

## **DNA Barcodes of Microlepidoptera Reared from Native Fruit in Kenya**

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NOTE

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This paper provides metadata for DNA barcode (COI) data in GenBank for a collection of small moths (microlepidoptera except Blastobasidae and Tortricidae) reared from native fruits throughout Kenya. 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](http://www.ibol.org)) and Kenya Barcode of Life projects, and to encourage enhancement in identifications, 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 for 251 sequences representing 114 barcode clusters (putative species) have been released on GenBank (accession numbers GU695820-GU695823, GU695866, HQ947262, JF847884-JF847887, JN284900, KF643008-KF643239, KF808331-KF808337) including the standard fields for the BARCODE data standard (Benson et al. 2012) and more data, including images and host plants, are available on BOLD ([www.boldsystems.org](http://www.boldsystems.org); Ratnasingham and Hebert 2007, 2013), in a dataset accessible using a DOI ([dx.doi.org/10.5883/DS-KFML1](https://dx.doi.org/10.5883/DS-KFML1)).

From 1999 to 2004, Copeland organized an insect-rearing program from native fruit collected at sites throughout Kenya

(Copeland et al. 2009). Although the study was focused on fruit flies (Tephritidae) and their parasitoids, the collections also yielded many Lepidoptera. This is the third in a series of papers focused on the Lepidoptera (Adamski et al. 2010, Razowski and Brown 2012), resulting from ongoing collaboration among the International Centre of Insect Physiology and Ecology (ICIPE), the Smithsonian Institution's National Museum of Natural History (USNM), the National Museums of Kenya (NMK), and International Barcode of Life project based at the University of Guelph. This is also a contribution to a series of papers documenting DNA barcodes for moths from Kenya (Martins et al. 2013, Miller et al. 2013).

MATERIALS AND METHODS

The 5-year survey reared insects from indigenous fruits in diverse localities throughout Kenya. A total of 3838 fruit collections were made representing 910 distinct plant taxa from 118 families. Lepidoptera were reared from 19% of the samples, representing 349 plant species. Copeland et al. (2009) provide a summary of the program and database of the rearings. Most specimens have a rearing lot number for the set of specimens that emerged from one sample of one species of fruit (prefaced by A&M or KIP), but some of the later collections were "ad hoc," made after the main sampling effort was completed, and thus lack lot numbers.

Three groups of Lepidoptera dominate the samples in both numbers of species and individuals; the family Blastobasidae, the family Tortricidae, and the subfamily

Phycitinae (Pyalidae), all of which include many species that are specialists in fruit, a niche not utilized by most Lepidoptera (Novotny et al. 2010). All of these, along with the remaining microlepidoptera reported here, are relatively small moths, mostly with drab colors, and often difficult to identify without reference to genitalic dissections or DNA sequences. We have approached analysis of these groups in different ways, depending on the state of taxonomic knowledge and the tools available to us. We approached Blastobasidae from a traditional morphological taxonomic perspective, dissecting most of the available specimens, comparing them to the type specimens of the named species, and providing a complete taxonomic framework (Adamski et al. 2010). During the process, high throughput DNA barcoding became available, so we superimposed barcode results onto the already finished morphological study, providing confirmation of identifications, and most importantly, the ability to match males and females (Adamski et al. 2010). Specimens of Tortricidae were examined by two taxonomic specialists on the family, John Brown and Jozef Razowski, the latter of whom had previously worked extensively on the African fauna. Thus, they were able to analyze the specimens in a morphological taxonomic framework, describing 13 new species in the process (Razowski and Brown 2012). For Phycitinae, we took a pragmatic approach and sequenced DNA from one to several specimens from each rearing lot (a collection of a single species of fruit from a single locality), depending on their morphological variability. We dissected the genitalia of at least one specimen from each cluster of DNA sequences, as putative morphospecies based on experience in other studies (Adamski et al. 2010, Craft et al. 2010, Ratnasingham and Hebert 2013), to test species concepts

and to link to taxonomic names where possible.

We have been able to identify some of the microlepidoptera by comparison to reference collections, the literature, or matching DNA sequences in BOLD, but because of the limited knowledge of African microlepidoptera (e.g., Mey 2011), this process will take considerable time. Thus, we are making these data available, while identifications are in progress. Where taxonomic names are not readily available from existing literature, the DNA cluster-based morphospecies can be used as species hypotheses that can be confirmed by future taxonomic studies in broader context of the African fauna (Schindel and Miller 2010, Ratnasingham and Hebert 2013).

Field methods and host plants: Rearing methods and sampling strategy for the native fruit-rearing program are provided by Copeland et al. (2006, 2009). All fruits were collected from native plant species except for *Passiflora mollissima* (Kunth) L. H. Bailey (Passifloraceae). Ripe and unripe fruits were collected from plants and occasionally from the ground; samples showing noticeable rotting were discarded. Because of the rearing conditions and multiple techniques involved, the quality of adult moths varied, and some specimens were in poor condition. Host plants were identified by Quentin Luke and are stored at ICIPE.

