From field courses to DNA barcoding data release for West Papua - making specimens and identifications from university courses more sustainable

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Abstract

The diversity of insects collected during entomological field courses at the University of West Papua (UNIPA), Indonesia, is studied using DNA barcoding tools. The results were compared with public data available for West Papua in the Barcode of Life Data System. During two training courses in 2013 and 2015, 1,052 specimens of insects were collected at eight sites near Manokwari in northern West Papua. The DNA sequences obtained from these specimens were assigned to 311 Barcode Index Numbers (BINs) and represent species in 27 families of Lepidoptera, Hymenoptera and Coleoptera. Of those BINs, 294 (95%) were new to West Papua. The study suggests that DNA barcoding applied to university courses achieves several goals, including capacity building and hands-on experience in molecular biodiversity assessment. In addition, it can provide valuable biodiversity data that are globally available to researchers for further studies.

Introduction

Carrying out field courses for students is a central aspect of any capacity building effort at universities, including and perhaps especially so in tropical countries (see Basset et al. 2000, Basset et al. 2004, Novotny et al. 2012, Tänzler et al. 2012, Rintelen et al. 2017). They serve mainly the purpose of demonstrating sampling, preparation and identification methods for insects and other arthropods. The scientific value of insect collecting during student courses can be dramatically increased if the specimens are adequately preserved

and/or mounted for long-term storage in a scientific collection (Lopez-Vaamonde et al. 2012, Schmidt et al. 2017). This applies even more if samples are then utilised for more sustainable methods of biodiversity assessment, such as DNA barcode analysis and if sequence and associated collecting data are generally accessible for researchers nationally and internationally (see Hebert et al. 2003, Hajibabaei et al. 2005, Janzen et al. 2009, Gwiazdowski et al. 2015, Wirta et al. 2015, Miller et al. 2016).

The present study makes an attempt to use specimens of insects collected during student courses in a more sustainable way. In particular, we tried to maximise the potential benefit of field and entomological training courses at the University of West Papua (UNIPA) on the western part of the island of New Guinea. The courses were conducted by a team of entomologists from the Zoologische Staatssammlung (ZSM) in Munich, Germany, which was followed up by a repeated staff exchange from UNIPA to the ZSM.

Material and Methods

The methods were described in Cancian de Araujo et al. (2017), with the following differences:

Specimen collecting and processing

In 2013 and 2015, 1,052 specimens of insects were collected during capacity building courses by lecturers and students of the State University of Papua (UNIPA) in Manokwari. The collections mainly served as a vehicle to demonstrate field survey methods and subsequent laboratory procedures for sustainable biodiversity inventory and discovery. Targeted field work was coordinated by RP and AK and part of the laboratory work was conducted by RP during her stay at the SNSB-Zoologische Staatssammlung München (ZSM, Bavarian State Collection of Zoology) in 2014 under the supervision of MB, SS and OS.

Samples were collected at eight sites in the Indonesian province West Papua, viz. Fumato, Kebar Village, Minyambo, Mubrani, Syoubri, Senopi, Gunung Meja and the Papua University Campus in Manokwari (Fig. 1). The latter was a short Malaise trapping exercise with one trap that was operated for three weeks. For more details about the field and lab protocols see Schmidt et al. (2015), Schmidt et al. (2017) and below. The specimen data are accessible on BOLD through the following doi: <u>10.5883/DS-INWPAPUA</u> and through GenBank (Accession nos MH094885-MH095566),

Data acquisition

The specimen data and result files generated for the present study were downloaded directly from the Barcode of Life Data Systems (BOLD, <u>http://www.boldsystems.org</u>) workbench. In addition, all other public records from the province West Papua and other Indonesian areas from the western half of the island of New Guinea present in BOLD were obtained through the REST API of the BOLD platform on 31-Jan-2018. We applied the

"Full Data Retrieval" parameters geo=Papua|West%20Papua|Papua%20Barat and marker=COI–5P in order to gather all public records from West Papua with the standard DNA barcoding marker (COI–5P).

Data processing

The files that were downloaded contained information on each record including the Barcode Index Number (BIN), collection data and taxonomy. The data were evaluated in terms of BIN diversity, spatial distribution of specimens, taxonomic identification depth and taxonomic diversity. The results were compared in terms of diversity of BINs, exclusive and shared BINs and BIN distribution. Analyses and comparisons were made using Microsoft Excel. The number of BINs shared by the two sources was evaluated and after that, the shared BINs were subtracted from our West Papua list in order to highlight the contribution of our case study for West Papuan records in general. The map with collecting records was created using Quantum GIS (vers. 2.8).

