

Molecular phylogeny of *Thraupis* Boie, 1826 (Aves: Passeriformes) and taxonomic review of the *Thraupis episcopus* (Linnaeus, 1766) – *Thraupis sayaca* (Linnaeus, 1766) species complex

RESUMO

O gênero *Thraupis* Boie, 1826 é um grupo monofilético composto por sete espécies. Entretanto, as relações entre estas espécies continuam obscuras. *Thraupis abbas* é a espécie irmã do clado *T. ornata*–*T. palmarum*. Um segundo grupo é composto pelo clado *T. episcopus*–*T. sayaca*. Por outro lado, *T. glaucocolpa* não foi incluída em nenhum dos trabalhos que utilizou dados moleculares, enquanto que a posição de *T. cyanoptera* continua ainda não é clara. O complexo de espécies *T. episcopus*–*T. sayaca*–*T. glaucocolpa* inclui 18 subespécies e uma grande variação morfológica, além de uma ampla distribuição de várias delas, com áreas de sintopia entre *T. episcopus* e *T. sayaca*, onde a identificação destas duas espécies é muito difícil. Estudos moleculares prévios só incluíram amostras de dois indivíduos de *T. episcopus* e uma de *T. sayaca*. Este complexo de espécies ainda apresenta uma grande instabilidade taxonômica. Na revisão deste gênero foram analisados 1.171 espécimes. As análises morfométricas mostraram que a massa é o parâmetro que apresenta a maior variação e que *T. cyanoptera* é a única mais claramente diferenciável dentre as unidades taxonômicas. Foi também realizada uma análise filogenética com base em dois marcadores mitocondriais (Cyt- β e ND2), além de três íntrons nucleares (íntron 3 do gen MUSK, íntron 5 do gen TGFB2 e uma parte do íntron 5 do gen BF5). Foi realizada uma análise RAxML e das redes de haplótipos independentemente para cada locus, e esta informação foi utilizada para agrupar as amostras em unidades taxonômicas genéticas. O RAxML e as redes de haplótipos mostraram uma relação próxima entre *T. episcopus* e *T. sayaca*, além de uma alta probabilidade de um processo de introgressão entre as espécies. Há também uma evidente estruturação genética em *T. episcopus*. As unidades taxonômicas genéticas foram utilizadas em uma análise multilocus de árvore de espécies, com um relógio molecular calibrado. A árvore de espécies sugere que a origem do gênero *Thraupis* se deu entre 5.5 e 7.7 milhões de

anos. *Thraupis glaucocolpa* é a linhagem mais antiga do gênero e a estrutura genética dentro de *T. episcopus* possui uma relação com as características morfológicas dessa espécie. Finalmente, são sinonimizadas várias subespécies e *T. episcopus cana* é elevada à espécie com base nos dados morfológicos e moleculares.

ABSTRACT

Currently, the genus *Thraupis* Boie, 1826 is a monophyletic group with seven species, all of which have high molecular and morphological support. Nevertheless, the phylogenetic relationship of the species still unclear: *T. abbas* is the sister species of *T. ornata*–*T. palmarum* clade, and a second group within the genus is composed by the *T. episcopus*–*T. sayaca* clade. Furthermore, in the remaining species group, one of the species, *T. glaucocolpa*, has not been included in any of the previous molecular studies, even it was believed to be close related with *T. sayaca*. Moreover, the last species within the genus, *T. cyanoptera*, has an uncertain position in the genus phylogenetic tree. The *T. episcopus*–*T. sayaca*–*T. glaucocolpa* species complex includes 18 subspecies and a high morphological variation and a wide distribution which includes overlapping zones of *T. episcopus* and *T. sayaca*, makes taxa identification almost impossible. Nonetheless, previous molecular studies had only used samples from two individuals of *T. episcopus* and one of *T. sayaca*. Furthermore, the group does not have taxonomic stability, as shown by the multiple changes, which occur at different levels: moving from one genus to another or from species to subspecies level etc. To check the genus, I analyze 1171 specimens. The morphometric analysis outcomes show the weight as the most variable and important measure and *T. cyanoptera* as the only clearly different species within taxonomic units. Finally, I did a phylogenetic analysis based on two mitochondrial genes (Cyt- β and ND2), in addition to three nuclear introns (intron 3 of MUSK gen, intron 5 of TGFB2 gen and a piece of the intron 5 of the BF5 gen). I performed the extractions from tissues collected at different localities around the natural distribution of the species, with emphasis on *T. episcopus* and *T. sayaca*. I ran independent locus RAxML analysis and haplotypes networks and used to group the

