DNA barcoding and DNA metabarcoding as tools for rapid inventory and high-throughput identification of Lepidoptera species in Amazonia

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The ability to identify the early stages of Lepidoptera and knowledge of their host-plant preferences is of fundamental importance in understanding their ecological interactions, biogeography and potential economic impact. In South Africa the early stages (or any part thereof) and host-plant associations are known for less than 10% of the Lepidoptera fauna estimated to be over 10 000 species. Images or illustrations of these early stages are available for only a fraction of the species for which at least some information is available. The advent of modern communication media such as nature websites, online databases, Virtual Museums and social media, as well as the free availability of good digital cameras, has sparked a renewed interest in the smaller fauna by the South African public. This prompted us to find ways of rapidly accelerating the discovery of the early stages of our Lepidoptera by exploiting this growing enthusiasm in the natural history of smaller creatures.

This presentation reported on the creation of a citizen-science project aimed at visually recording the 'caterpillar – host-plant – adult' associations of African Lepidoptera, which culminated in the formation of the now active Caterpillar Rearing Group under the auspices of The Lepidopterists' Society of Africa.

In the first eighteen months the project has yielded some spectacular results: Number of valid entries received: 1168. Number of species for which we now have the minimum criteria: 725. Number of host-plant associations new to science: 568. Minimum number of species never reared before: 214.

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In the fields of ecology and conservation biology, when information requiring species-level identification is needed, many groups of living organisms are generally excluded de facto from the studies, merely because of the lack of access to the scarce specific taxonomic expertise and because of the largely unexplored and overwhelming diversity of some taxa, in particular in the species-rich inter-tropical regions. In French Guiana, we used standard Sanger-based DNA barcoding to document this relatively unknown tropical Lepidopteran fauna, revealing an outstanding local diversity. Our efforts resulted in the assembly of a large DNA barcode library

comprising expert-identified species when possible as well as a large portion of unidentified species only characterized to date by their Barcode Index Number (BIN) in BOLD.

As an alternative approach and to demonstrate the usefulness of DNA barcode libraries, we carried out a community ecology study in Brazilian and Colombian Amazonia where we used a DNA metabarcoding approach to identify bulk samples of Saturniidae and Sphingidae moths along a gradient of land-uses. This study used Next Generation Sequencing technology (454 Roche) to expedite the process of sequencing a large number of samples (ca. 1700) in many collecting sites (54).

The flowchart of taxonomy – right pieces in the right order

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Challenge

Numerous insect groups include a great amount of unknown species, and many of them lack present-day taxonomic expert. One such group is the Australian Hypertrophinae (Gelechioidea: Depressariidae) with 51 described and tens of undescribed species. Very little is known about these endemic moths, mainly due to their insufficient taxonomy. To be able to gather knowledge on hypertrophines, their species boundaries need to be defined, phylogenetic relationships studied, and newly discovered species described. However, their small size, somewhat cryptic wing patterns, and the lack of present-day experts pose a considerable challenge.

Solution

A flowchart of taxonomy was recently presented to describe the workflow of the study of the Hypertrophinae (Kekkonen 2014). The flowchart includes four steps, starting from the phylogenetic analyses concentrating on the hierarchical level above the focal group (for further details, see Heikkilä et al. 2013). The next phase introduces DNA barcode-based delineation of putative species (i.e., operational taxonomic units, OTUs) (for further details, see Kekkonen & Hebert 2014), and the third part returns to the phylogenetics, but this time, at the level of the focal group. The last step includes the validation of OTUs based on all available data (e.g., morphological characters, nuclear loci), and subsequent association of type specimens with defined species by applying DNA barcodes.

Reason

A rationale behind the flowchart of taxonomy is to choose the most suitable source of data and the set of methods for each phase. The flowchart presents a viewpoint where initial boundaries of putative species are formed based on DNA barcodes. This approach offers many benefits