

SPIXIANA	42	1	47–59	München, September 2019	ISSN 0341–8391
----------	----	---	-------	-------------------------	----------------

Towards creating a DNA barcode reference library of geometrid moths from western Himalaya, India

(Lepidoptera, Geometridae)

Pritha Dey, Axel Hausmann & V. P. Uniyal

Dey, P., Hausmann, A. & Uniyal, V. P. 2019. Towards creating a DNA barcode reference library of geometrid moths from western Himalaya, India (Lepidoptera, Geometridae). *Spixiana* 42 (1): 47–59.

The taxonomy of the family Geometridae (Lepidoptera: Geometroidea) is rather well established for most temperate regions of our earth, but tropical areas still need large-scale revisions. The Indian Himalayan region with its unique physical attributes is home to a distinctive faunal diversity but there is a lack of comprehensive study on its Geometridae moth fauna. In the present study a total of 113 species of the family Geometridae collected in the Nanda Devi Biosphere Reserve (a UNESCO World Heritage Site), Uttarakhand, India have been subjected to DNA barcoding (CO1). Distinct Barcode Gaps (difference between intraspecific and interspecific nucleotide divergence) exist between most of the species studied, thus confirming their discrimination. Sequences of species from the Lepidoptera Collection of the Zoologische Staatssammlung München collected from western Himalaya (Himachal Pradesh and Kashmir) and eastern Himalaya (Sikkim) were included into the analysis. Four species from Sikkim show deep genetic (K2P distance) divergence from conspecifics collected in the western Himalaya (Uttarakhand), indicating potential cryptic diversity. Our preliminary assessment was based on DNA barcoding in combination with standard taxonomy, and revealed one new species record for India and distribution extensions of 15 other species within India. The baseline data generated through this study will provide scope to do further extensive research on moths in this region, providing effective geographical distribution data, ecological understanding and foundation for their conservation in the face of habitat degradation and climate change.

Pritha Dey (corresponding author) & V.P. Uniyal, Wildlife Institute of India, Chandrabani, Dehradun-248001, Uttarakhand, India;
e-mail: dey.pritha126@gmail.com

Pritha Dey & Axel Hausmann, SNSB – Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 Munich, Germany

Introduction

The Barcode of Life initiative (Hebert et al. 2003a,b), which started more than a decade ago, has been established as a standardized method for identifying species and for reducing the morphological taxonomic burden, by using a single gene, cytochrome c oxidase subunit 1 (CO1). DNA-based taxonomy – as initially proposed by Tautz et al. (2003) – has,

since then, proven to be an immensely useful tool to solve taxonomic problems of lesser known groups (cf. Miller et al. 2016). DNA barcoding adds genetic information to the already established Linnean taxonomy, but sometimes the barcode analysis reveals a mismatch with existing Linnean binomials (Pons et al. 2006). This mismatch or incongruency is an effect of either cryptic species (Hebert et al. 2004a,b); or a lack of expert taxonomic species delineation

(Agapow et al. 2004, Wiens & Penkrot 2002). DNA barcoding, thus far, has been successfully applied across taxa and across countries, including plants (Group et al. 2009), fishes (Ward et al. 2005, Hubert et al. 2008), mammals (Clare et al. 2007), birds (Kerr et al. 2007), insects (Hebert et al. 2004a, Hastings et al. 2008, Hendrich et al. 2014, Morinière et al. 2014, Schmidt et al. 2015, Hawlitschek et al. 2016), crustaceans (Hou et al. 2009) among others. The order Lepidoptera has received particular attention (Janzen et al. 2005, 2009, Hajibabaei et al. 2006, Burns et al. 2008, Silva-Brandão et al. 2009, Hausmann et al. 2011a,b, 2013, 2016, Mutanen et al. 2016 and many more) with currently 1.09 million barcode records on BOLD, the Barcode of Life Data System (Ratnasingham & Hebert 2007). The success of DNA barcoding has encouraged efforts to build DNA barcode libraries across various groups and regions globally (Ekrem et al. 2007, Kerr et al. 2007, Zhou et al. 2009, 2011, deWaard et al. 2010, 2011, Dincă et al. 2011, Kuzmina et al. 2012, Webb et al. 2012, Raupach et al. 2014) also using natural history collections in museums (Strutzenberger 2012, Hebert et al. 2013, Hausmann et al. 2016). DNA barcodes in recent years have shown promising new prospects in answering fundamental ecological questions that govern community assemblage, macroevolutionary processes and conservation of species (Joly et al. 2014, Veldman et al. 2014). The ecological information coupled with the understanding of evolutionary histories from DNA barcodes across taxa can assist in answering complex questions relevant to species assembly and distribution in the era of bioinformatics (Joly et al. 2014). Moreover, it is a valuable tool to understand feeding habits (González-Varo et al. 2014) and target protection of habitats (Kress et al. 2015).

Despite their fundamental roles in nature as selective herbivores, pollinators, detritivores, and prey for birds, e.g. migratorial passerines, forest indicator taxa (Summerville et al. 2003a,b, Kitching et al. 2000, Beck et al. 2002, 2017, New 2004) and potential in the definition of conservation priority areas, moths have been strongly neglected in conservation studies. Geometridae, together with the Erebidae, are the most species-rich family of moths worldwide. Geometridae is a cosmopolitan family with the exception of Antarctica and shows diversity peaks in tropical South America, Africa and South-east Asia. This group is a well-established model group for biodiversity studies in temperate and tropical regions (Brehm et al. 2013, 2003a,b, Axmacher et al. 2004, 2009, Barlow, 1982, Barlow & Woiwod 1989 and many more). Geometrids are known to associate with the prevailing environmental conditions while other families like Erebidae include larger portions of species which can colonize human-affected habitats

replacing forest species (Summerville 2004, Winfree et al. 2011). Large parts of the diverse Geometridae family may be at the risk of extinction due to destruction and fragmentation of their habitats in the tropics. The taxonomy of geometrid moths is replete with ambiguities. A global review suggests that there is some degree of synonymy at the species level in this family (Scoble et al. 1995) which suggests that taxonomic revisions are impending. There is no comprehensive morphology-based phylogeny available for geometrid moths, but the relationships between subfamilies and many tribes were recently assessed from the analysis of several nuclear genes (Young 2006, Yamamoto & Sota 2007, Regier et al. 2009, Öunap 2011, Sihvonen et al. 2011) largely confirming the traditional classification derived from morphological traits.