samples on genetic taxonomic units. RAxML and haplotypes analysis shows a close relationship between *T. episcopus* and *T. sayaca* with high probably introgression process within. Furthermore, exposed a genetic structure within *T. episcopus*. I used this genetic taxonomic units to ran a multilocus species tree with a calibrated molecular clock. The species tree suggests that the origin of the genus *Thraupis* was between 5.5 and 7.5 million years before present, in the Messinian age. Also recovers *T. glaucocolpa* is the oldest lineage in the genus and shows a relation between the morphological traits with the genetic structure within *T. episcopus*. Finally, I suggest synonymizing several subspecies and elevating to species level the subspecies *T. episcopus cana*, based on the morphological and molecular data.

1. INTRODUCTION

1.1 Taxonomic diversity within Thraupidae

The Thraupidae is a family of nine-primary oscine Passeriformes. Currently 377 species (3.6% of all birds) are included in the family, making it the second largest family of birds (Clements *et al.*, 2017). It is a family restricted to the Americas (Hilty, 2011), and its origins, diversification and highest richness are concentrated in the Neotropics (Sedano and Burns, 2010; Hilty, 2011). Birds belonging to this family are small-medium sized, usually with colorful and contrasting plumages (Hilty, 2011).

This family represents one of the most diverse radiations of birds, not only in the number of taxa but also in plumage types, colors, ecological traits, morphology, and behavior (Hilty, 2011; Burns, Unitt and Mason, 2016). Genera as *Diglossa*, *Coereba* and *Cyanerpes* are good examples of it. Representatives of these genera have specialized bill morphology adapted for foraging from nectar flowers, although they are not closest relatives (Burns, Unitt and Mason, 2016). *Coereba* and *Cyanerpes* function as pollinizers, showing similar ecological strategies. On the other hand, *Diglossa* exhibits a parasitic behavior by piercing the corolla and sucking the nectar without pollinating the flower (Hilty and Brown, 1986;

Rojas-Nossa, 2007). Besides this nectar-eaters, the Thraupidae also includes seed-eater birds such as the ones in the genus *Sporophila*, insectivores as those species in *Conirostrum*, and fruit-eaters as the genera *Thraupis* and *Tangara* (Hilty, 2011; Burns, Unitt and Mason, 2016). Each group has particular adaptations in bill morphology for their specific diets. Due to this enormous variation, relationships within the family have remained unclear and debated for a long time. Some of its current members were previously placed in other families, such as Coerebidae and Emberizidae. This taxonomic assumptions were based on morphology and feeding behavior of the birds (Hilty, 2011). Only recently (1990 to present) the advent of molecular data allowed attaining a better understanding of the relationships within the family (Hilty, 2011; Burns, Unitt and Mason, 2016).

1.2 A brief summary on taxonomic history of the genus *Thraupis*

The genus *Tanagra* was first described by Linnaeus in 1766 (Linné, 1766) with *Tanagra episcopus* as the type species. Thus, the genus *Tanagra* became the type genus of the family Tanagridae. Nevertheless, in 1908 it was pointed out that Linnaeus used the same name for a different group of birds (currently *Euphonia*, Fringillidae) in 1764 (CON, 1963; Hilty, 2011). Since then, a group of ornithologists (mainly from North America) started using *Thraupis* Boie (1826) instead of *Tanagra*, but old-world ornithologists kept using *Tanagra*. At the end of the 1950's, both names became widely used for the same taxon. In 1968, the International Committee of Zoological Nomenclature (ICNZ) decided to suppress the name *Tanagra* based on two proposals from Dr. Dean Amadon and Dr. Ernst Mayr. As the name of the type genus was a not valid name, the family name Tanagridae was not either. The next available name for the genus was *Thraupis* Boie (1826) and based on the “principle of coordination” (Ride, 1999) the family became Thraupidae as required by this principle (ICZN, 1968; Hilty, 2011).

