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A phylogenetic analysis of species in the *Bufo crucifer* group (Anura: Bufonidae), based on indolealkylamines and proteins from skin secretions

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Abstract

This research tested the utility of two classes of skin secretion compounds to the phylogeny of the *Bufo crucifer* group. Skin secretions from specimens of nine populations of *B. crucifer* group were obtained and submitted to qualitative analysis. We observed a clear difference in the composition of the skin secretion molecules obtained from the species of *Bufo* studied. Fifty-nine molecules, 16 indolealkylamines and 43 proteins, were used as characters, and 39 of these were parsimonious informative. The tree topology of the skin secretion combined data showed areas of congruence and conflict when compared to an mtDNA phylogeny of the *B. crucifer* group. We used the Templeton test to evaluate the heterogeneity between the skin secretion and mtDNA data. Although not recommended, we performed a combined analysis with the two partitions. The skin secretion characters from the species of *Bufo* studied have phylogenetic signal. These data are indicative, at least as a preliminary study, of the phylogenetic relationships among the *B. crucifer* group taxa.

Keywords: *Bufo*; *Bufo crucifer* group; Toad; Skin secretion; Proteins; Indolealkylamines; Alternative character to infer phylogeny

1. Introduction

The genus *Bufo* Laurent, 1768 is cosmopolitan and encompasses more than 250 species, arranged in approximately 40 phenetical groups (Frost, 2004). One of these, the *Bufo crucifer* group, occurs in Atlantic Rain Forest and adjacent

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areas from the state of Ceará to Rio Grande do Sul in Brazil and possibly in northeastern Argentina (Baldissera et al., 2004). The group is presumably most closely related to the *Bufo marinus* group (Blair, 1972; Low, 1972; Maxson, 1984; Baldissera et al., 1999), from which it differs by having relatively lower cranial crests, smoother dorsal skin, and smaller parotoid glands (Duellman and Schulte, 1992). Wied-Neuwied (1821) described the epithet *crucifer* as a triple cross, formed by a dark dorsal pattern that follows the vertebral line. This character is extremely polymorphic and has contributed to some taxonomic confusion in the group.

In the past, different forms in the *B. crucifer* group were regarded as species or subspecies (Lutz, 1934; Cochran, 1955) but were placed under the synonymy of *B. crucifer* by Cochran (1955). Cei (1980) redescribed *B. crucifer* using a specimen from São Paulo state (Brazil), and some recent works suggest the existence of more than one species in the group (Haddad and Sazima, 1992; Izecksohn and Carvalho-e-Silva, 2001). Recently, Baldissera et al. (2004) revised the *B. crucifer* group on the basis of external morphological and morphometric characters and recognized five species: *B. crucifer* Wied-Neuwied, 1821 (ranges from Ceará to southern Espírito Santo and northeastern Minas Gerais); *Bufo ornatus* Spix, 1824 (occurs from southern Espírito Santo, through Rio de Janeiro and São Paulo to northern Paraná and possibly in northeastern Argentina, in the provinces of Misiones and Corrientes); *Bufo henseli* A. Lutz, 1934 (southern Santa Catarina to the coast of Rio Grande do Sul); *Bufo abei* Baldissera, Caramaschi, and Haddad, 2004 (Paraná through Santa Catarina to northern Rio Grande do Sul); and *Bufo pombali* Baldissera, Caramaschi, and Haddad, 2004 (occurs in transitional areas between the Atlantic Rain Forest and ‘‘cerrados’’ of Minas Gerais). Additionally, Baldissera (2001) proposed a phylogeny of the group based on 12S and 16S rRNA mitochondrial gene sequences.

Several data sets have been used in phylogenetic analysis of amphibians, including DNA or RNA sequence (Liu et al., 2000; Chek et al., 2001; Read et al., 2001; Pramuk et al., 2001; Dawood et al., 2002; Wilkinson et al., 2002; Symula et al., 2003; Moriarty and Cannatella, 2004; Hillis and Wilcox, 2005), hormones (Conlon et al., 2000), morphology (Ford, 1993; Mendelson et al., 2000; Faivovich, 2002; Haas, 2003), protein sequences (Alrubaian et al., 2002), skin secretions (Maciel et al., 2003), and sperm ultrastructure (Garda et al., 2002). These different data sets have been used in isolation or in combined analyses (e.g. Pramuk, 2002).

The granular glands of amphibian skin produce a great variety of substances responsible for the noxious or poisonous character of this tissue. These substances have been grouped according to their chemical structure as biogenic amines, steroids, alkaloids, peptides, and proteins (Daly et al., 1987; Clarke, 1997; Erspamer, 1994). They act as passive defense mechanisms against predators and microorganisms, and they have evolved independently in different groups of amphibians as a result of their interaction with the environment (Toledo and Jared, 1995).

Three groups of aromatic amines are found in amphibian skin (Erspamer, 1971): indolealkylamines, imidazolealkylamines, and hydroxyphenylalkylamines. Owing to their variety, abundance, and widespread distribution, indolealkylamines are by far the most important and characteristic biogenic amines of amphibian skin (Erspamer, 1994). According to Cei et al. (1972), the skin of *Bufo* provides a spectacular representation of indolealkylamines. Since the occurrence of a given indole derivative implies the occurrence of the enzyme systems catalyzing its biosynthesis and several of these enzyme systems are likely highly specific, these substances might trace species evolution and provide information about phylogenetic relationships. Several works describe differences in indolealkylamine composition from species of *Bufo* (e.g. Cei et al., 1972), and they have been used in phylogenetic analyses of species of *Bufo* (Ceriotti et al., 1989; Maciel et al., 2003).

Herein we evaluate the utility of indolealkylamines and proteins from skin secretions in a phylogenetic analysis of species in the *B. crucifer* group, and conduct partitioned and combined analyses of the different forms (as recognized by Baldissera et al., 2004), using data from skin secretions and gene sequences.

2. Materials and methods

Specimens from nine populations of the *B. crucifer* group were collected in different regions in eastern Brazil (Table 1). Specimens of *B. marinus*, *Bufo granulosus*, and *Bufo* gr. *margaritifer* were also collected and used as outgroups in phylogenetic analyses (see below). The surveys were done under Centro de Conservação e Manejo de Anfíbios e Répteis/Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (RAN/IBAMA) license numbers 12/2001 and 054/02. Voucher specimens were deposited in the Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília, DF, Brazil – *B. marinus* – Belém do Pará (PA): CHUNB 35659; *B.* gr. *margaritifer* – Balsas (MA): CHUNB 35660; *B. granulosus* – Porto de Galinhas (PE): CHUNB 35661, 35662; *Bufo* cf. *crucifer* – Manhumirim (MG): CHUNB 35661–35669; *B. crucifer* – Itacaré (BA): CHUNB 35670–35672; *B. pombali* – Nova Lima (MG):