Coming to the Indian scenario, Gaikwad et al. (2012) and Kumar et al. (2019) barcoded the butterflies from the Western Ghats and the geometrid moths from the eastern Himalaya respectively. DNA barcoding of groups other than Lepidoptera from India has also been attempted in recent years (Kumar et al. 2007, 2012, Lakra et al. 2011, Dubey et al. 2011, Laskar et al. 2013, Gaikwad et al. 2016, Kaur & Sharma 2017). The integration of molecular tools and morphology for taxonomic research, especially for Lepidoptera is still in a nascent state. How do we conserve species with limited knowledge of which species are endangered or even how many species there are? There is lack of a comprehensive inventory of moths from western Himalaya, despite being one of the most biodiverse regions. The Himalayas provide an array of habitats and forest types comprising an excellent scenario to understand the diversity and the related evolutionary processes in this mosaic landscape. Moreover, we find Himalayan-Chinese and Indo-Malayan faunal dominance in the eastern Himalayas as compared to the western Himalayas (Mani 1968). The factors governing the distribution of high altitude insects differ among different mountains systems, depending partly on the present ecological conditions and historical distribution. The wide variations of the geological conditions provide diversified edaphic factors (Mani 1968). Western Himalaya, where the Oriental and Palearctic elements merge (Meinertzhagen 1928), has a unique biodiversity and interesting patterns can be expected. Faunistic inventories are the basis of biodiversity conservation, particularly in threatened and fragmented landscapes like the western Himalaya. An inventory of the moth fauna based on both morphological and molecular characteristics from this area would definitely add valuable information to the existing data and help us look into moth diversity in a comprehensive manner.

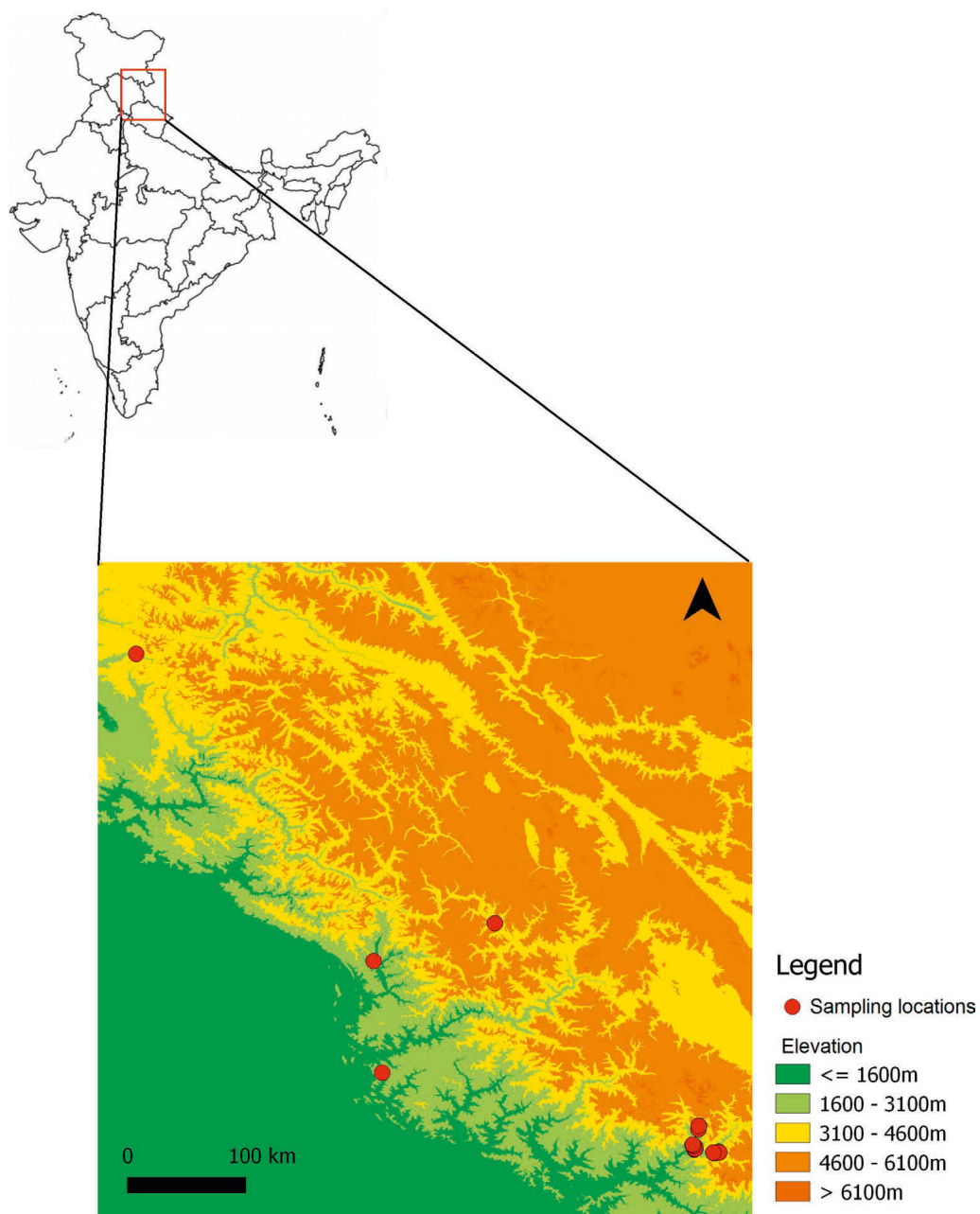


Fig. 1. The elevational gradient map shows the different sampling points from parts of states of Uttarakhand, Himachal Pradesh and Kashmir in the western Himalaya.

The intention behind this study is to combine the exploration of unstudied biodiversity of the study area and using DNA barcoding as an established

tool for improving classifications and understand diversity patterns for highly diverse groups like the geometrid moths in India.

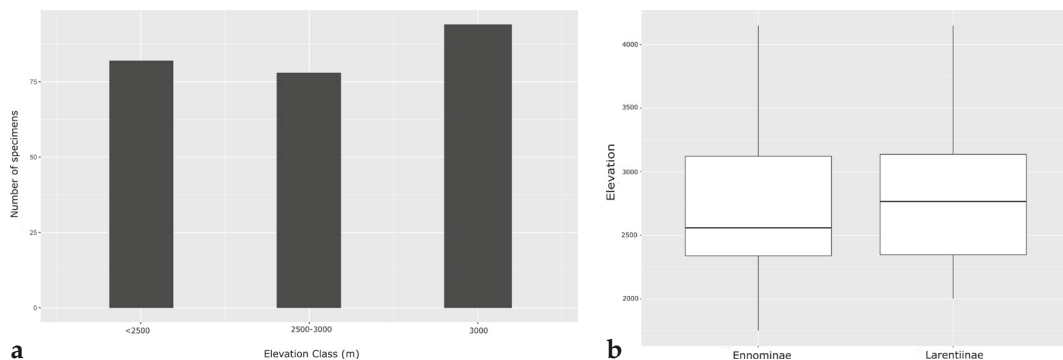


Fig. 2. a. The graph shows that specimens of Geometridae moths from different altitudes in western Himalayan states of Uttarakhand, Himachal Pradesh and Kashmir were collected with almost equal number of specimens across the elevational classes. b. The box-plot shows the elevational distribution of the two most speciose subfamilies (Ennominae and Larentiinae) of Geometridae in the present study. The highest altitude of collected specimens in both of the subfamilies was 4150 m a.s.l.