As defined by recent studies, *Thraupis* is a monophyletic genus comprised by seven

species: *Thraupis episcopus* (Linnaeus, 1766), *T. sayaca* (Linnaeus, 1766), *T. ornata* (Sparman, 1789), *T. cyanoptera* (Vieillot, 1817), *T. palmarum* (Wied-Neuwied, 1821), *T. abbas* (Deppe, 1830), and *T. glaucocolpa* Cabanis 1850 (Sedano and Burns, 2010; Hilty, 2011; Burns *et al.*, 2014; Burns, Unitt and Mason, 2016; Rensen *et al.*, 2017). Previous phylogenetic hypotheses of the Thraupidae partially resolved the relationships within *Thraupis* by using DNA markers (Sedano and Burns, 2010; Burns *et al.*, 2014). However, there are taxonomic and phylogenetic uncertainties that have not been solved yet. For instance, the phylogenetic position of *T. cyanoptera* remains uncertain due to low maximum-likelihood and posterior probability support values (Burns *et al.*, 2014); Fig. 1). Additionally, the phylogenetic position of *T. glaucocolpa* remains unknown because no samples have been assessed in a molecular framework (Burns *et al.*, 2014; Burns, Unitt and Mason, 2016) and its placement in *Thraupis* is based solely on morphology. Thus, *T. glaucocolpa* is assumed to be the sister taxon of *Thraupis sayaca* due to similarities in plumage coloration (Hellmayr, 1936; Burns *et al.*, 2014). Moreover, *T. glaucocolpa*'s taxonomic category is debated. After its description as a species, *T. glaucocolpa* was treated as a subspecies of *Thraupis sayaca*, mainly because of their similarity on plumage coloration (Hellmayr, 1936; Rensen *et al.*, 2017). More recently, it was suggested to be part of a superspecies with *T. episcopus* and *T. sayaca* (Hilty, 2011). Currently *T. glaucocolpa* is considered as a full species by the South American Classification Committee (SACC) based on the continued use in several classifications, but not as the result of a taxonomic revision (Hilty, 2011; Rensen *et al.*, 2017).

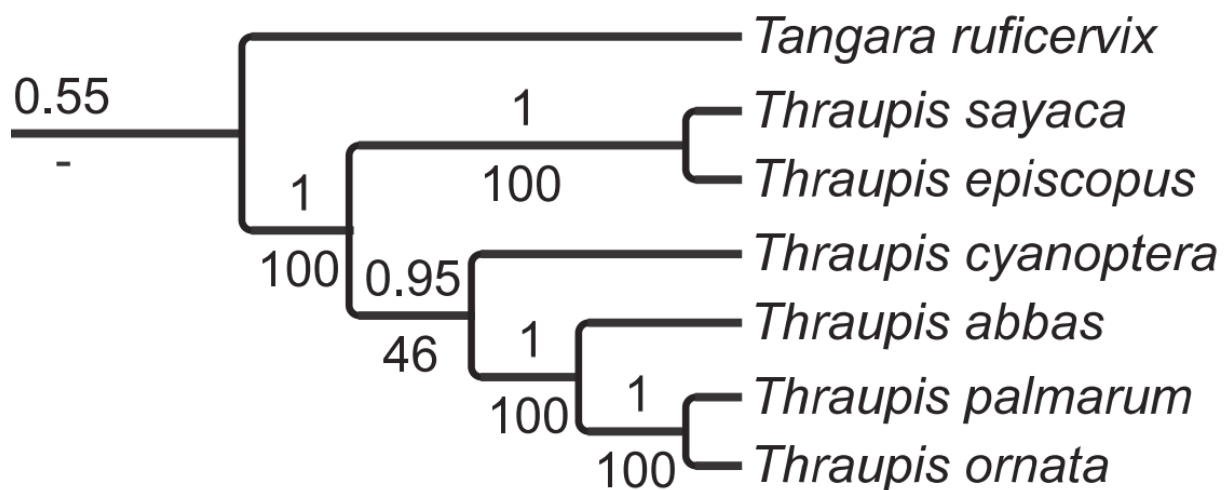


Figure 1. Phylogenetic tree of *Thraupis* based on two mitochondrial markers ND2 and Cyt- β .

