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Erythrocyte nuclear size as a better diagnostic character than cell size in the identification of live cryptic polyploid species

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Abstract

It is well documented in anurans the cryptic condition of many species complexes involving polyploids. In these complexes the character that clearly differentiates them is the number of chromosome complements. The blood cells of amphibians conserve their nucleus, and so the erythrocyte size is correlated with the DNA content. We analyzed two cryptic-polyploid complexes occurring in the center of Argentina: *Odontophrynus cordobae* (2n)/*O. americanus* (4n) and *Pleurodema kriegi* (4n)/*P. cordobae* (8n). Our aim was evaluate the efficiency in the utilization of nuclear area with respect to cellular area of the erythrocytes to define the limits values for the identification of cryptic-polyploid species. We studied 110 individuals of *Pleurodema* and 116 individuals of *Odontoprhynus*. For each individual, we measured the cellular and nuclear length (L) and width (A) of 40 erythrocytes (Area= L*A*π/4) and boundary values were calculated using distribution curves. In both complexes studied, the erythrometric parameters showed significant differences between related species. Moreover, in both complexes the nuclear area was more efficient for identifying the species (*Pleurodema*: 34.39 μm² (probability=99.96%) and *Odontophrynus*: 24.02 μm² (99.075%)) than the cell area (*Pleurodema*: 273.08 μm² (97.55%) y *Odontophrynus*: 197.69 μm² (97.94%)). Greater efficiency found using nuclear area is novel and significant because most studies use only the cell area to differentiate polyploid complexes.

Key words: Erythrometry, Octoploid, Tetraploid, *Pleurodema*, *Odontophrynus*, Polyploidy

Introduction

The occurrence of natural bisexual polyploids in amphibians provides evidence that polyploidization is an extensive mechanism of speciation in several families of anurans (Bogart 1980; King 1990; Tymowska 1991; Otto & Whitton 2000; Stöck *et al*. 2002; Martino & Sinsch 2002; Rosset *et al*. 2006; Valetti *et al*. 2009). It is well documented in anurans the morphological similarity, or cryptic condition, of species within complex involving polyploids (i.e.; Ralin 1968; Bogart & Wasserman 1972; Bogart & Tandy 1976; Mahony & Robinson 1980; Valetti *et al*. 2009). Two or more species are considered 'cryptic' if they are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable (Bickford *et al*. 2007). Such cryptic species can only be identified by nonmorphological characteristics, such as differences in ecology, behavior, cytogenetics, or biochemistry (Borkin *et al.* 2001). Species delimitations and identification have implications for both estimating species richness and assessing conservation needs (Angulo & Reichle 2008). In cryptic complexes that include species with different ploidy levels, the character that clearly differentiates them is the number of chromosome complements. However, the chromosome studies require the sacrifice of the animal, and a significant processing time in laboratory to know the ploidy level of an individual. Consequently, novel diagnostic characters should be found for a correct, simple and rapid distinction of the species without sacrificing individuals (Grenat *et al*. 2009b). It is well known that the blood cells of amphibians conserve their nucleus, and so the erythrocyte size is correlated with the DNA content (Uzzell 1964; Stöck &

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