



Short Communication

A multilocus analysis provides evidence for more than one species within *Eugenes fulgens* (Aves: Trochilidae)[☆]

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ABSTRACT

The status of subspecies in systematic zoology is the focus of controversy. Recent studies use DNA sequences to evaluate the status of subspecies within species complexes and to recognize and delimit species. Here, we assessed the phylogenetic relationships, the taxonomic status of the proposed subspecies, and the species limits of the monotypic hummingbird genus *Eugenes* (*E. fulgens* with traditionally recognized subspecies *E. f. fulgens*, *E. f. viridiceps*, and *E. f. spectabilis*), using nuclear (Beta Fibrinogen *BfIb*, Ornithine Decarboxylase *ODC*, and Muscle Skeletal Receptor Tyrosine Kinase *MUSK*) and mitochondrial (NADH dehydrogenase subunit 2 *ND2*, NADH dehydrogenase subunit 4 *ND4*, and Control Region *CR*) markers. We performed Bayesian and Bayesian Phylogenetics and Phylogeography analyses and found genetic differences between the three groups, suggesting the existence of two cryptic species (*E. fulgens* and *E. viridiceps*) and one phenotypically differentiated species (*E. spectabilis*). Our analyses show that the *E. viridiceps* and *E. fulgens* groups are more closely related with one another than with *E. spectabilis*.

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1. Introduction

For many years, there has been an important debate regarding the use of the subspecies concept to describe geographic variation in birds (Patten, 2010; Zink, 2004). The use of different species concepts has been one of the main reasons for this controversy. Whereas some authors defend the biological species concept, others argue that taxonomic decisions should be made exclusively from the point of view of independence in evolutionary histories (James, 2010).

In an influential paper, De Queiroz (2007) proposed that different types of data (morphological, ethological, ecological, molecular, etc.) are needed to operationally determine whether the lineages under study are evolving separately, i.e. whether they can be considered different species. De Queiroz (2007) also argued that species differentiation is affected by the time elapsed since the diversification event, a problem that must be taken into account in species delimitation studies.

Much research in recent years has focused on implementing molecular tools to complement studies of morphological variation and geographic isolation in taxonomic evaluations of bird species

complexes (Navarro-Sigüenza et al., 2001; Cadena et al., 2007; Bonaccorso et al., 2008). The systematic situation of several bird families remains unresolved, and such is the case in Trochilidae. The number of phylogenetic studies of different taxa within this family has increased recently (e.g. García-Moreno et al., 2006; McGuire et al., 2007, 2009, 2014), but only a few of them deal with the taxonomic status of proposed subspecies using molecular markers (Arbeláez-Cortés and Navarro-Sigüenza, 2013; Cortés-Rodríguez et al., 2008; García-Deras et al., 2008). The monotypic *Eugenes* (Gould, 1861) represents useful model to explore this basic taxonomic issue.

Eugenes fulgens (Swainson, 1827, cited in Schuchmann, 1999) is a sexually dimorphic hummingbird species that has phenotypic differences across its geographic range, from the southeastern USA to the Central American highlands (Howell and Webb, 1995; Schuchmann, 1999). In particular, differences in color and body size between populations have led to four different hypotheses regarding the subdivision of *E. fulgens*. The first hypothesis is that *E. fulgens* consists of two subspecies: *E. f. fulgens* (Swainson 1827, cited in Schuchmann, 1999), ranging from the southeastern USA to the Nicaraguan highlands, and *E. f. spectabilis* (Lawrence, 1867), found in the highlands of Costa Rica and Panama (Johnsgard, 1984; Schuchmann, 1999). The second hypothesis proposes a third subspecies, *E. f. viridiceps* (Boucard, 1878, cited in Schuchmann, 1999), found from the highlands of Chiapas to Nicaragua (Peters, 1945). Other researchers propose the existence

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of more than one species within *E. fulgens*. Renner and Schuchmann (2004) propose two species, *E. fulgens* (including *viridiceps*) and *E. spectabilis*, whereas a fourth hypothesis suggests the existence of three full species: *E. fulgens*, *E. viridiceps* and *E. spectabilis* (Navarro-Sigüenza and Peterson, 2004; Ridgway, 1911).

According to Renner and Schuchmann (2004), the males of the original *fulgens* and *viridiceps* subspecies have completely black feathers on the chest, breast, and abdomen, in contrast to those of *spectabilis* which are green (Fig. 1-II). Additionally, Schuchmann (1999) mentions that the gorget of *fulgens* and *viridiceps* is green, whereas that of *spectabilis* is blue-green (Fig. 1-II). The females of *spectabilis* are in general more yellow-green than those of *fulgens* and *viridiceps*, and the tips of the rectrices of *fulgens* and *viridiceps* are less white (Renner and Schuchmann, 2004). No color differences between *viridiceps* and *fulgens* have been reported, and their separation is based on non-overlapping geographic ranges (Navarro-Sigüenza and Peterson, 2004).

The purpose of the present work is to test the above mentioned hypotheses by evaluating phylogenetic relationships among the subspecies proposed for *E. fulgens*, and to provide a systematic reevaluation using a multilocus molecular dataset.

2. Methods

2.1. Taxon sampling and laboratory procedures

We used tissue samples from *E. fulgens* specimens in the collections of the Museo de Zoología Alfonso L. Herrera (Universidad Nacional Autónoma de México), Natural History Museum (University of Kansas), The Burke Museum (University of Washington), and Museum of Natural Science (Louisiana State University).