Posterior probability from Bayesian framework analysis above the branch, maximum likelihood value below the branch. Modified from Burns *et al.* (2014).

Another taxonomic problem pertains the widespread species *T. episcopus*. It exhibits high morphological variation (described as 14 subspecies) that in some cases is structured geographically (Hilty, 2011). Within *T. episcopus*, there are individuals with white lesser-coverts and others with blue lesser-coverts. The first group is distributed east of the Andes and the second group in the west Andes and inter-andean valleys within the northern (Hilty, 2011). Other individuals that show light purple lesser-coverts are located in the Llanos of eastern Colombia and western Venezuela (Fig. 2). This area is where the two aforementioned morphs get into contact. Also, the subspecies *T. episcopus ehrenreichi* seems to be embedded within the distribution of *T. episcopus mediana* (Hilty, 2011). Moreover, the species limits between *T. episcopus* and *T. sayaca* are not clear. Some individuals ascribed to *T. episcopus* show similar plumage to *T. sayaca* and in some regions of southeastern Peru and northwestern Bolivia it is difficult to differentiate them even when hand-held (Schulenberg *et al.*, 2007). Finally, the subspecies *T. sayaca boliviana* (from the borders among Peru, Brazil and Bolivia) shows intermediate traits and some authors suggest those individuals are of intergrades between *T. episcopus* and *T. sayaca* (Zimmer, 1944; Hilty, 2011; Remsen *et al.*, 2017) and others already categorize them as a hybrids (McCarthy, 2006).



Figure 2. Color variation of lesser-coverts within *Thraupis episcopus*: a) *Thraupis episcopus cana* (ICN-AO33361), Colombia, west of the Andes; b) *Thraupis episcopus* cf. *nesophila* (ICN-38869), Llanos of eastern Colombia; c) *Thraupis episcopus leucoptera* (ICN-38786), Colombian Amazon, east of the Andes.

The use of DNA sequence data has generated a reappraisal of traditional morphology-based taxonomy and has greatly improved our understanding of phylogenetic relationships

at different taxonomic levels (Reddy, 2011; Isler, Bravo and Brumfield, 2013). Also, the use of DNA has been an important instrument to promoting the development of phylogeography studies (Avice, 2000), which, in turn, have proven useful to define species limits (e.g., Cadena *et al.* 2007, Isler *et al.* 2012) and to identify cryptic lineages i.e., established evolutionary lineages with little (or none) external morphological differences (e.g., Carneiro *et al.* 2012). Furthermore, phylogeographic studies bring us closer to a better understanding of mechanisms and patterns behind speciation and diversification (e.g., Moritz *et al.* 2000, Aleixo 2004, Ribas & Miyaki 2004, Cadena & Cuervo 2010). Consequently, using the outcomes of phylogeographic analyses in concert with phenotypic, behavioral, and ecological data is advised toward robust and stable taxonomic classifications (e.g., Isler *et al.* 2012, Cadena *et al.* 2007, Cadena & Cuervo 2010, Carneiro *et al.* 2012). Ultimately, adequate species delimitation procedures will have important implications on species conservation (Dénes *et al.*, 2011).

Here, I integrate information from mitochondrial and nuclear DNA markers with a morphological analysis to define the species limits within the *T. episcopus*–*T. sayaca*–*T. glaucocolpa* species complex and to suggest a new phylogenetic hypothesis for the genus *Thraupis* based on DNA sequence data.

5. Conclusions

The *Thraupis glaucocolpa* – *T. sayaca* – *T. episcopus* species complex is composed by four species. Adding to the aforementioned taxa we included *T. cana* (Swainson, 1834), elevated from subspecies to the species level.

T. glaucocolpa is the oldest lineage within *Thraupis*; the phylogenetic relationships between *T. cyanoptera*, *T. abbas*, *T. palmarum*, *T. ornata*, *T. sayaca*, *T. episcopus* and *T. cana* were investigated in detail.

We found introgression within the representatives of the genus *Thraupis* at different levels and between distinct species.

It is necessary to sample at genome level to clarify the phylogenetic relationships and

hybridization process in Cerrado-Amazon ecotone, Colombian–Venezuelan Grasslands, and the possible speciation by hybridization in Trinidad and Tobago.

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