Materials and methods

Abbreviations

BOLD Barcode of Life Data Systems
 CCDB Canadian Centre for DNA Barcoding
 ZSM SNSB – Zoologische Staatssammlung München, Germany (Bavarian State Collection of Zoology, Munich)

Collecting

Moth specimens were collected in the Nanda Devi Biosphere Reserve, Uttarakhand, India using light-trapping (vertical sheet method). Specimens were collected across the elevational and vegetation gradient of the study area. A part of the collected specimens was pinned, labelled, mounted and identified at the ZSM to provide a reference collection for the DNA barcode library initiative for the western Himalaya. Not all specimens could be used due to legal restrictions in transferring biological material outside the country of collection. Locations of all the specimens used for DNA analysis are shown in Figure 1. Figure 2 shows the elevational distribution.

DNA sequencing

One dry leg was removed from each individual from the specimens collected by the first author and some specimens from the Herbulot collection stored in the ZSM, with sterile forceps and transferred to a 96-well microplate preloaded with one drop of 95 % ethanol in each well. DNA extraction and sequencing were performed at the CCDB, University of Guelph, following standardized high-throughput protocols for DNA barcode amplification and sequencing (Ivanova et al. 2006, deWaard et al. 2008).

Detailed information on the voucher specimens, genetic sequences, images, taxonomic classification, collection data and other relevant information for the

specimens collected by the first author is available in the public data set DS-INDIAGEO in the Barcode of Life Datasystems (BOLD; www.boldsystems.org) (Ratnasingham & Hebert 2007, 2013).

DNA sequence analysis

Additional sequences from the specimens collected in Himachal Pradesh, Kashmir and Sikkim regions of India, stored in the Herbulot collection at the ZSM and available (unpublished) in BOLD were included into some analysis steps. Sequence divergences for the barcode region were calculated for only the 224 specimens collected by the first author, using the analytical tools on BOLD Barcode Gap Analysis with BOLD alignment (pairwise deletion) using the Kimura 2 Parameter model (Kimura, 1980). Distances between species are presented as minimum pairwise distances, intraspecific variation as the mean and maximum pairwise distance within that species. BOLD assigns a Barcode Index Number (BIN) (Ratnasingham & Hebert 2013) automatically to sequences >500 bp which belong to the related sequence cluster. BINs very often coincide with the traditional species concepts but can reveal genetic splits within an “established” species or can reveal BIN-sharing (“merge”) of two taxa which in previous studies were thought to be two different species. In cases of discrepancy between the sequence based and morphological taxonomy, the specimens were re-examined morphologically to ascertain their identification.

Results

In the present study, we were able to generate 224 CO1 sequences from 250 specimens (90 %). 46 sequences out of 68 specimens (67 %) were added from the collection Herbulot in the ZSM.

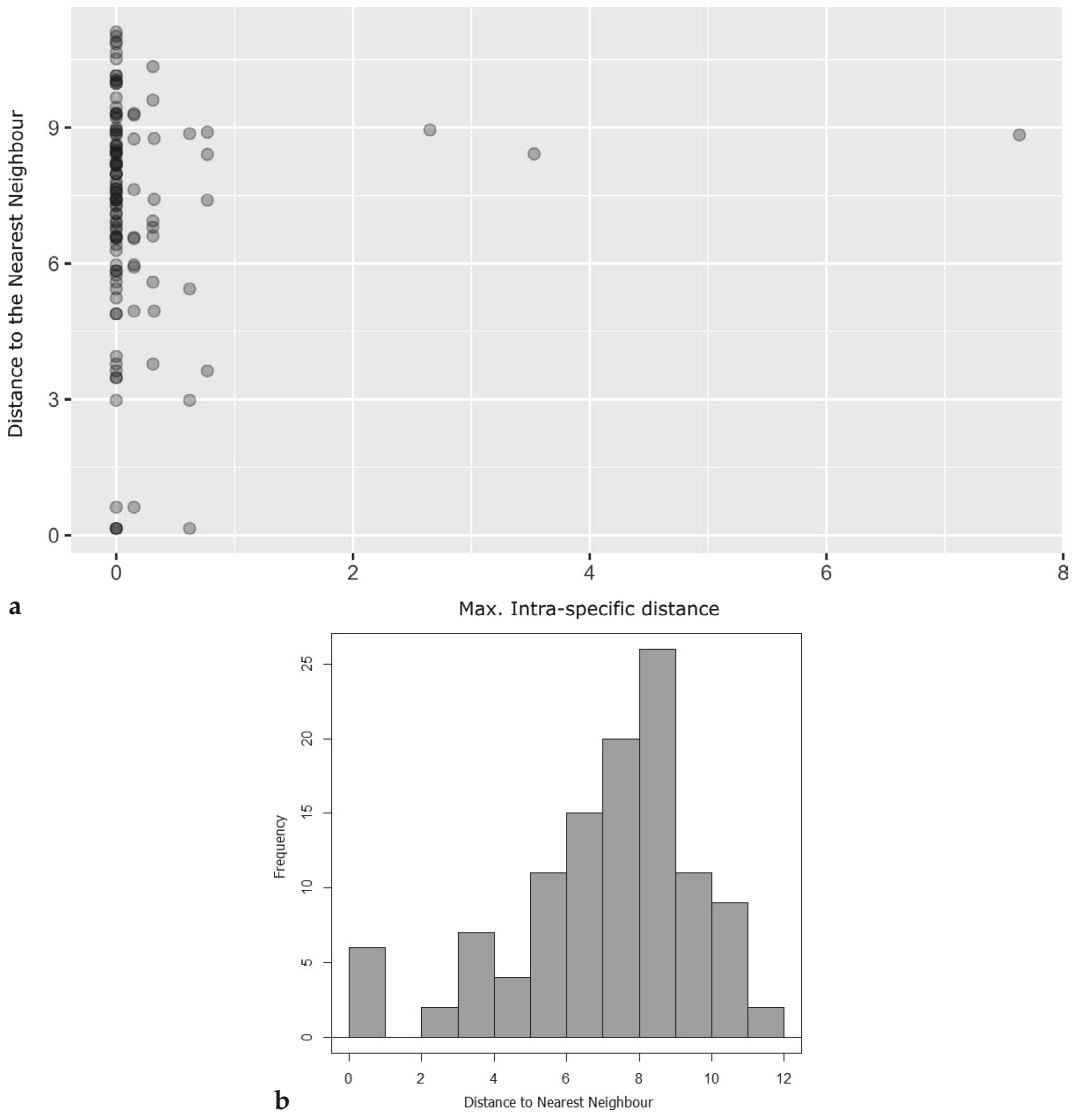


Fig. 3a,b. Barcode Gap Analysis performed with BOLD analytical tool shows the max. intra-specific distances in relation to the distances to the Nearest Neighbour and frequency distribution of the Nearest Neighbour distances in all the specimens collected from Uttarakhand by the first author.

