PROC. ENTOMOL. SOC. WASH.
117(2), 2015, pp. 247–250

NOTE

# DNA barcodes of Lepidoptera reared from Yawan, Papua New Guinea

DOI: 10.4289/0013-8797.117.2.247

This paper provides metadata for DNA barcodes (cytochrome coxidase I sequences, COI) in GenBank for a collection of specimens from ecological studies in Yawan, Morobe Province, Papua New Guinea (PNG), sampled as part of long term research on the ecology and taxonomy of herbivorous insects in PNG (Miller et al. 2003, Craft et al. 2010, Novotny et al. 2010, Whitfeld et al. 2012). This paper aims to make DNA barcode data available to document ongoing research, to contribute to the International Barcode of Life (iBOL; www.ibol.org) project, and to encourage enhancement in species identification, in line with the concept of DNA barcode data release papers and the Fort Lauderdale principles for genetic data (Schindel et al. 2011). Many of these records represent undescribed species, and we have purposefully refrained from assigning new names until the relevant taxa can be studied in sufficient detail. Under the Fort Lauderdale principles, we ask others to refrain from assigning new species names to these records outside of the context of proper systematic study. Data released on GenBank (accession numbers KP849894-KP851000) includes the standard fields for BARCODE data (Benson et al. 2012), while more data, including images and host plants, are available on BOLD (www.boldsystems.org; Ratnasingham and Hebert 2007), accessible from the project YAWAN using a DOI (dx.doi.org/10.5883/DS-YAWAN).

## MATERIALS AND METHODS

The study site is adjacent to Yawan village in the Saruwaged Mountains at 1,700 m elevation (146.83°E, 6.167°S). It is within the YUS Conservation Area (YUS is an acronym for Yopno-Uruwa-Som, the local areas), designated by the Government of Papua New Guinea in 2009, and in turn, part of the Huon Peninsula (Freeman et al. 2013). Yawan is a small, isolated village (population ~200 people), surrounded by valleys of anthropogenic grassland and montane rain forest. The villagers, who are the customary owners of the surrounding lands, rely to a great extent on forest resources for their subsistence (Rappaport 1984). Slash-and-burn agriculture, pig husbandry, and, to a lesser extent, hunting are the major sources of food. Once a year, family groups plant gardens by clearing vegetation in grassland or forest areas. The gardens are planted with mixed crops including sweet potato, taro, and banana. After several years of planting and harvesting, the gardens are abandoned. These traditional practices in rural New Guinea provide a unique opportunity for destructive sampling of forest plots without adding to the existing level of deforestation.

Field methods were similar to our previous study at Wanang (Miller et al. 2013, Whitfeld et al. 2012). Ten 45 x 45 m (0.2025 ha) plots were surveyed for woody stems with a diameter at breast height (DBH) ≥ 5 cm, along with woody epiphytes with stems ≥ 1 cm. Enumeration

was followed by the gradual felling of trees where local landowners had planned to clear the sites for subsistence gardens. After mapping and clearing adjacent vegetation, trees were individually felled and immediately inspected for caterpillars and leaf miners. Live caterpillars were hand-collected and placed in plastic vials for processing. Host plant data are linked to the BOLD records by the plant collection number in the form "YS1A0001." All plants were vouchered, and specimen data are available through the Digital Flora of New Guinea (http://ng.atrium-biodiversity.org/atrium).

Methods for handling the Lepidoptera are described by Miller (2015), Miller et al. (2003), and Novotny et al. (2010). DNA sequencing (COI barcode) followed standard methods at the Biodiversity Institute of Ontario, University of Guelph (Craft et al. 2010, Wilson 2012), using legs and the LepF1 and LepR1 primers. A total of 1,161 vouchers were sampled for DNA, resulting in 1,107 successful sequences, a 95% success rate.