We isolated genomic DNA from tissues using Qiagen DNAeasy kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. We sequenced 34 individuals collected in 16 localities throughout the entire range of *E. fulgens* (Table 1; Fig. 1-I), including individuals representing three geographic regions: (1) northern and central Mexico, (2) southern Mexico and northern Central America, and (3) southern Central America. We defined these geographic regions according to the three subspecies proposed for this complex (*fulgens*, *viridiceps* and *spectabilis*). We included five samples from *Lamprolaima rhami*, the sister group of *Eugenes* (García-Moreno et al., 2006), and two samples from *Heliomaster constantii* as outgroups (McGuire et al., 2014).

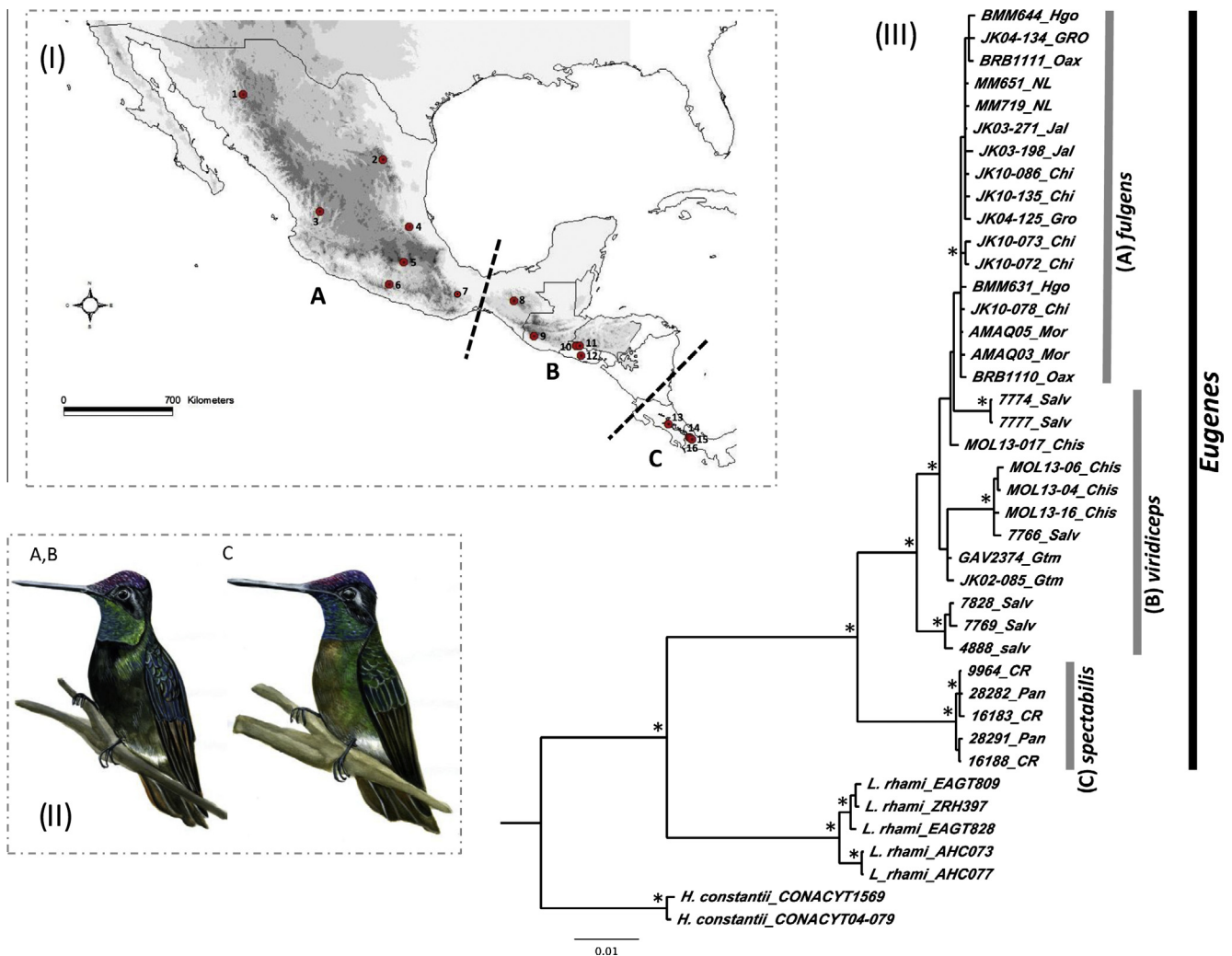


Fig. 1. I) Sampled localities for this study (see Table 1). II) The morphological variation is illustrated according with previous reported descriptions; the first morpho corresponds to *fulgens* and *viridiceps* subspecies (A, B), and the second one corresponds to *spectabilis* subspecies (C). III) Phylogenetic Bayesian Inference reconstruction from 34 individuals from *Eugenes fulgens* complex using mitochondrial and nuclear markers (*ND2*, *ND4*, *RC*, *Fbif*, *MUSK*, and *ODC*). Posterior probabilities $P \geq 0.95$ are shown (*). Above right is represented the main different groups recovered on the phylogenetic reconstruction according to their geographic distribution and the subspecies proposed (A: *fulgens*, B: *viridiceps*, C: *spectabilis*).

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