Present study specimens from prov. Uttarakhand, western Himalaya: Out of the 224 DNA barcoded specimens, 35 specimens have only been identified to genus level, the rest (184=82.14 %) were assigned morphologically to a Linnean species name, while 5 specimens remain unidentified. Out of the 250 specimens subjected to DNA barcoding, 148 specimens belong to Larentiinae, while 90 belong to the subfamily Ennominae, 8 and 4 specimens to Sterrhinae and Geometrinae respectively. A total of

113 morphological species belonging to 51 genera were assigned to 116 BINs, out of which 63 BINs were singletons. All pre-identified species with multiple records/specimens were placed in a single BIN and successfully discriminated with a distinct Barcode Gap without any overlap in the study area except for *Alcis nudipennis* – *Alcis leucophaea* (BOLD:AAJ3727), *Opisthograptis mimulina* – *Opisthograptis* PD01 (BOLD:AAK5702) sharing their BINs. Some species were assigned to more than one BIN – *Loxaspilates obliquaria*

(BOLD:ADF4071, BOLD:ABA2651), *Trichopterigia* PD01 (BOLD:ADF3716, BOLD:ADF2951) and *Euphyia subangulata* (BOLD:AAL5265, BOLD:ADF3104). Figure 3a and b show the distance to nearest neighbour in relation to the max. intraspecific distance and the frequency distribution of nearest neighbour distances obtained from Barcode Gap Analysis on BOLD. 12 BINs (10.3 %) include a different morphological species from outside the study area (requiring further study). There was overlap of BINs (as currently present on BOLD Data Systems) including specimens from mainly the countries of the Indian subcontinent: Pakistan (24 BINs shared), Bhutan (10 BINs), Nepal (6 BINs), while from a biogeographical point of view 50 BINs are shared with Indo-Malayan realm and only 4 BINs are shared with Palearctic countries. Comparison of conspecific CO1 sequences from Himachal Pradesh, Kashmir and Eastern Himalaya (Sikkim, Kanchenjunga) as generated from ZSM (specimens from Herbulot collection) revealed high intraspecific divergence in four species between western (Uttarakhand) and eastern (Sikkim) Himalayas: *Arichanna flavinigra*, *Heterolocha falconaria*, *Abraxas superpicaria* and *Ecliptopera substituta* with intraspecific minimum pairwise genetic distance (Kimura-2-Parameter) generated from CO1 sequences in MEGA 7 varying from 3 to 6 % as shown in Figure 4. These four species will be subjected to further detailed taxonomic examination and molecular evidence based on more samples. The highest divergence was in *Heterolocha falconaria* (5.9 %) followed by *Ecliptopera substituta* (5 %), *Abraxas superpicaria* (4.1 %) and *Arichanna flavinigra* (3.1 %). These species show a very characteristic morphology so any chance of misidentification can be ruled out.

Three male and one female specimens of a taxon, initially identified as *Prometopidia conisaria* Hampson, 1902 (new distribution for the state of Uttarakhand) showed 6.6 % genetic divergence and with further investigation of genitalia and type specimen comparison, it was found that the female specimen is possibly a new species in the genus *Prometopidia*. The morphological analysis of these specimens is beyond the scope of this paper and is discussed in detail in another paper (Dey et al. in prep.).

Alcis paghmana Wiltshire, 1967 was identified through sequence matching in BOLD and thereafter morphological confirmation, representing a new distribution record for India. Correlating intra-/inter-species sequence divergence results and re-examining morphology revealed the following species with new distribution records for Uttarakhand: *Alcis nudipennis*, *Alcis perspicuata*, *Arichanna sparsa*, *Peetula stramineata*, *Entephria poliotaria*, *Perizoma variabilis*, *Costicoma exangulata*, *Triphosa venimacu-*

lata, *Perizoma variabilis*, *Perizoma conjuncta*, *Perizoma seriata*, *Electrophaes zaphenges*, *Entephria punctatissima*, and *Pennithera comis*.

Discussion

This study is a first initiative for assembling a DNA barcode reference library for geometrid moths from western Himalaya, India. Recently, Kumar et al. 2019 has barcoded some geometrid moths from the eastern Himalaya. But our study shows that investigation of DNA barcode variation and BIN-based species delimitation was successful for 113 species of Geometridae and reconfirming the reliability of DNA barcoding as an effective tool for species discrimination in Lepidoptera. Except for two species pairs with <2 % divergence from the nearest neighbour, intraspecific divergence was found to be lower than the distance to the nearest neighbour, thus allowing successful separation. Similarly high success rates for lepidopteran species re-identifications were found for Germany (Hausmann et al. 2011b), Pakistan (Ashfaq et al. 2013, 2017) and Costa Rica (Hajibabaei et al. 2006). Recent studies have explored continent-wide samples also with high (~93 %) re-identification success, e.g. for European geometrids (Hausmann et al. 2013) and North American Noctuoidea (Zahiri et al. 2014, 2017). Since our study area holds much higher species richness than other studies cited here, our results strongly support the importance of molecular taxonomy in the geometrid moth taxonomy in the country where the majority of the fauna awaits genetic analysis.

The success of DNA barcoding depends on the match between the BIN assignments and the taxonomic pre-identification. Neighbour-joining clustering analysis showed high incidence of monophyly in our study results, but the study area is restricted only to western Himalaya, and the sample size was comparatively small. Thus, the relationship between successful species differentiation and geographical range of the samples could not be exploited. We had four species showing intraspecific divergences of 3–6 % collected from two different parts of India, Uttarakhand and Sikkim (at a distance of about 1000 km). Ashfaq et al. (2013, 2017) showed maximum faunal overlap between Pakistan and India, likewise our study reveals the highest number of BINs (24) shared with Pakistan. The case of BIN-sharing of distinct species like that of *Alcis leucophaea* and *Alcis nudipennis* might indicate ancestral polymorphisms with subsequent incomplete lineage sorting (Zahiri et al. 2017) or just a lack of sound taxonomic diagnosis. Discrepancies between DNA barcodes and standard taxonomy

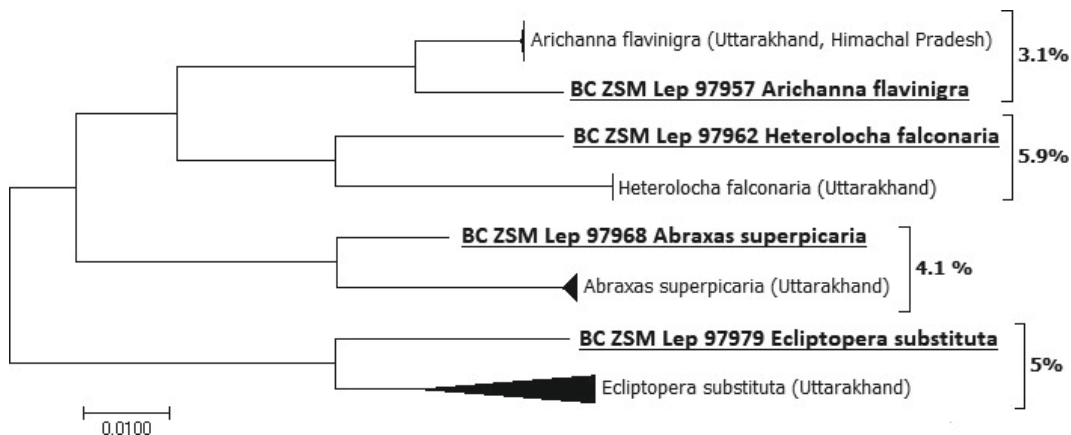


Fig. 4. Neighbour-joining tree (unrooted) showing the genetic divergence between specimens from western and eastern Himalaya found in four species. The names of the branches (bold and underlined) are the specimens from eastern Himalaya (Sikkim) from Collection Herbulot stored in ZSM. Locations of the rest from western Himalaya are given in parentheses.

