

Clarifying the Taxonomy of the *Taygetis virgilia* Species Complex

(Lepidoptera: Nymphalidae: Satyrinae: Euptychiina)

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Abstract

A study of DNA 'barcodes' and adult morphology was conducted to explore species diversity within two formerly recognized species of *Taygetis* butterflies, *T. virgilia* and *T. rufomarginata*. Molecular data revealed the likely existence of multiple cryptic species occurring sympatrically, and wing pattern, wing shape and male genital characters showed consistent differences among a number of these putative species. We conclude that there are at least 6 species among the specimens we studied, and we are now working to identify existing names and describe new taxa where necessary.

Introduction

Butterflies and moths are one of the most numerous and diverse groups of species on our planet, yet much of their biology and evolution remains to be studied. Our research focused on the genus *Taygetis*, a diverse group of large Neotropical butterflies with about 30 species, and in particular on one of the largest, most common and apparently most variable species, *Taygetis virgilia*, and the similar *Taygetis rufomarginata* (Lamas, 2004). We attempted to clarify how many different species are contained in this group based on a study of geographical distribution, genital structures, DNA sequences, and wing pattern.

Objectives

- Dissect male genitalia and examine wing pattern of specimens representing observed morphological variation
- Sequence COI 'barcode' gene to help identify diagnostic morphological characters
- Integrate morphological and molecular data to clarify the taxonomy

Materials and Methods

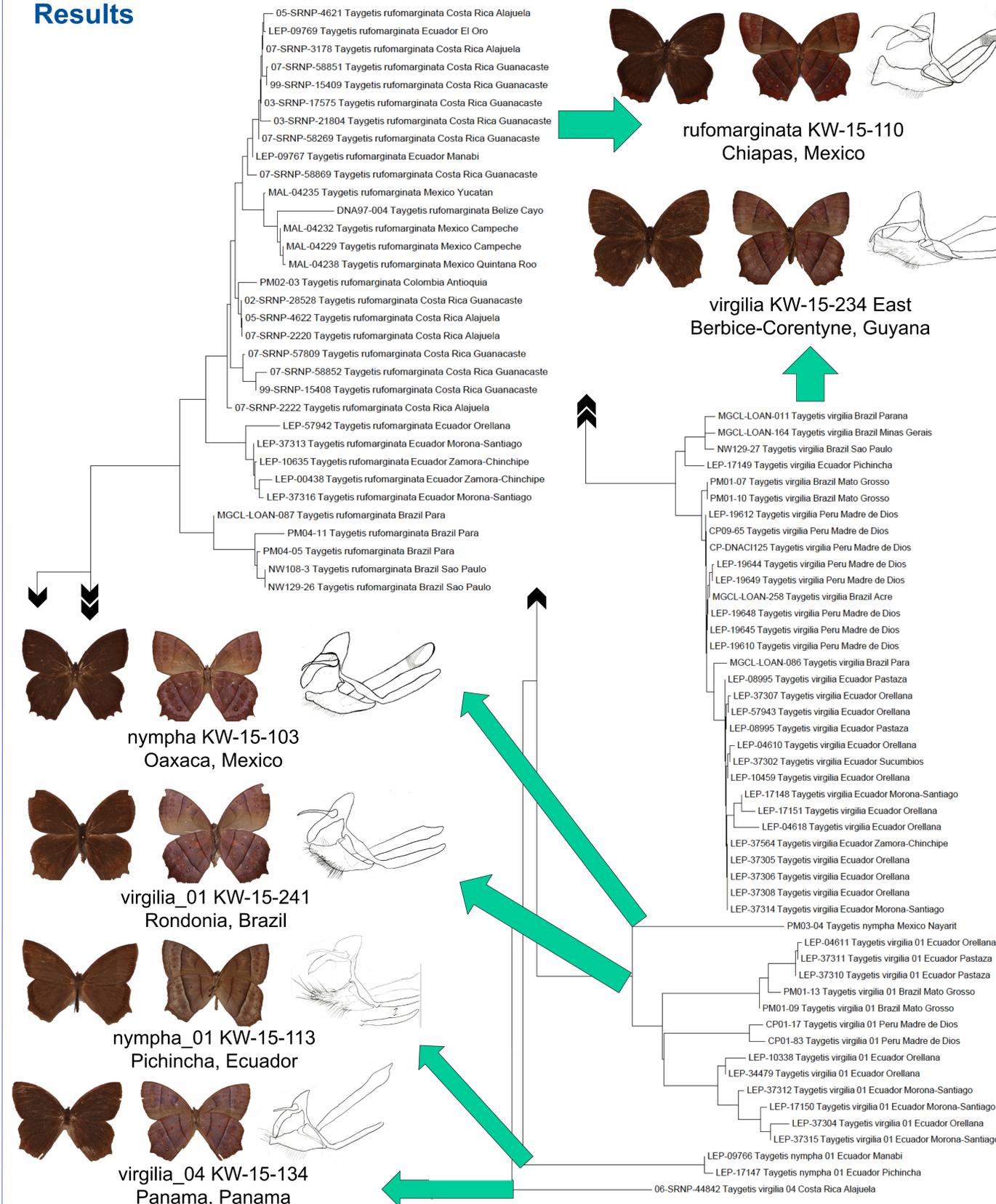
Morphological

Male specimens from throughout the Neotropics representing observed wing pattern variation were selected from the collection of McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History. Genitalia was examined using standard methods, placing the abdomen in hot 10% KOH for 15 mins before dissecting and cleaning the genitalia. The genitalia was examined and drawn under a stereomicroscope with a drawing tube, then stored in a glass vial in glycerol.

Molecular

Genomic DNA was extracted from ground legs of dried specimens and the first half of the mitochondrial gene cytochrome oxidase I (COI), also known as the barcode region for animals, amplified and sequenced using standard methods. The resulting 50 sequences were aligned using BioEdit v. 7.1.3 (Hall, 1999), with 32 additional sequences obtained from online databases. The final aligned sequences were of length 668 bp. To test for consistent genetic differences between phenotypes we did a Neighbor-joining (NJ) analysis using MEGA 7.0.18 (Kumar et al., 2016), with the Kimura 2-parameter substitution model, partial deletion of sites with missing data, and other default settings.

Results



Results and conclusions

The comparison of DNA sequences, as indicated in the NJ tree, suggest that there are at least six species within what was formerly treated as *T. virgilia* and *T. rufomarginata*. Putative diagnostic wing pattern and genitalic characters are listed below. Additional research might indicate that some divergent groups, such as eastern and western *T. rufomarginata*, should also perhaps be treated as distinct species.

virgilia

- Dark shading around postdiscal line on ventral wings
- Broad, concave valva
- Valva protrudes beyond tip of uncus

virgilia_01

- Round spot on ventral hind wing discocellular veins
- Tapering, upturned valva
- Uncus tip protrudes beyond valva

virgilia_04

- Dash on ventral hind wing discocellular veins
- Narrow-tipped valva not upturned
- Short brachia

nympha

- Forewing apex acute
- Valva similar to *virgilia_01*

nympha_01

- Short, narrow valva
- Dark line ventral hind wing bordered with pale scaling

rufomarginata

- Orange edging along dark lines on ventral wings
- Flaring, wide-tipped valva
- Valva protruding farther than tip of uncus

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