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Inter- and intra-island divergence in *Odorrana ishikawae* (Anura, Ranidae) of the Ryukyu Archipelago of Japan, with description of a new species

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Abstract

The endangered frog, *Odorrana ishikawae* (Anura, Ranidae), is a species endemic to the Amami and Okinawa Islands of the Ryukyu Archipelago, Japan. Segmentation of these islands has been considered to occur middle or upper Pleistocene. Our morphometric analyses revealed obvious differences between the Amami and Okinawa populations. Two distinct morphotypes were also recognized from the Amami Island (Amami common and Amami large types). Furthermore, the Amami and Okinawa populations could be distinguished clearly by coloration and dorsal tuberculation. Based on 16S rRNA gene data, the Okinawa and Amami populations were phylogenetically separated but the genetic divergence (1.44–2.16%) was lower than the value suggested as species threshold in anurans (> 3% in *16S*). Individuals of the Amami common and large types were nested within a single clade. Artificial hybridization experiments revealed normal hybrid viability between the two Amami types, with one exception. By contrast, between Okinawa females and two Amami type males, complete hybrid inviability was observed at early embryonic stages in the hybrids contrary to expectations from their low divergence in *16S*. The reciprocal hybrids between two Amami type females and Okinawa males were viable, but spermatogenesis in the hybrid males showed some degree of abnormality. These results strongly indicate specific separation of the Amami population from the Okinawa population of *O. ishikawae*. Thus, we describe the Amami population as a new species, which is readily distinguishable from *O. ishikawae* by smaller ruggedly edged dorsal spots and an immaculate ventral surface.

Key words: *Odorrana splendida* **sp. nov.**, *Odorrana ishikawae*, Ranidae, Ryukyu Archipelago, Japan

Introduction

Ishikawa's frog, *Odorrana ishikawae*, is a species endemic to mountain stream regions in the Amami and Okinawa Islands of the Ryukyu Archipelago, Japan. It is a large and brightly colored frog, and the species is occasionally referred to as the most beautiful frog in Japan (Maeda & Matsui 1999). Unfortunately, environmental destruction and over hunting for pet trade purpose have recently devastated the populations of this species. As a result, *O. ishikawae* has been listed as a class B1 endangered species on the IUCN Red List of Threatened Species (IUCN 2010), and subsequently protected by law in Okinawa and Kagoshima prefectures. The taxonomic history of *O. ishikawae* is somewhat complicated. Originally, this frog was described as a rhacophorid species, *Buergeria ishikawae* (Stejneger 1901). Then it was reclassified into several ranid genera, such as *Rana* and *Huia*, and most recently it has been classified as *Odorrana* (Frost 2010). No sister species of *O. ishikawae* is known to date (Maeda & Matsui 1999), and several molecular phylogenetic studies have suggested a basal position within *Odorrana* (Matsui *et al*. 2005; Cai *et al*. 2007; Kurabayashi *et al*. 2010).

The breeding ecology of *O. ishikawae* was revealed by Katsuren *et al*. (1977), Utsunomiya *et al*. (1979), and Utsunomiya and Utsunomiya (1983). Eggs are typically deposited in underground water in holes located along the slopes of rocky upper streams in forest regions. Advertisement calls are composed of a single long note with conspicuous frequency modulation (Kuramoto 1980). Eggs are pigmentless and very large; ca. 4.3 mm in ovum diam-

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