ON THE USE AND LIMITATIONS OF BARCODES IN MODERN TAXONOMY

JEAN HAXAIRE

Correspondent of the MNHN of Paris, Associate Professor of Biological Sciences. Le Roc, 47310 Laplume, France.

jeanhaxaire@sfr.fr

(*The author, among the foremost international experts on hawkmoths (Sphingidae), shares his pioneering experience on the importance given to DNA barcoding in taxonomy, based on his own experience.*)

I remember that phone call in early spring 2006, when Dr. Rodolphe Rougerie, postdoctoral fellow in the laboratory of the Canadian Centre for DNA Barcoding at the University of Guelph (Ontario, Canada), told me that they were working on a "perfect" tool to assess the species level of any specimens. He was the first to tell me about the concept of barcoding, even though I was aware that it had been proposed that a short sequence of a mitochondrial gene could give a good indication for identification at species level. Rodolphe was planning to test the validity of barcoding on a whole family of moths. He was facing a major problem. Most of the larger institutions were not willing to permit someone to spend months working on a collection, taking DNA samples of specimens of each species including very rare (if not unique) ones. In summary, he was looking for someone who had a global collection, with more than 90% of the known species, mostly recent specimens (important for DNA) and would be willing to accept his presence for weeks. To my great pleasure, he had thought about me.

He was correct, I immediately accepted, and there followed a very exciting experience. Rodolphe took about 4660 samples (legs of Sphingidae) during his 6 week stay with me. Almost all genera were represented, and more than 95% of the South American fauna was sequenced during his stay. He told me that it would be very useful if I could keep on working with the unsequenced species in the following years, and I did it.

I was very impatient to see the first results, and they were fine. For most of the species, the identification trees were very significant, isolating the taxa with a good percentage of genetic distance. The first and logical question was of course, how many percent distance is required to be sure that we have two different species?. In fact, we have never had any answer to that question, and it is still the most frequently asked one. The good news was that overall, the tool was fine and useful. When we had doubts about a cryptic species, the results of analyzing their DNA were sometimes spectacular, providing clinching evidence to supplement evidence gathered by the traditional approach. For instance, when you think that within a well known, widespread and common species, there are, in fact, two (or

more) hidden species, you may have some evidence based on morphology, anatomy, or just biology (flight-time, host-plant, larval pattern), but you need confirmation. In that special case, the barcodes were more than useful in providing clear and final evidence that you were right (or not).

The situation has not been that idyllic with allopatric populations, isolated on different islands, mountains or valleys. In that situation, it was common to see notable barcode differences (2 or 3 percent) between populations, even though you are certain that they really belong to the same species. No difference in the ecology, biology, morphology, but 3% of difference in CO1 mitochondrial gene. What could we do? Then start the problems, with two different answers. Mine was to do nothing, and to consider that it was just a small, normal, genetic variation due to a significant genetic isolation. But some authors decide to treat the divergent populations as new taxa, and sometimes in large numbers, with a simplistic concept: a different barcode = a new species. First, find a difference in the DNA, and after that, do your best to find a morphologic/anatomic difference. It is axiomatic that when one searches enough for something, you usually find it, in this case, some minor morphological or other difference. In some groups, the number of descriptions has been incredible. When you see the number of new taxa described during the last ten years, you can really estimate the power of the "barcoding effect". The number of Asiatic or South American taxa has more than doubled in some families. It means that there have been more species described during the last ten years than in more than 250 years since 1756! And for most of those "new" taxa, it is impossible to identify them if you do not know the origin of the specimens. Of course, it does not mean that those species are invalid, and it is not unlikely that most of them are correct, but it has definitely changed our species concept. A very normal question now, when someone submits a photo of a specimen in an entomological forum, asking for determination is, "Where does it come from?" It means clearly "no data, no name". This is really a new taxonomical concept. And it has changed a lot of things, including in my own work. I was quite confident with the fact that I was able to put a name to most of the Sphingidae of the world with a good recto/verso picture (with the exception of some very difficult genera like the *Macroglossum* or *Cypa*, for which dissection of the specimen is generally necessary for a reliable determination). Now, I need to have the origin of the moth, and even with that, nothing is simple. The best is to have the DNA sequence of a small part of the CO1 mitochondrial gene (658bp) but this is unaffordable for most of the entomologists, and that's another serious problem of the method. It is an entomology for rich people.

Within some African genera that belong to the Smerinthinae subfamily, with very fragmented populations, the situation is even worse. Almost each population presents a different barcode, and following that logic, should be named as a different species. This is unacceptable.

In my experience, therefore, barcodes have been a very good additional tool to check the validity of a species, but only one tool among many others, and not the perfect one. We have described *Daphnusa zythum* Haxaire & Melichar, even though its barcode is similar to the one of *Daphnusa ocellaris* (Walker, 1856), and we strongly believe in the validity of our new species because we have

morphological evidence. We have seen some South American species like *Nyceryx hyposticta* (Felder, 1874) and *Pachylia ficus* (L., 1758), showing two (or more) very distinct barcodes, but so far, we haven't been able to find any morphological difference justifying the split of *Nyceryx hyposticta* into different species. And last but not least, when the new technique was developed, my hope was that it could help clarify the status of species in some very complex genera, like *Perigonia* or *Neogene*. Unfortunately, this has not been the case; I am sorry to say that it is worse than before.

Now that the novelty has worn off and the confusion has set in, we have to consider the limitations of the barcode approach and recognize it as only one more approach, supplementing traditional approaches to distinguishing taxa within the species concept. It cannot be ignored, it helps a lot with cryptic species, sympatric twin species, but failed to clarify some very difficult genera, and in such cases, it can be misleading if overly relied upon. It has also been used to inflate the number of known species of some families, but that situation will be clarified in due course, with probably a lot of new synonymies being recognized among species described on the basis of over-reliance upon or the misinterpretation of DNA barcode data.

FIRST REPORT OF *SATURNIA CIDOSA* MOORE, 1865 (LEPIDOPTERA: SATURNIIDAE) FROM ARUNACHAL PRADESH AND NAGALAND, INDIA

ALFRED J. DANIEL[1*], SANKARARAMAN. H[2], J.M. SAMRAJ[3] AND ALKA VAIDYA[4]

[1]Biosystematics Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu

**Corresponding author: danieljalfred@gmail.com*

[2]Parasitoid Taxonomy and Biocontrol Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, India

[3]Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand

[4] J 145 Lokmanya Nagar, Kataria Marg, Mahim, Mumbai 400 016

Reviewer: Stefan Naumann

The saturniid moth *Saturnia cidosa* is hitherto reported only from "N.E. India" (Type Locality), Nepal (Moore 1865; Naumann & Loffler, 2005) and Bhutan (Irungbam & Irungbam, 2019). Although Hampson (1892) synomised *S. cidosa* with *S. pyretorum* Westwood, [1847], Naumann & Loffler (2005) revised the genus *Saturnia* Schrank,