Results

Between 2013 and 2015, 1,052 specimens from West Papua were processed. The records are distributed in six areas in West Papua and were collected at altitudes between 80 and 1,555 meters above sea level. The taxa belong to three insect orders: Coleoptera (108), Hymenoptera (217) and Lepidoptera (727). The geographic distribution per site and taxon are presented in Fig. 1. Out of these 1,052 specimens, the CO1-5P barcode sequence was recovered from 686 specimens, corresponding to 311 BINs from at least 27 families of insects (Fig. 2).

When searching for public data of arthropods from West Papua in BOLD, we recovered 1,268 records that were assigned to 584 BINs. The records belong to 10 orders with the most common being Lepidoptera (910 records, 441 BINs), Coleoptera (214 records, 68 BINs) and Decapoda (60 records, 30 BINs). When comparing our records with the public data available on BOLD, only 17 BINs (5%) had been recorded before from West Papua, whereas 294 BINs (95%) were new records for this area in BOLD.

A comparison of BINs recorded in our study that were also recorded from elsewhere showed that 74 BINs (24%) were already present in BOLD from regions outside of West Papua, mainly from Australia (39 BINs), Papua New Guinea (23 BINs), French Polynesia (6 BINs) and Indonesian locations other than West Papua (23 BINs) (Table 1). The species is given if the BIN was associated with a species (or genus) name in BOLD, but it should be stressed that, for many species, the barcode-based species level identification require verification based on morphology by a specialist.

Two taxa, *Spolodea recurvalis* (Lepidoptera, Crambidae) and a species of *Cotesia* (Hymenoptera, Braconidae), had a wider distribution with records from five or more countries (Table 1). The remaining 237 BINs (76%) were recorded excusively from West Papua.

Discussion

The high number of BINs that are exclusive to an area with comparatively well studied surroundings, highlights the urgency of studying the biodiversity of tropical regions. Analysis of 1,052 specimens increased the diversity of known species in this particular area 1.5-fold, from 583 to 877 species, as expressed by BINs that have been shown to closely relate to biological species. This was achieved by analysing a handful of randomly collected samples obtained by students during field courses under the supervision of entomologists from the ZSM (Munich).

Even for well studied groups like Lepidoptera, the study led to an increase of 205 BINs, corresponding to nearly one third (32%) of all species known so far for this insect order from West Papua. For less well known groups like Hymenoptera and Coleoptera, all BINs were new to West Papua. It is important to stress that nearly all Coleoptera and Hymenoptera specimens of the present study were collected at one site, the campus of the Papua University at Manokwari, showing the potential for a significantly higher number of discoveries with a broader sampling regime across different elevations and actually investigating primary forest areas. The Geometridae (Lepidoptera) was a target group for collecting which explains the predominance of geometrid moths, representing nearly half (48%) of the BINs.

Our study suggests that DNA barcoding applied to university courses achieves several goals, including capacity building, hands-on experience in molecular biodiversity assessment and it provides valuable data that are globally available by researchers for further studies (see also Vernooy et al. 2010). Specimens that would usually only be identified to order or perhaps genus level (and then often forgotten) can now serve to provide data in a sustainable manner. The data have become a community resource and are available for local researchers to benefit their research. Ideally, the next steps would include more focussed and specific project orientated field- and laboratory work that could strongly support the analysis of large scale patterns of diversity as outlined by Tänzler et al. (2012).

In a very similar context, DNA barcoding applied to samples obtained through activities of citizen scientists in remote localities (Janzen and Hallwachs 2011, Miller et al. 2014, Wilson et al. 2015, Jisming-See et al. 2016, Loos et al. 2015, Schilthuizen et al. 2017, Suprayitno et al. 2017, Freitag et al. 2018) could make significant, objective contributions to our understanding of the patterns of global biodiversity.

Finally, this simple experiment provided additional occurrence records for virtually cosmopolitan species like the Lepidoptera, Crambidae: *Spoladea recurvalis* and a widespread species of braconid wasps (Hymenoptera, Braconidae, *Cotesia* sp.), confirming the usefulness of DNA barcoding for the large scale assessment of global distribution patterns and also for monitoring the distribution and spread of invasive species.

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Conflicts of interest

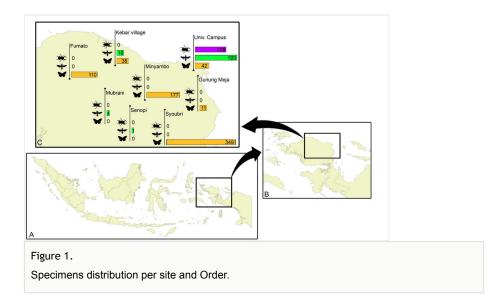
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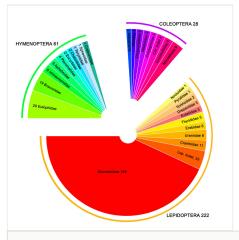


Figure 2. BINs distribution per Order and Family.