(once misidentifications are outruled) can be treated as a scope to go in depth both in taxonomy and in the underlying evolutionary processes. Low or no interspecific divergence may suggest potential synonymy (Yang et al. 2016) and species with remarkably high intraspecific divergence demand further in-depth taxonomic analysis as they might include cases of cryptic diversity. However, in our case, low sample sizes and lack of sister species sampling might have affected the intra- and inter-specific distances in some cases giving poorly informative values (Meyer & Paulay 2005). A sample size of 20 samples per species is suggested for a robust DNA barcode analysis (Bergsten et al. 2012, Zhang et al. 2010, Luo et al. 2015).

We have shown that some (4 out of 113) eastern and western Himalayan species show considerable intraspecific divergence which can be explained by the ecological distinctiveness of these two regions. Intraspecific variations in DNA barcodes of species are a key to their unexploited ecological modifications (Joly et al. 2014). The western Himalaya was formed more recently than the eastern Himalaya, and hence there are ecological anomalies in the biogeographical distribution of different species, especially Lepidoptera (Mani 1968) perhaps due to subsequent climatic fluctuations. The environmental factors like the mean elevation, humidity, forest cover, and precipitation are also higher in the eastern than in the western Himalaya (Price et al. 2011). Intraspecific genetic distances might increase with geographic distance but not significantly enough to challenge the efficacy of DNA barcoding (Huemer et al. 2014) and species with discontinuous distribution

not necessarily show effects on genetic divergence correlated with geography (Mutanen et al. 2012). Habitat-induced low dispersal due to discontinuity of physical, geological, ecological and microclimatic conditions might have led to their geographical isolation. Similarly, adaptation to ecological niches is known to cause genetic divergence (Papadopoulou 2008). Study on birds (Price et al. 2003) have shown that species composition varies in different parts of the Himalaya mountains due to local extinctions of populations and that species numbers in the Himalaya decline with the latitudinal gradient. While studies on Himalayan butterflies have shown a significant rarefaction of humid-tropical-forest species westwards of the Sikkim state contrary to the significant increase of the temperate-forest species towards the west (reviewed in Mani 1968). The abundance and number of individuals per species was found to decrease by about five times from the east to the west between western Nepal and Pakistan, where the mountains take a northwards track (Mani 1968, Price et al. 2003, Ghosh-Harihar 2013). Detailed examination of host plant relationships and extending the sampling region to cover more of the distribution range of the investigated species is required to further tease apart the effect of elevation and habitat types from species ecology on cryptic diversity.

A detailed study of past geological and climatic events coupled with phylogeny can probably explain many of these cases of BIN discordance. The prevailing ecological conditions in the Himalaya mountain system favour a high rate of speciation with higher numbers of endemics than any other mountain chain

(e.g. the Alps, where there are more relict species). Apparently, this can be attributed to the more intensive Pleistocene glaciations in Europe than in Asia (Mani 1968). Past evolutionary patterns were directed towards ecological specializations while at present the high altitude fauna is evolving towards increasing species 'enrichment' (Mani 1968). As for the ecology and habitat requirements of largely unexplored biota, like the Himalayan fauna, it is a difficult task to elucidate even a small fraction of the existing life histories and species interactions.

Need and benefits of a DNA barcode library for India

In tropical regions the taxonomy of hyper-diverse groups of arthropods is a neglected field and, often, morphospecies are surrogates for species (Basset et al. 2012, Ashton et al. 2015), with major constraints due to little availability of taxonomic expertise and resources (Zenker et al. 2016). Thus, an ambitious insect inventory project can quickly overwhelm taxonomists with too many species and specimens. Adding to this, the concentration of expertise to only a few well-known species, leads to 'taxonomic impediment' (de Carvalho et al. 2005). As a result, there is unavailability of fine scale data for conservation for many groups. Along with species diversity, functional diversity and phylogenetic diversity are new dimensions to biodiversity studies but they are not yet particularly well studied in tropical regions among species-rich insect groups (Brehm et al. 2013).

India has a lot of unexplored biodiverse areas since the British left the country. Fragmentary taxonomic records (often very old done by British taxonomists, Hampson 1892–1896, Cotes & Swinhoe 1886, Warren 1889 to name a few) exist for moths from different parts of the country. From Uttarakhand state, some prominent works include Smetacek (2008), Sanyal et al. (2011, 2013, 2017) and Sondhi & Sondhi (2016). Sanyal et al. (2011, 2013) and Dey et al. (2015, 2017) have looked into the diversity and distribution of moth assemblages but none of the studies so far have ventured into the molecular phylogenetics area of research.

The way ahead

Based on the results of the present study, it is clear that the taxonomy of geometrid moth species from the study area is currently obscured in some degree of uncertainty. We need to further investigate correlations between the genetic divergence and morphological characteristics and to try to uncover overlooked species (Janzen et al. 2009) which have not been included in previous taxonomic work.

Species at lower elevations show a wider range of distribution than those at the higher elevations, which also may be reflected and thus measurable by genetic variation. For addressing questions like "how many species are there", geographically large scale reference libraries are required. A logical, great step forward will be to use DNA barcodes to understand large scale ecological patterns in intraspecific variation, and to explore the causes and the outcomes of such variations (Joly et al. 2014). As Janzen et al. (2009) predicted, DNA barcoding will bridge the gap between what is already known and what can be found out.

DNA barcoding has furthermore been shown as a valuable tool for unveiling host-plant associations (Smith et al. 2006, Miller et al. 2007, Matheson et al. 2008, Jurado-Riviera et al. 2009), when collected larvae were fogged or cannot be reared to adult because of food-plant supply constraints or failed rearing (Hausmann & Scalercio 2016). This paper thus opens up possibilities to detect insect-plant relationships using barcodes to connect life histories with species in large scale, which was earlier not possible.

Lastly, very often the conservation efforts are directed towards the Himalayas as a unit, but because of the inherent diverse nature of this mountain system, area-specific conservation should be targeted (Price et al. 2003). A characterisation of such poorly studied groups of insects (e.g. moths) at a molecular level, will allow a large step forward in resolving moth taxonomy using a valuable, integrative approach.