#### Lepidoptera methods

This study is based on the analysis of approximately 675 reared specimens, over 30 genitalic dissections, and 251 cytochrome c oxidase I (COI) sequences. We attempted to analyze at least one sequence from each morphologically distinctive species from each rearing lot, but up to 5 specimens from some rearing lots. Genitalic dissections follow Robinson

(1976). Morphological comparisons were made to literature and collections of the National Museum of Natural History (USNM), Natural History Museum, London (BMNH), National Museums of Kenya (NMK), and Kenya Agriculture Research Institute, Nairobi (KARI). Additional context was provided by intensive samples of moths from light at Mpala Research Centre in central Kenya, made from 1998 to 2011 by Scott Miller and Tina Kuklenski (Adamski et al. 2010). Vouchers are retained by USNM, NMK and ICIPE. General field and laboratory methods for Lepidoptera are described in Adamski et al. (2010) and Copeland et al. (2009). DNA sequencing (COI barcode) followed standard methods at the Biodiversity Institute of Ontario, University of Guelph between 2008 and 2012 (Craft et al. 2010, Hrcek et al. 2011, Wilson 2012), using legs from pinned specimens selected for analysis. We sampled 252 vouchers for DNA, resulting in 251 successful sequences, including two added from Nigeria for comparison.

#### RESULTS

Identifications for all specimens are provided in BOLD ([dx.doi.org/10.5883/DS-KFML1](https://dx.doi.org/10.5883/DS-KFML1)) and GenBank. While many specimens remain unidentified, some identifications are worth comment. This collection is rich in Carposinidae, Epermeniidae, and Yponomeutidae, all families that are very poorly known in Africa. Several species of Sesiidae, which are rarely recorded from fruit (Harms and Aiello 1995), were also reared.

#### Blastobasidae

Several records supplemental to Adamski et al. (2010; GenBank GQ330121-GQ330289) are included and will be treated in more detail by David Adamski in the future.

#### Cosmopterigidae

We appear to have up to six species tentatively placed in the genus *Stilbosis*, reared from several species of *Garcinia* (Clusiaceae) – lots A&M 953, 1076, 1192, 1513, 1612, 1687, 1832, 2116, 2378 and KIP 269. They are similar to the species reared by Ghesquière (1940: 76) from *Garcinia* in the Democratic Republic of Congo, and published as *Stilbosis firma* (Meyrick), a species described from the Seychelles. Unfortunately the type of *Stilbosis firma* in BMNH is missing its abdomen, but Legrand (1965: 63) published a figure of the genitalia of the species (as *Pyroderces firma*), which is similar to, but not the same as, our genitalia slides USNM 93136 and 125921. It is also doubtful that the Congo species is conspecific with *Stilbosis firma*.

#### Gelechiidae

*Encolpotis xanthoria* Meyrick was reared from *Monanthotaxis parvifolia* Verdc. (Annonaceae, lot A&M 2313) and *Agelanthus sansibarensis* (Engl.) Polhill and Wiens (Loranthaceae, lot A&M 2383). Krüger (1998: 59) suggested this species inhabits *Acacia* galls as a predator, and the species was described from South African specimens reared from larvae feeding on *Icerya* sp. (Homoptera: Coccidae). The species was also figured by Janse (1960: 158) and Clarke (1969: 55). LePelly (1932: 76) recorded it from bored coffee berries in Kiambu, Kenya (voucher specimens in BMNH and KARI), and BMNH has additional specimens reared from coffee berries at Kiambu in 1957 by D.J. McCrae, as well as specimens from Malawi reared by C. Mason in 1914 with the notation “larvae predaceous on mulberry scale 822.”

*Mometa*—We have three apparently undescribed species of this genus, related

to the supposedly widespread African cotton pest *Mometa zemiodes* Durrant, which was described from Nigeria (Ghesquière 1940: 49, Russo 1940: 145, Common 1958, Janse 1963: 147) – lots A&M 1068 from *Acanthus pubescens* Engl. (Acanthaceae), 1617 from *Sterculia africana* (Lour.) Fiori (Malvaceae) and 2455 from *Turraea robusta* Gurke (Meliaceae).

*Palumbina guerinii* (Stainton) was reared from *Pistacia aethiopica* Kokwaro (Anacardiaceae) (lot A&M 2521 and a 2008 record from Mt Kulal). The biology was reviewed by Sattler (1982). Our sequences match those from European specimens of this species (P. Huemer, unpublished data in BOLD). This appears to be the first record from the Afrotropics.

*Pectinophora gossypiella* was reared from *Gossypioides kirkii* Mast. (Malvaceae) (lot A&M 1564), and its sequence matches JF815075-JF815081 from Arizona, Texas and Israel (Hughes and Moore 2011). Previously known from Kenya (Prior 1985: map 260).

#### Oecophoridae

*Stathmopoda auriferella* (Walker) were reared from *Lannea welwitschii* (Hiern.) Engl. (Anacardiaceae), *Antidesma venosum* J.J. Sm. (Euphorbiaceae), *Psydrax polhilli* Bridson (Rubiaceae), and *Tetracera boiviniana* Baill. (Dilleniaceae) (lots KIP 567, KIP 641, A&M 1266, A&M 1954 respectively) and specimens from Nigeria (female genitalia slide USNM 125801) identified by comparison to types at BMNH and Kasy (1973: fig. 43) and Prevett (1963). Although this has been considered a widespread polyphagous species (Robinson et al. 1994: 55), the difference between the Nigeria and Kenya sequences (98.47% similar) suggests that more sampling may prove this taxon to be a species complex.

#### Yponomeutidae

*Prays citri* (Millière) was reared from *Vepris nobilis* (Delile) Mziray (Rutaceae) (lot A&M 873). Our sequences match European sequences (P. Huemer, unpublished data in BOLD). A widespread pest of citrus, although evidently not previously recorded from Kenya (CABI 2013).

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