Table 1.

Taxa with BOLD records from locations outside West Papua. Given are insect order, family, Barcode Index Number (BIN) and species (or genus) in case of a BOLD BIN match and country from where the species was recorded.

| Order | Family | BIN / Species | Country |
|-------------|---------------|---|---|
| Coleoptera | Chrysomelidae | BOLD:ADG9196 | Indonesia (West Sumatera) |
| Coleoptera | Coccinellidae | BOLD:AAH3306 (Scymnus mitior) | Australia |
| Coleoptera | Coccinellidae | BOLD:ADC0638 | Indonesia (West Sumatera) |
| Coleoptera | Mordellidae | BOLD:ADC2466 | Malaysia |
| Coleoptera | Nitidulidae | BOLD:ADG5133 (<i>Epuraea ocularis</i>) | Indonesia (West Sumatera), French Polynesia |
| Hymenoptera | Aphelinidae | BOLD:ADD0593 | Indonesia (West Java) |
| Hymenoptera | Braconidae | BOLD:AAH1084 (<i>Cotesia</i> sp.) | Australia, French Polynesia, Indonesia (West Sumatera), Pakistan, Papua New Guinea, United Arab Emirates |
| Hymenoptera | Braconidae | BOLD:AAH1349 | Indonesia (West Java), South Korea |
| Hymenoptera | Braconidae | BOLD:ADJ6741 | Indonesia (West Java) |
| Hymenoptera | Chalcididae | BOLD:AAW0748 | Australia |
| Hymenoptera | Crabronidae | BOLD:ACV0318 | Indonesia (West Sumatera) |
| Hymenoptera | Encyrtidae | BOLD:ACP0359 | Indonesia (Bali, West Sumatera), Australia |
| Hymenoptera | Eulophidae | BOLD:ADE0821 | Indonesia (West Java) |
| Hymenoptera | Ichneumonidae | BOLD:AAH2022 (<i>Enicospilus</i> sp.) | Australia, Indonesia (West Sumatera) |
| Hymenoptera | Ichneumonidae | BOLD:ADG2435 | Indonesia (West Java) |
| Hymenoptera | Ichneumonidae | BOLD:ADK3119 | Indonesia (West Java) |
| Hymenoptera | Sphecidae | BOLD:AAH3486 | Australia |
| Lepidoptera | Crambidae | BOLD:AAA3666 (<i>Spoladea recurvalis</i>) | Australia, Canada, China, French Polynesia, Israel, Japan, Pakistan, Seychelles, South Africa, South Korea, United States |
| Lepidoptera | Crambidae | BOLD:AAB5972 (Prophantis adusta) | Australia, Papua New Guinea |
| Lepidoptera | Crambidae | BOLD:AAC4723 (Cnaphalocrocis poeyalis) | Australia, French Polynesia |
| Lepidoptera | Crambidae | BOLD:AAD1174 (<i>Eurrhyparodes bracteolalis</i>) | Australia |

| Lepidoptera | Crambidae | BOLD:AAD7675 (Tabidia insanalis) | Australia, Papua New Guinea |
|-------------|-------------|--|--|
| Lepidoptera | Crambidae | BOLD:AAE0808 (Agrioglypta eurytusalis) | French Polynesia, Australia, Indonesia (West Java) |
| Lepidoptera | Crambidae | BOLD:AAE5257 (Herpetogramma hipponalis) | Australia |
| Lepidoptera | Crambidae | BOLD:AAl4942 (<i>Palpita uedai</i>) | Australia |
| Lepidoptera | Crambidae | BOLD:AAL8459 (<i>Parotis</i> sp.) | Papua New Guinea |
| Lepidoptera | Crambidae | BOLD:ABU8109 | Indonesia (West Sumatera) |
| Lepidoptera | Drepanidae | BOLD:AAD3754 (<i>Tridrepana lunulata</i>) | Australia |
| Lepidoptera | Erebidae | BOLD:AAB3896 (<i>Cyme</i> nr <i>pyraula</i>) | Australia |
| Lepidoptera | Erebidae | BOLD:AAF0763 (Polypogon fractalis) | Australia |
| Lepidoptera | Erebidae | BOLD:AAZ1754 (<i>Harita nodyna</i>) | Australia |
| Lepidoptera | Erebidae | BOLD:ACE6049 (<i>Pogonia umbrifera</i>) | Australia |
| Lepidoptera | Euteliidae | BOLD:AAK0923 (Anigraea cinctipalpis) | Australia, Malaysia |
| Lepidoptera | Euteliidae | BOLD:ABA5144 (Anigraea deleta) | Malaysia |
| Lepidoptera | Geometridae | BOLD:AAA9837 (Eucyclodes pieroides) | Australia |
| Lepidoptera | Geometridae | BOLD:AAA9900 (<i>Hyposidra talaca</i>) | Indonesia (West Java), Australia, China, Papua New Guinea |
| Lepidoptera | Geometridae | BOLD:AAB0565 (<i>Idaea simplex</i>) | Australia |
| Lepidoptera | Geometridae | BOLD:AAB1570 (Comostola leucomerata) | Australia |
| Lepidoptera | Geometridae | BOLD:AAB8518 | Australia, French Polynesia |
| Lepidoptera | Geometridae | BOLD:AAC1659 (Pingasa chlora) | Papua New Guinea |
| Lepidoptera | Geometridae | BOLD:AAD4641 (Symmacra ochrea or S. solidaria) | Papua New Guinea |
| Lepidoptera | Geometridae | BOLD:AAE2192 (Chloroclystis cissocosma) | Indonesia (West Java, East Java), Australia |
| Lepidoptera | Geometridae | BOLD:AAE7089 (Agathiopsis basipuncta) | Australia |