Acknowledgements

We are thankful to the Director and the Dean of Wildlife Institute of India for providing the opportunity to carry out this study in India. We are grateful to the Uttarakhand Forest Department for granting the permission for collecting specimens and the Divisional Forest Officer, Joshimath and his staff for providing logistic help and support. The study was a part of a project funded by the Department of Science and Technology, Govt. of India, and German Academic Exchange Service (DAAD). The authors are grateful to Paul Hebert and Sujeevan Ratnasingham and their competent teams (CCDB and BIO, University of Guelph) for their help in sequencing the specimens and for providing the bioinformatic platform for sequence analysis. The authors also wish to extend their gratitude to the National Biodiversity Authority (India) for giving necessary permission to bring out moth samples on loan to ZSM to fulfil the study. PD wishes to thank Rohit Chakravarty for his valuable inputs for improving the quality of an earlier draft of the manuscript.

References

- Agapow, P. M., Bininda-Emonds, O. R., Crandall, K. A., Gittleman, J. L., Mace, G. M., Marshall, J. C. & Purvis, A. 2004. The impact of species concept on biodiversity studies. *The quarterly review of biology* 79(2): 161–179.
- Ashfaq, M., Akhtar, S., Khan, A. M., Adamowicz, S. J. & Hebert, P. D. N. 2013. DNA barcode analysis of butterfly species from Pakistan points towards regional endemism. *Molecular Ecology Resources* 13(5): 832–843.
- , Akhtar, S., Rafi, M. A., Mansoor, S. & Hebert, P. D. 2017. Mapping global biodiversity connections with DNA barcodes: Lepidoptera of Pakistan. *PLoS One* 12(3): e0174749.
- Ashton, L. A., Barlow, H. S., Nakamura, A. & Kitching, R. L. 2015. Diversity in tropical ecosystems: the species richness and turnover of moths in Malaysian rainforests. *Insect Conservation and Diversity* 8(2): 132–142.
- Axmacher, J. C. & Fiedler, K. 2009. Habitat type modifies geometry of elevational diversity gradients in geometrid moths (Lepidoptera Geometridae) on Mt Kilimanjaro, Tanzania. *Tropical Zoology* 21(2): 243–251.
- , Holtmann, G., Scheuermann, L., Brehm, G., Müller-Hohenstein, K. & Fiedler, K. 2004. Diversity of geometrid moths (Lepidoptera: Geometridae) along an Afrotropical elevational rainforest transect. *Diversity and Distributions* 10: 293–302.
- Barlow, H. S. 1982. *An introduction to the moths of South East Asia*. 292 pp., Kuala Lumpur (The Malayan Nature Society).
- & Woiwod, I. P. 1989. Moth diversity of a tropical forest in peninsular Malaysia. *Journal of Tropical Ecology* 5(1): 37–50.
- Basset, Y., Cizek, L., Cuénoud, P., Didham, R. K., Guilhaumon, F., Missa, O., Novotny, V., Ødegaard, F., Roslin, T., Schmid, J. & Tishechkin, A. K. 2012. Arthropod diversity in a tropical forest. *Science* 338(6113): 1481–1484.
- Beck, J., Schulze, C. H., Linsenmair, K. E. & Fiedler, K. 2002. From forest to farmland: diversity of geometrid moths along two habitat gradients on Borneo. *Journal of Tropical Ecology* 18: 33–51. doi:10.1017/S026646740200202X
- , McCain, C. M., Axmacher, J. C., Ashton, L. A., Bärtschi, F., Brehm, G., Choi, S. W., Cizek, O., Colwell, R. K., Fiedler, K., Francois, C. L., Highland, S., Holloway, J. D., Intachat, J., Kadlec, T., Kitching, R. L., Maunsell, S. C., Merckx, T., Nakamura, A., Odell, E., Sang, W., Toko, P. S., Zamecnik, J., Zoi, Y. & Novotny, V. 2017. Elevational species richness gradients in a hyperdiverse insect taxon: a global meta-study on geometrid moths. *Global Ecology and Biogeography*: 1–13. doi:10.1111/geb.12548
- Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G. N. & Ribera, I. 2012. The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology* 61(5): 851–869. doi:10.1093/sysbio/sys037
- Brehm, G., Homeier, J. & Fiedler, K. 2003a. Beta diversity of geometrid moths (Lepidoptera: Geometridae) in an Andean montane rainforest. *Diversity and Distributions* 9: 351–366.
- , Sussenbach, D. & Fiedler, K. 2003b. Unique elevational diversity patterns of geometrid moths in an Andean montane rainforest. *Ecography* 26: 456–466.
- , Strutzenberger, P. & Fiedler, K. 2013. Phylogenetic diversity of moths decreases with elevation in the tropical Andes. *Ecography* 36: 1247–1253.
- Burns, J. M., Janzen, D. H., Hajibabaei, M., Hallwachs, W. & Hebert, P. D. N. 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservacion Guanacaste, Costa Rica. *Proceedings of the National Academy of Sciences USA* 105: 6350–6355.
- Clare, E. L., Lim, B. K., Engstrom, M. D., Eger, J. L. & Hebert, P. D. N. 2007. DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes* 7: 184–190. doi:10.1111/j.1471-8286.2006.01657.x
- Cotes, E. C. & Swinhoe, C. 1886. *A catalogue of moths of India*. 878 pp., Calcutta (Order of the Trustees of the Indian Museum).
- de Carvalho, M. R., Bockmann, F. A., Amorim, D. S., de Vivo, M., de Toledo-Piza, M., Menezes, N. A., de Figueiredo, J. L. & McEachran, J. D. 2005. Revisiting the taxonomic impediment. *Science* 307(5708): 353.
- deWaard, J. R., Ivanova, N. V., Hajibabaei, M. & Hebert, P. D. N. 2008. Assembling DNA barcodes: analytical protocols. Pp. 275–293 in: Martin, C. (ed.) *Methods in molecular biology: environmental genetics*. Totowa (Humana Press).
- , Hebert, P. D. N. & Humble, L. M. 2011. A comprehensive DNA barcode library for the looper moths (Lepidoptera: Geometridae) of British Columbia, Canada. *PLoS One* 6(3): e18290.
- , Mitchell, A., Keena, M. A., Gopurenko, D., Boykin, L. M., Armstrong, K. F., Pogue, M. G., Lima, J., Floyd, R., Hanner, R. H. & Humble, L. M. 2010. Towards a global barcode library for *Lymantria* (Lepidoptera: Lymantriinae) tussock moths of biosecurity concern. *PLoS One* 5(12): e14280.
- Dey, P., Uniyal, V. P. & Sanyal, A. K. 2015. Moth assemblages (Lepidoptera: Heterocera) as a potential conservation tool for biodiversity monitoring – study in western Himalayan protected areas. *Indian Forester* 141(9): 985–992.
- , Uniyal, V. P. & Chandra, K. 2017. A prefatory estimation of diversity and distribution of moths in Nanda Devi Biosphere Reserve, Western Himalaya, India. *National Academy Science Letters* 40(3): 199–203. doi:10.1007/s40009-016-0534-1
- Dincă, V., Zakharov, E. V., Hebert, P. D. N. & Vila, R. 2011. Complete DNA barcode reference library for a country's butterfly fauna reveals high performance for temperate Europe. *Proceedings of the Royal Society of London B, Biological Sciences* 278(1704): 347–355.

