

Erwin (Lucky et al. 2002; Erwin et al. 2006), who monitored the fauna of 200 trees in 9 replicates from 1994-1996 in north-eastern Ecuador. Identity of all the fogged trees, and their neighbours is known. In two pilot studies we could prove, that larvae can be identified to species by their 'barcode sequences' (mtDNA), and that sequencing of gut content is possible too, in order identify the larval plant meal and to prove feeding on the fogged host-tree, rather than on epiphytes or on the neighbouring tree (Miller et al. 2006; Matheson et al. 2006). Identification of the larvae was performed by analysis of the complete sequence of the mitochondrial gene cytochrome c oxidase I (COI) and comparison with sequences of collection specimens. The effectiveness of the 'barcoding' tool for species identification had already been shown in many other studies (cf e.g. Hebert & Mitchell 2006). Gut contents were successfully identified by comparing sequence of a 157 bp long fragment of the chloroplast gene *rbcl* with that of the pre-identified host-plant and a wide set of other plants of the study area. Plant meals could be detected, when the insects were killed and preserved in Ethanol up to 12 hours after the last feeding (Matheson et al. submitted). For large sets of possible host-plants and for discrimination of closely related plant species, e.g. in tropical countries, additional markers (fragments/genes) may be necessary.

Results from the planned research project will provide, for the first time, comprehensive informa-

tion on host-plant relationships and host specificity for a large group of phytophagous insects in the neotropical rain forest canopy. With these data the estimations of total species numbers in Geometridae and insects may be extrapolated and refined. Similar projects are planned for geometrid moth larvae in Israel.

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## DNA barcoding of Australian Lepidoptera

Paul Hebert & Andrew Mitchell

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Corresponding author: Dr. Andrew Mitchell, Agricultural Scientific Collections Unit, Orange Agricultural Institute, NSW Department of Primary Industries, Forest Rd, Orange NSW 2800, Australia; e-mail: andrew.mitchell@dpi.nsw.gov.au

DNA barcodes are short (658bp) sequences from a standardized region of the mitochondrial gene cytochrome c oxidase I (COI or *cox1*). Past work has revealed that sequence diversity in this gene region is an effective tool for species identification and discovery. As a result, large-scale DNA barcoding programs are now underway, including efforts to assemble barcodes for all fish and all bird species. We intend to develop a comprehensive barcode library for Australian lepidopterans as a complement to a similar project underway in North America.

We now present results of a pilot study that has barcoded 3500 specimens representing over 800 species collected from sites in north-eastern Queens-

land and the Central West of New South Wales. All specimens were databased and photographed before DNA was extracted from a single leg. DNA barcodes were subsequently gathered from the specimens and analysed using the Barcode of Life Data System ([www.barcodinglife.org](http://www.barcodinglife.org)).

Levels of intra-specific variation at COI averaged just 0.2 %, while congeneric species showed sequence divergences that were, on average, 20 times higher. As with studies in other geographic regions, more than 95 % of the species that we examined possessed unique DNA barcodes, allowing their easy identification. Although there was little overlap in species coverage between our two sampling regions, our

results suggest that geographic variation in barcode sequences will not be an important complication in species recognition.

We expect to obtain barcode coverage for all common species of Australian Lepidoptera through intensive collecting at a few well-chosen sites. How-

ever, we also hope to broaden our network of collaborators so that more extensive sampling coverage is possible. As well, we expect that advances in sequencing technology will soon permit the analysis of museum collections, allowing rapid growth in sequence coverage for uncommon taxa.

## Successful extraction of eggs from dry geometrid moth collection specimens

Axel Hausmann, Sławomir Kuczkowski & Marius Junker

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Corresponding author: Dr. Axel Hausmann, Zoologische Staatssammlung München, Münchhausenstr. 21, D-81247 München, Germany; e-mail: Axel.Hausmann@zsm.mwn.de

Though modern techniques (scanning electron microscopy, SEM) offer very promising perspectives for the study of egg morphology, this kind of research has not achieved much attention in geometrid moth systematics, apart from a few publications (cf. Salkeld 1983; Young 2006).

SEM studies of egg morphology are generally thought to require fresh material. Very often, however, living females are unavailable due to rareness or restricted distribution areas in tropical countries, they may be hardly stimulated to egg deposition or their life cycles may not coincide with the study period.

In this contribution we present a way to get access to suitable egg material from dry female collection specimens. The method is based on enzymatic digestion of the abdomens and it is the same, which was recently proposed (Knölke et al. 2005) as a combined procedure for obtaining both DNA for sequence analysis and mazerated tissues for the preparation of the genitalia. Hence, this the new method can provide, simultaneously, three completely different data sets for taxonomic and phylogenetic research.

We analysed the influence of various parameters on the quality of the results, e.g. protease concentration, duration of digestion, humidity, and age of voucher (collection date).

In most cases the results are highly satisfying and provide clear SEM photographs of the chorionic sculpturing, which are very similar to those from fresh egg material of the same species. We got good results also from old collection specimens (up to >100 years). A number of examples was shown in the presentation, detailed results are published in Junker et al. (2006). The method is applied in a research program on Sterrhinae phylogeny, which was shortly presented, too.

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