| Indication(Episothalma obscurate)LepidopteraGeometridaeBCD:AAF9586 (Aeolochroma sp.)Papua New GuineaLepidopteraGeometridaeBOLD:AAF9622Indonesia (West Java, East Java)LepidopteraGeometridaeBOLD:AAI6447AustraliaLepidopteraGeometridaeBOLD:AAI7614 (Idaae elaphrodes)AustraliaLepidopteraGeometridaeBOLD:AAR3997 (Chloroclystis semiscripta)Papua New GuineaLepidopteraGeometridaeBOLD:AAR3997 (Chloroclystis semiscripta)Indonesia (West Java)LepidopteraGeometridaeBOLD:AR3997 (Chloroclystis semiscripta)Papua New GuineaLepidopteraGeometridaeBOLD:ABX6397 (Craspedosis auriguta)Papua New GuineaLepidopteraGeometridaeBOLD:ABX7397 (Craspedosis auriguta)Papua New GuineaLepidopteraGeometridaeBOLD:ABX7397 (Craspedosis auriguta)Papua New GuineaLepidopteraGeometridaeBOLD:AC80570 (Craspedosis auriguta)Papua New GuineaLepidopteraGeometridaeBOLD:AC80570 (Draspedosis Sp.)Papua New GuineaLepidopteraGeometridaeBOLD:AC80570 (Draspedos duplicipuncta of N.mysolati)Papua New GuineaLepidopteraGeometridaeBOLD:AC80570 (Draspedos duplicipuncta of N.mysolati)Papua New GuineaLepidopteraGeometridaeBOLD:AC80570 (Draspedos duplicipuncta of N.mysolati)Papua New GuineaLepidopteraGeometridaeBOLD:AC20577 (Tradspedos duplicipuncta of N.mysolati)Papua New Guine | Lepidoptera | Geometridae | BOLD:AAF3254 | Australia |
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| Lepidoptera Geometridae BOLD:ACZ0867 Indonesia (West Java) | Lepidoptera | Geometridae | BOLD:ACZ0473 | Indonesia (East Java) |
| | Lepidoptera | Geometridae | BOLD:ACZ0547 | Indonesia (Bali) |
| Lepidoptera Geometridae BOLD:ACZ1181 Indonesia (West Java) | Lepidoptera | Geometridae | BOLD:ACZ0867 | Indonesia (West Java) |
| | Lepidoptera | Geometridae | BOLD:ACZ1181 | Indonesia (West Java) |

| Lepidoptera | Noctuidae | BOLD:AAO8880 (Argyrolepidia thoracophora) | Australia |
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| Lepidoptera | Thyrididae | BOLD:AAA8776 (<i>Mellea ordinaria</i> species complex) | Papua New Guinea |
| Lepidoptera | Thyrididae | BOLD:AAG6033 (<i>Canaea hyalospila</i> or <i>C. rusticata</i>) | Australia, Papua New Guinea |
| Lepidoptera | Tortricidae | BOLD:AAA9084 (<i>Adoxophyes templana</i> species comples) | Australia, Papua New Guinea |
| Lepidoptera | Tortricidae | BOLD:AAB4056 (Homona trachyptera) | Australia, Papua New Guinea |
| Lepidoptera | Uraniidae | BOLD:AAC9235 (<i>Cathetus euthysticha</i>) | Australia |
| Lepidoptera | Uraniidae | BOLD:AAD1023 (Phazaca mutans) | Australia |