## Results

The 1,107 successful sequences group into 315 barcode clusters (BINs), using the RESL algorithm as implemented in BOLD (Ratnasingham and Hebert 2013). Identifications are in progress, using voucher specimens in the Smithsonian National Museum of Natural History and Natural History Museum, London, and the BOLD identification tool, primarily against over 18,000 sequences generated for New Guinea Lepidoptera, and a larger library for Australian Lepidoptera, including more than 40,000 specimens from the Australian National Insect Collection (Hebert et al. 2013). Many species remain unidentified, and we welcome comments on all identifications. Where taxonomic names are not readily available from existing literature, BINs (DNA cluster-based morphospecies) can be used as species hypotheses that can be confirmed by future taxonomic studies (Schindel and Miller 2010, Ratnasingham and Hebert 2013). We are currently preparing ecological analyses of the caterpillar communities, their host plants, and their parasites (e.g., Hrcek et al. 2011).

The four most diverse families in the sample are Tortricidae (82 putative species), Geometridae (76 putative species), Erebidae (35 putative species), and Crambidae (34 putative species). Tortricidae include distinctive New Guinea mid-montane elements, e.g., *Adoxophyes* in the complex around *Adoxophyes marmarygodes* (Hulcr et al. 2007: 551), *Isotenes*, and the colorful genus *Zacorista* (including *Chionotremma* following Razowski 2013: 31).

Biogeographic analysis must await detailed identifications, but it is worth noting that, as expected, very few species (as hypothesized by barcode clusters) match those from the lowlands around Madang and Wanang (e.g., Miller et al. 2013), while many match those found at Mu, Chimbu Province (6.08°S 145.03°E, 1,800 m, Novotny et al. 2005); Wau Ecology Institute, Morobe Province (7.34°S 146.72°E, 1,200 m); and Biaru Road, Kuper Range Field Station, Morobe Province (7.49°S 146.80°E, 2,000 m).

## Acknowledgments

We thank the staff at the New Guinea Binatang Research Center for field assistance, Yawan landowners for access to field sites and assistance, Kipiro Damas and PNG Forest Research Institute for plant species identification. John Brown, Jeremy Holloway, Jadranka Rota, and Alberto Zilli have assisted with identifications for these moths.

Karolyn Darrow, Lauren Helgen, and many taxonomists have assisted in building the broader DNA barcode library used for Lepidoptera identifications. The Natural History Museum, London, has provided access to historical collections. Field work was supported by the U.S. National Science Foundation grant DEB 0841885 and the Czech Science Foundation grant 14-04258S. DNA sequencing was supported by a grant from the Government of Canada through Genome Canada and the Ontario Genomics Institute in support of the iBOL project.

## Literature Cited

Benson, D. A., I. Karsch-Mizrachi, K. Clark, D. J. Lipman, J. Ostell, and E. W. Sayers. 2012. GenBank. Nucleic Acids Research 40(D1): D48–D53. doi: 10.1093/nar/gkr1202.

Craft, K. J., S. U. Pauls, K. Darrow, S. E. Miller, P. D. N. Hebert, L. E. Helgen, V. Novotny, and G. D. Weiblen. 2010. Population genetics of ecological communities with DNA barcodes: An example from New Guinea Lepidoptera. Proceedings of the National Academy of Science USA 107: 5041–5046. doi:10.1073/pnas.0913084107.

Freeman, B. G., A. Class, J. Mandeville, S. Tomassi, and B. M. Beehler. 2013. Ornithological survey of the mountains of the Huon Peninsula, Papua New Guinea. Bulletin of the British Ornithologists' Club 133: 4–18.

Hebert, P. D. N., J. D. de Waard, E. V. Zakharov, S. W. Prosser, J. E. Sones, J. T. McKeown, B. Mantle, and J. La Salle. 2013. A DNA 'barcode blitz': rapid digitization and sequencing of a natural history collection. PloS ONE 8(7): e68535. doi: 10.1371/journal.pone.0068535

Hrcek, J., S. E. Miller, D. L. J. Quicke, and M. A. Smith. 2011. Molecular detection of trophic links in a complex insect host–parasitoid food web. Molecular Ecology Resources 11: 786–794. doi: 10.1111/j.1755-0998.2011.03016.x.

Hulcr, J., S. E. Miller, G. P. Setliff, K. Darrow, N. D. Mueller, P. D. N. Hebert, and G. D. Weiblen. 2007. DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae). Molecular Ecology Notes 7: 549–557. doi:10.1111/j.1471-8286.2007.01786.x.

Miller, S. E. 2015. DNA barcoding in floral and faunal research. Pp. 296–311. In M. F. Watson, C. Lyal, and C. Pendry, eds, Descriptive taxonomy: The foundation of biodiversity research. Cambridge University Press, Cambridge, x + 324 pp.

