

Family tree of 'boring' butterflies reveals they're anything but

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Even distantly related euptychiines can look nearly identical, which has led to several cases of mistaken identity that have only just recently been resolved with the help of DNA analysis. Credit: Keith Willmott

Walk a short distance through the Amazon Rainforest, and you might



witness what look like dead leaves launch from the ground and fly off into the understory. These masters of disguise are euptychiines, one of the most diverse and least understood groups of butterflies in the American Tropics.

There are as many as 100 co-occurring euptychiine species in the rainforests of Peru and Brazil, but even the most seasoned butterfly experts have a hard time telling them apart.

"They're one of the groups that often get called 'brown, boring butterflies," said André Freitas, a biology professor at the State University of Campinas in Brazil. "They aren't very attractive to collectors or researchers, and even distantly <u>related species</u> can look very similar. The early naturalists had no way to accurately classify them."

Freitas is a co-author on a new study that adds some much-needed definition to what has remained, up until now, a black hole of butterfly diversity. The German entomologist Jacob Hübner was the first to describe the group in the early 1800s, when he lumped the few species then known into a handful genera based on similar appearance.

Using DNA, Freitas and his colleagues show there are at least 70 Euptychiina genera, containing more than 500 species. Their results also suggest there are at least 130 unnamed species in the group awaiting scientific description.

The study is the result of a project more than a decade in the making, initially conceived by Keith Willmott, director of the McGuire Center for Lepidoptera and Biodiversity at the Florida Museum of Natural History. In 2009, Willmott reached out to Freitas and other researchers who'd taken a stab at individually sorting through euptychiine butterflies piecemeal and proposed they instead combine their efforts.



Before researchers could make heads or tails of euptychiine diversity, they first needed a sense of just how many groups there were and how they were related to each other.

"The way people would typically work on this kind of problem would be to divide and conquer, but that doesn't work for euptychiines, because there are very few unifying features among species that you can use to define groups," Willmott said.

Instead, a coalition of international researchers focused on studying as many euptychiine species as they could lay their hands on. They examined more than 60,000 specimens from museums in Europe and North and South America and collected euptychiine butterflies throughout their range, from the foothills of the Andes in Ecuador to the Atlantic Forest in Southeastern Brazil.

In the process, they discovered more than 100 <u>new species</u>, many of which were hiding in plain sight, concealed by their close resemblance to each other.

"A recent example is a large butterfly that used to be known as Pseudodebis celia from western Ecuador, which turned out to be four separate species," Willmott said. "These are big butterflies. It's hard to imagine these kinds of species are still escaping detection."

Not all euptychiines have evolved to blend in. Several species have bright blue scales or blazing orange eyespots, which might seem like it'd make them easy to classify. But closer inspection reveals these color patterns can be deceptive as well. Results of the study's genetic analysis show, for example, that multiple, Euptychiines have transformed their wings into blue frescoes, making them appear superficially similar.

Mimicry is often the primary suspect when unrelated butterflies have a



similar appearance. Predators learn to avoid species with toxic, bittertasting compounds, like Monarchs (Danaus plexippus). With a little false advertising, species that lack these compounds can still deter predators by copying the colors and patterns of genuinely toxic butterflies.

But according to Willmott, this likely isn't the case for euptychines. "As far as we know, they're not unpalatable or protected against predators in any way. It looks like mimicry, but there's really no basis for it. It's a fascinating mystery that needs study."

Blue euptychiines can play further tricks on butterfly experts—sometimes, the color is only present in some individuals of a given species.

"In most cases, the males are colorful, and the females are brown," said Marianne Espelend, a curator at the Leibniz Institute for the Analysis of Biodiversity and lead author on the study.

This mismatch has led to several cases of mistaken identity. A brown species from French Guiana described in 2012 was <u>later determined to be the incognito female half</u> of a well-known species discovered a century earlier. This triggered inspection of other blue species, and discovery of similar problems.

The new classification provided by this study will help researchers pin down the exact identity of familiar euptychiines and shorten the long queue of species in the group that have yet to be given a scientific name.

It also sets the stage for scientific forays into other aspects of euptychiine biology that experts are just now beginning to understand, said Freitas, reciting a litany of unknowns that can now be thoroughly investigated.



"We know that several species have scales that release scents to attract females, but we have no idea what types of chemicals are involved; the males of some species make an audible clicking sound, but we don't know how they do it; and I can count on my hand the number of times I've been able to find euptychiine caterpillars in the wild, of which we know very little."

According to Espeland, the study is a rough but robust sketch of butterflies that are among the Amazon's most abundant and overlooked inhabitants. "They've been largely ignored because people didn't think they were interesting, historically, but I find them really beautiful. We now have a framework we can use to learn more about them."

The authors published their study in the journal *Systematic Entomology*.

More information: Marianne Espeland et al, Combining target enrichment and Sanger sequencing data to clarify the systematics of the diverse Neotropical butterfly subtribe Euptychiina (Nymphalidae, Satyrinae), *Systematic Entomology* (2023). DOI: 10.1111/syen.12590

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