- Dubey, B., Meganathan, P. R. & Haque, I. 2011. DNA mini-barcoding: an approach for forensic identification of some endangered Indian snake species. *Forensic Science International, Genetics* 5(3): 181–184.
- Ekrem, T., Willassen, E. & Stur, E. 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution* 43(2): 530–542.
- Gaikwad, S., Warudkar, A. & Shouche, Y. 2016. Efficacy of DNA barcoding for the species identification of spiders from Western Ghats of India. *Mitochondrial DNA, Part A* 28(5): 638–644.
- Gaikwad, S. S., Ghate, H. V., Ghaskadbi, S. S., Patole, M. S. & Shouche, Y. S. 2012. DNA barcoding of nymphalid butterflies (Nymphalidae: Lepidoptera) from Western Ghats of India. *Molecular Biology Reports* 39(3): 2375–2383.
- Ghosh-Harihar, M. 2013. Distribution and abundance of foliage-arthropods across elevational gradients in the east and west Himalayas. *Ecological Research* 28(1): 125–130.
- González-Varo, J. P., Arroyo, J. M. & Jordano, P. 2014. Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods in Ecology and Evolution* 5(8): 806–814.
- Group, C. P. W., Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., Chase, M. W., Cowan, R. S., Erickson, D. L. & Fazekas, A. J. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences USA* 106(31): 12794–12797.
- Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W. & Hebert, P. D. N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences USA* 103: 968–971. doi:10.1073/pnas.0510466103.
- Hampson, G. F. 1892. *Fauna of British India moths 1*. 527 pp., London (Taylor and Francis).
- 1894. *Fauna of British India moths 2*. 528 pp., London (Taylor and Francis).
- 1895. *Fauna of British India moths 3*. 517 pp., London (Taylor and Francis).
- 1896. *Fauna of British India moths 4*. 595 pp., London (Taylor and Francis).
- Hastings, J. M., Schultheis, P. J., Whitson, M., Holliday, C. W., Coelho, J. R. & Mendell, A. M. 2008. DNA barcoding of New World cicada killers (Hymenoptera: Crabronidae). *Zootaxa* 1713: 27–38.
- Hausmann, A., Godfray, H. C. J., Huemer, P., Mutanen, M., Rougerie, R., van Nieukerken, E. J., Ratnasingham, S. & Hebert, P. D. N. 2013. Genetic patterns in European geometrid moths revealed by the Barcode Index Number (BIN) system. *PLoS One* 8(12): e84518.
- , Haszprunar, G. & Hebert, P. D. N. 2011a. DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. *PloS One* 6(2): e17134.
- , Haszprunar, G., Segerer, A. H., Speidel, W., Behounek, G. & Hebert, P. D. N. 2011b. Now DNA-barcoded: the butterflies and larger moths of Germany. *Spixiana* 34(1): 47–58.
- , Miller, S. E., Holloway, J. D., deWaard, J., Pollock, D., Prosser, S. W. J. & Hebert, P. D. N. 2016. Calibrating the taxonomy of a megadiverse insect family: 3000 DNA barcodes from geometrid type specimens (Lepidoptera, Geometridae). *Genome* 59(9): 671–684. doi:10.1139/gen-2015-0197
- & Scalerio, S. 2016. Host-plant relationships of 29 Mediterranean Lepidoptera species in forested ecosystems unveiled by DNA barcoding (Insecta: Lepidoptera). *SHILAP Revista de Lepidopterología* 44(175): 463–471.
- Hawlitschek, O., Morinière, J., Lehmann, G. U. C., Lehmann, A., Kropf, M., Dunz, A., Glaw, F., Detchaen, M., Schmidt, S., Hausmann, A., Szucsich, N., Caetano-Wyler, S. A. & Haszprunar, G. 2016. DNA barcoding of crickets, katydids and grasshoppers (Orthoptera) from Central Europe with focus on Austria, Germany and Switzerland. *Molecular Ecology Resources* 17: 1037–1053. doi:10.1111/1755-0998.12638
- Hebert, P. D. N., Cywinska, A., Ball, S. L. & de Waard, J. R. 2003a. Biological identification through DNA barcodes. *Proceedings of the Royal Society London B, Biological Sciences* 270: 313–322.
- , Ratnasingham, S. & deWaard, J. R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergence among closely related species. *Proceedings of the Royal Society London B, Biological Sciences*: 96–99. doi:10.1098/rsbl.2003.0025
- , Penton, E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences USA* 101(41): 14812–14817.
- , Zemplak, T. S., Stoeckle, M. Y. & Francis, C. M. 2004b. Identification of birds through DNA barcodes. *Public Library of Science, Biology* 2(10): e312.
- , deWaard, J. R., Zakharov, E. V., Prosser, S. W. J., Sones, J. E., McKeown, J. T. A., Mantle, B. & La Salle, J. 2013. A DNA ‘Barcode Blitz’: rapid digitization and sequencing of a natural history collection. *PLoS One* 8(7): e68535.
- Hendrich, L., Morinière, J., Haszprunar, G., Hebert, P., Hausmann, A., Köhler, F. & Balke, M. 2014. A comprehensive DNA barcode database for Central European beetles: adding more than 3,500 identified species to BOLD. *Molecular Ecology* 15(4): 795–818. doi:10.1111/1755-0998.12354
- Hou, Z. E., Li, Z. & Li, S. Q. 2009. Identifying Chinese species of *Gammarus* (Crustacea: Amphipoda) using DNA barcoding. *Current Zoology* 55(2): 158–164.
- Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J. & Bernatchez, L. 2008. Identifying Canadian fresh water fishes through DNA barcodes. *PLoS One* 3(6): e2490.
- Huemer, P., Mutanen, M., Sefc, K. M. & Hebert, P. D. N. 2014. Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic

