



Lineage divergence in *Odorrana graminea* complex (Anura: Ranidae: *Odorrana*)

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Abstract

The confusing and unstable taxonomy of *Odorrana livida* (*Rana livida*) since its first record has made it a focal frog complex for systematics. In China, four species, *Odorrana nebulosa*, *O. graminea*, *O. sinica*, *O. leporipes*, were described to closely resemble *O. livida* or *O. chloronota* based on their morphological similarities, accompanied by much taxonomic confusion because of ambiguities in the wide distribution and morphological variations. Currently *O. graminea* is being used as the name of a provisional monotypic species group to include all the populations in China that closely resemble *O. livida* or *O. chloronota*. Here, we conducted a range-wide molecular phylogeographic analysis of the large green odorous frog (*Odorrana graminea*) complex across the majority of its range in China, based on 2780 bp DNA sequences of three mitochondrial genes (12S, 16S, ND2) in 107 samples from 20 sites. Our data recognized three distinct phylogeographic lineages of the *Odorrana graminea* (lato sensu) complex in China, and they together with a Thailand lineage formed a monophyletic group. Among the four lineages within *O. graminea* complex, the average genetic distances based on the concatenated sequences of 12S, 16S and ND2 were 7.5–8.8% and those based on 16S rRNA alone were 4.2–5.5%. Furthermore, canonical discriminant functions in morphometric analyses showed significant separations of all the paired lineage comparisons in China. The aforementioned genetic divergence and mismatched phenotypes among the lineages within the *Odorrana graminea* complex, in addition to their non-overlapping geographic distributions, imply extensive lineage diversification. However, precise taxonomic status of these lineages needs more studies based on adequate type information and more thorough species delimitation based on analysis of differentiation in bioacoustic and nuclear genetic characters especially regarding gene flow and admixture in geographical contact zones.

Key words: *Odorrana livida*, *O. chloronota*, *O. graminea*, phylogeny, lineage delineation

Introduction

The process of defining species boundaries is more than academic, for understanding which species occurs where is essential to mitigate loss of biodiversity (Nair *et al.* 2012). With the limited resources, we usually take into consideration of conservation of species with smaller geographic ranges, and consequently, greater vulnerability to extinction (Jablonski & Chaloner 1994; Gaston & Blackburn 1996; Purvis *et al.* 2000). Given the conservative morphological evolution of amphibians (Cherty *et al.* 1978; Stuart *et al.* 2006), as well as many other taxa (Austin 1995; Fondon & Garner 2004), cryptic species (multiple genetically distinct species classified as a single widespread species on the basis of similar morphology) pose substantial conservational challenges as underestimation of the importance of cryptic species may lead to the lower values of biodiversity and erroneous strategy of species conservation (Bickford *et al.* 2007; Fouquet *et al.* 2007; Vieites *et al.* 2009; Rowley *et al.* 2010). Recently, with advances in DNA sequencing and molecular phylogenetic methods, many new cryptic amphibian lineages have been described from various biodiversity hotspots in East and Southeast Asia (Bain *et al.* 2003; McLeod 2010; Yang *et al.* 2011; Nair *et al.* 2012; Shen *et al.* 2012; Yang *et al.*, 2014), indicating that the amphibian diversity within this area may be heavily underestimated. This is certainly the case for *livida/chloronota* complex in the genus *Odorrana* Fei, Ye, and Huang, 1990 (Bain *et al.* 2003; Matsui & Jaafar 2006; Stuart *et al.* 2006).

The genus *Odorrana* is a speciose group of ranid frogs that inhabit swift montane torrents throughout East and Southeast Asia. Recently more species of *Odorrana* have been described in this area (Fei *et al.* 2007a; Fei *et al.* 2007b; Li *et al.* 2008; Yang & Rao 2008; Bain *et al.* 2009; Chen *et al.* 2010; Kuramoto *et al.* 2011). Among the 55