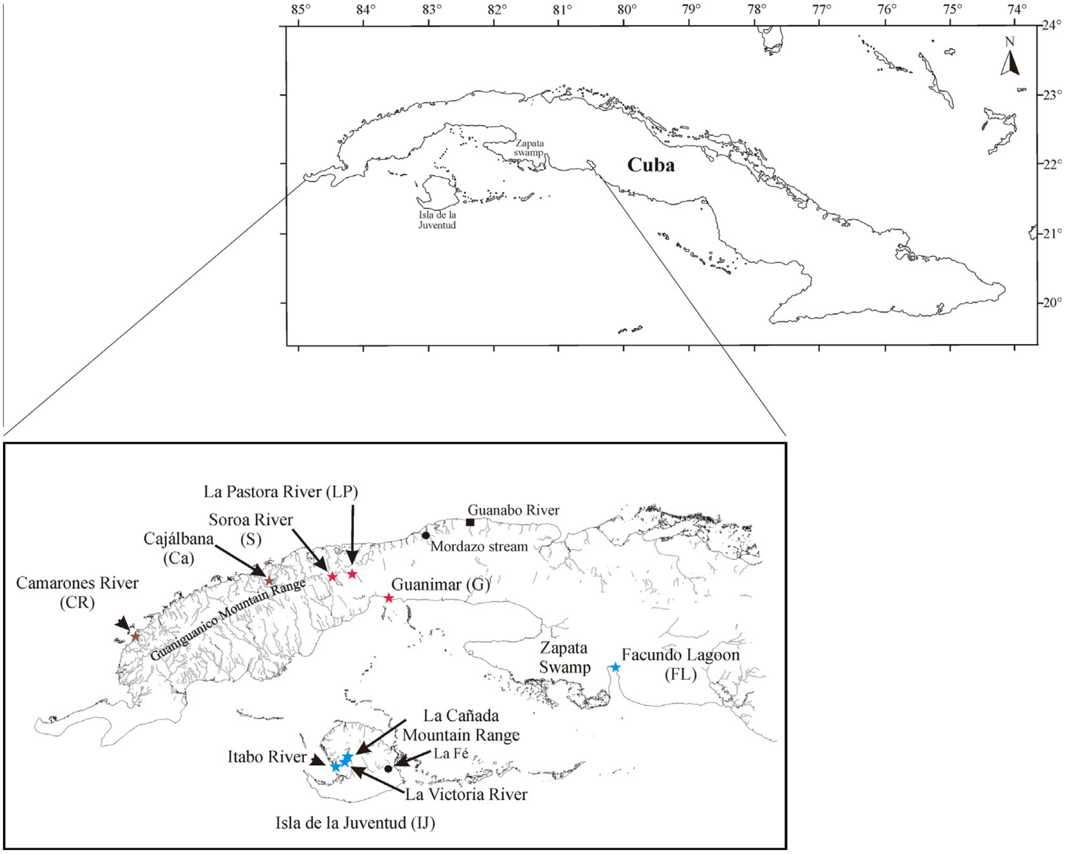

Fig. 1. Sampling sites of the genus *Rivulus* in Cuba. The sampling sites are indicated by stars. Circles indicate the type localities of *R. cylindraceus* (Mordazo stream) and *R. insulaepinorum* (La Fé), and a square indicates the sampling site for *Kryptolebias marmoratus*. The colour of the stars corresponds to that of the clades in Fig. 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

low and a COI phylogeny did not indicate reciprocal monophyly (Lara et al., 2010). A population aggregation analysis (PAA) (Davis and Nixon, 1992) also failed to find diagnostic nucleotide sites for *R. insulaepinorum*.

The delimitation of species has important consequences since biodiversity assessment and conservation programs are largely based on species identification (Agapow et al., 2004). The definition of a species is a contentious field of theoretical discussion in the evolutionary literature, mainly due to the lack of consensus about which properties of a species and the speciation process are universally valid criteria (de Queiroz, 2007). In recent years, several approaches have been proposed to improve species delimitation (Puorto et al., 2001; Templeton, 2001; Wiens and Penkrot, 2002; Sites and Marshall, 2003; Knowles and Carstens, 2007; Rissler and Apodaca, 2007; La Salle et al., 2009; Puillandre et al., 2012). The vast majority of authors recognize that species should be assessed within an explicit statistical framework in which a species represents a hypothesis that needs to be corroborated by supplementary data. For instance, the null hypothesis that organisms being sampled come from a single evolutionary lineage can be tested using phylogenetic and phylogeographic analysis (e.g. Templeton, 2001). Mitochondrial DNA (mtDNA) has often been the marker of choice in spite of controversy about some of its characteristics (Ballard and Whitlock, 2004; Galtier et al., 2009). Species delimitation based on mtDNA analysis can then be further assessed using independent and relevant characters such as nuclear genes, morphology, behavior, mating preference and dispersal ability (Dayrat, 2005; Will et al., 2005; Rissler and Apodaca, 2007; Yeates et al., 2011; Puillandre et al., 2012).

The distribution of tropical freshwater fishes is a function of both the current physical characteristics of the habitat, such as, temperature, water level, and stream order (Moyle and Cech, 1996), as well as the effectiveness of barriers and historical climatic changes. Barrier efficiency appears to vary as function of several intrinsic (e.g. tolerance to salinity) and extrinsic (e.g. lack of corridors, presence of predators, distance between favourable habitats, episodic flooding due to strong rain and storms) factors. Under certain conditions even highly habitat – specialised species have been reported to be able to disperse and colonise new territories (Bossuyt et al., 2004; Bruyn and Mather, 2007; García-Machado et al., 2011; Walter et al., 2011). Historical variations in climate and landscape can have profound effects on species distribution and diversification by creating temporary corridors that promote species dispersion (Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006) or by imposing barriers or fragmenting territories (Jones and Johnson, 2009).

The current distribution of *R. cylindraceus* is highly fragmented, due to human impact as well as natural factors. In the former case, the destruction of the type locality, the Mordazo stream in Havana City is an obvious example. To our knowledge, *R. cylindraceus* and *R. insulaepinorum* have low tolerance to salinity. Climate changes during the last few million years has had a profound impact on sea levels and the shape of the Cuban archipelago (Iturralde-Vinent, 2006), resulting in periods where the Isla de la Juventud has been connected to parts of the main island. The distribution of *R. cylindraceus* and *R. insulaepinorum* on Isla de la Juventud as well as on the main island probably reflects these past changes in the landscape. Similarly, low tolerance to salinity may have