Miller, S. E., J. Hrcek, V. Novotny, G. D. Weiblen, and P. D. N. Hebert. 2013. DNA barcodes of caterpillars (Lepidoptera) from Papua New Guinea. Proceedings of the Entomological Society of Washington 115(1): 107–109. doi: 10.4289/0013-8797.115.1.107.

Miller, S. E., V. Novotny, and Y. Basset. 2003. Studies on New Guinea moths. 1. Introduction (Lepidoptera). Proceedings of the Entomological Society of Washington 105: 1035–1043.

Novotny, V., S. E. Miller, Y. Basset, L. Cizek, K. Darrow, B. Kaupa, J. Kua, and G. D. Weiblen. 2005. An altitudinal comparison of caterpillar (Lepidoptera) assemblages on *Ficus* trees in Papua New Guinea. J. Biogeog. 32(8): 1303–1314. doi: 10.1111/j.1365-2699.2005.01225.x

Novotny, V., S. E. Miller, L. Baje, S. Balagawi, Y. Basset, L. Cizek, K. J. Craft, F. Dem, R. A. I. Drew, J. Hulcr, J. Leps, O. T. Lewis, R. Pokon, A. J. A. Stewart, G. A. Samuelson, and G. D. Weiblen. 2010. Guild-specific patterns of species richness and host specialization in plant–herbivore food webs from a tropical forest. Journal of Animal Ecology 79: 1193–1203. doi: 10.1111/j.1365-2656.2010.01728.x.

Rappaport, R. 1984. Pigs for the Ancestors: Ritual in the Ecology of a New Guinea People. Yale University Press, New Haven, 502 pp.

Ratnasingham, S. and P.D.N. Hebert. 2007. The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. doi: 10.1111/j.1471-8286.2007.01678.x.

Ratnasingham, S., and P. D. N. Hebert. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS ONE 8(7): e66213. doi: 10.1371/journal.pone.0066213

Razowski, J. 2013. An assessment of the Tortricid (Lepidoptera: Tortricidae) fauna of Seram Island, Indonesia. Acta Zoologica Cracoviensia 56(2): 29–89. doi:10.3409/azc.56_2.29.

Schindel, D. E. and S. E. Miller. 2010. Provisional nomenclature: The on-ramp to taxonomic names. Pp. 1091–1115. In A. Polaszek, ed., Systema Naturae 250: The Linnaean Ark. CRC Press, Boca Raton, Florida.

Schindel, D., M. Stoeckle, C. Milensky, M. Trizna, B. Schmidt, C. Gebhard, and G. Graves. 2011. Project Description: DNA barcodes of bird species in the National Museum of Natural History, Smithsonian Institution, USA. ZooKeys 152: 87–91. doi: 10.3897/zookeys.152.2473.

Whitfeld, T. J. S., V. Novotny, S. E. Miller, J. Hrcek, P. Klimes, and G. D. Weiblen. 2012. Predicting tropical insect herbivore abundance from host plant traits and phylogeny. Ecology 93(supplement): S211–S222. doi: 10.1890/11-0503.1.

Wilson, J. J. 2012. DNA barcodes for insects. Pp. 17–46. In W. J. Kress, and D. L. Erickson, eds., DNA Barcodes: Methods and Protocols. Springer, New York.

Scott E. Miller, Margaret E. Rosati, Bradley Gewa, Vojtech Novotny, George D. Weiblen and Paul D.N. Hebert, *(SEM, MER) National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC 105, Washington, D.C. 20013-7012, U.S.A. (e-mail: millers@si.edu); (BG) Binatang Research Center, P. O. Box 604, Madang, Papua New Guinea; (VN) Biology Center, Academy of Sciences of the Czech Republic, and University of South Bohemia, Faculty of Science, Branisovska 31, 37005 Ceske Budejovice, Czech Republic; (GDW) University of Minnesota, Bell Museum and Department of Plant Biology, 250 Biological Sciences Center, 1445 Gortner Avenue, St. Paul, Minnesota 55108-1095, U.S.A.; and (PDNH) Department of Integrative Biology and the Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario N1G 2W1, Canada*