- distances have small genetic impacts. *PLoS One* 9(12): e115774.
- Ivanova, N. V., deWaard, J. R. & Hebert, P. D. N. 2006. An inexpensive, automation friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998–1002.
- Janzen, D. H., Hajibabaei, M., Burns, J. M., Hallwachs, W., Remigio, E. & Hebert, P. D. N. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 360(1462): 1835–1845.
- , Hallwachs, W., Blandin, P., Burns, J. M., Cadiou, J., Chacon, I., Dapkey, T., Deans, A. R., Epstein, M. E., Espinoza, B. & Franclemont, J. G. 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources* 9(S1): 1–26.
- Joly, S., Davies, T. J., Archambault, A., Bruneau, A., Derry, A., Kembel, S. W., Peres-Neto, P., Vamosi, J. & Wheeler, T. A. 2014. Ecology in the age of DNA barcoding: the resource, the promise and the challenges ahead. *Molecular Ecology Resources* 14(2): 221–232.
- Jurado-Rivera, J. A., Vogler, A. P., Reid, C. A. M., Petitpierre, E. & Gomez-Zurita, J. 2009. DNA barcoding insect-host plant associations. *Proceedings of the Royal Society B, Biological Sciences* 276(1567): 639–648.
- Kaur, H. & Sharma, K. 2017. COI-based DNA barcoding of some species of Pentatomidae from North India (Hemiptera: Heteroptera). *Mitochondrial DNA Part A* 28(5): 756–761.
- Kerr, C. R., Stoeckle, M. Y., Dove, C. J., Weight, L. A., Francis, C. M. & Hebert, P. D. N. 2007. Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Resources* 7(4): 535–543. doi:10.1111/j.1471-8286.2007.01670.x
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. doi:10.1007/BF01731581
- Kitching, R. L., Orr, A. G., Thalib, L., Mitchell, H., Hopkins, M. S. & Graham, A. W. 2000. Moth assemblages as indicators of environmental quality in remnants of upland Australian rain forest. *Journal of Applied Ecology* 37: 284–297. doi:10.1046/j.1365-2664.2000.00490.x
- Kress, W. J., García-Robledo, C., Uriarte, M. & Erickson, D. L. 2015. DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution* 30(1): 25–35.
- Kumar, N. P., Rajavel, A. R., Natarajan, R. & Jambulingam, P. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* 44(1): 1–7.
- , Srinivasan, R. & Jambulingam, P. 2012. DNA barcoding for identification of sand flies (Diptera: Psychodidae) in India. *Molecular Ecology Resources* 12(3): 414–420.
- Kumar, V., Kundu, S., Chakraborty, R., Sanyal, A., Raha, A., Sanyal, O., Ranjan, R., Pakrashi, A., Tyagi, K. & Chandra, K. 2019. DNA barcoding of Geometridae moths (Insecta: Lepidoptera): a preliminary effort from Namdapha National Park, Eastern Himalaya. *Mitochondrial DNA Part B* 4(1): 309–315.
- Kuzmina, M. L., Johnson, K. L., Barron, H. R. & Hebert, P. D. N. 2012. Identification of the vascular plants of Churchill, Manitoba, using a DNA barcode library. *BMC Ecology* 12(1): 12–25.
- Lakra, W. S., Verma, M. S., Goswami, M., Lal, K. K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K. V., Ward, R. D. & Hebert, P. D. N. 2011. DNA barcoding Indian marine fishes. *Molecular Ecology Resources* 11(1): 60–71.
- Laskar, B. A., Bhattacharjee, M. J., Dhar, B., Mahadani, P., Kundu, S. & Ghosh, S. K. 2013. The species dilemma of northeast Indian mahseer (Actinopterygii: Cyprinidae): DNA barcoding in clarifying the riddle. *PLoS One* 8(1): e53704.
- Luo, A., Lan, H., Ling, C., Zhang, A., Shi, L., Ho, S. Y. & Zhu, C. 2015. A simulation study of sample size for DNA barcoding. *Ecology and Evolution* 5(24): 5869–5879.
- Mani, M. S. 1968 (republished 2013). *Ecology and biogeography of high altitude insects*, Vol. 4. 539 pp., Springer Science & Business Media.
- Matheson, C. D., Müller, G., Junnila, A., Vernon, K., Hausmann, A., Miller, M. A., Greenblatt, C. & Schlein, Y. 2008. A PCR method for detection of plant meals from the gut of insects. *Organisms, Diversity & Evolution* 7(4): 294–303.
- Meinertzhagen, C. R. 1928. Some biological problems connected with the Himalaya. *Ibis* 70: 480–502. doi:10.1111/j.1474-919X.1928.tb08733.x
- Meyer, C. P. & Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology* 3: 2229–2238.
- Miller, M. A., Müller, G. C., Kravchenko, V. D., Junnila, A., Vernon, K. K., Matheson, C. D. & Hausmann, A. 2007. DNA-based identification of Lepidoptera larvae and plant meals from their gut content. *Russian Entomological Journal* 15(4): 427–432.
- Miller, S., Hausmann, A., Hallwachs, W. & Janzen, D. 2016. Advancing taxonomy and bioinventories with DNA barcodes. *Philosophical Transactions of the Royal Society London B* 371(1702): 20150339. doi:10.1098/rstb.2015.0339
- Morinière, J., Hendrich, L., Hausmann, A., Haszprunar, G. & Gruppe, A. 2014. Barcoding Fauna Bavaria: 78 % of the Neuropterida fauna barcoded! *PLoS One* 9(10): e109719.
- Mutanen, M., Hausmann, A., Hebert, P. D. N., Landry, J. F., de Waard, J. R. & Huemer, P. 2012. Allopatry as a Gordian knot for taxonomists: patterns of DNA barcode divergence in arctic-alpine Lepidoptera. *PLoS One* 7(10): e47214.
- , Kivelä, S. M., Vos, R. A., Doorenweerd, C., Ratnasingham, S., Hausmann, A., Huemer, P., Dincă, V., van Nieukerken, E. J., Lopez-Vaamonde, C. & Vila, R. 2016. Species-level para- and polyphyly in

- DNA barcode gene trees: strong operational bias in European Lepidoptera. *Systematic Biology* 65(6): 1024–1040.
- New, T. R. 2004. Moths (Insecta: Lepidoptera) and conservation: background and perspective. *Journal of Insect Conservation* 8:79–94. doi:10.1007/s10841-004-1329-0
- Öunap, E., Javois, J., Viidalepp, J. & Tammaru, T. 2011. Phylogenetic relationships of selected European Ennominae (Lepidoptera: Geometridae). *European Journal of Entomology* 108: 267–273.
- Papadopoulou, A., Bergsten, J., Fujisawa, T., Monaghan, M. T., Barraclough, T. G. & Vogler, A. P. 2008. Speciation and DNA barcodes: testing the effects of dispersal on the formation of discrete sequence clusters. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 363(1506): 2987–2996.
- Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D. & Vogler, A. P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55(4): 595–609.
- Price, T., Zee, J., Jamdar, K. & Jamdar, N. 2003. Bird species diversity along the Himalayas: a comparison of Himachal Pradesh with Kashmir. *Journal of Bombay Natural History Society* 100: 394–409.
- Price, T. D., Mohan, D., Tietze, D. T., Hooper, D. M., Orme, C. D. L. & Rasmussen, P. C. 2011. Determinants of northerly range limits along the Himalayan bird diversity gradient. *The American Naturalist* 178(S1): S97–S108.
- Ratnasingham, S. & Hebert, P. D. N. 2007. BOLD: the barcode of life data system (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364.
- , Hebert, P. D. N. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS One* 8(7): e66213.
- Raupach, M. J., Hendrich, L., Küchler, S. M., Deister, F., Morinière, J. & Gossner, M. M. 2014. Building-up of a DNA barcode library for true bugs (Insecta: Hemiptera: Heteroptera) of Germany reveals taxonomic uncertainties and surprises. *PLoS One* 9(9): e106940.
- Regier, J. C., Zwick, A., Cummings, M. P., Kawahara, A. Y., Cho, S., Weller, S., Roe, A., Baixeras, J., Brown, J. W., Parr, C. & Davis, D. R. 2009. Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BMC Evolutionary Biology* 9(1): 280.
- Sanyal A. K., Uniyal, V. P., Chandra, K. & Bhardwaj, M. 2011. Diversity and indicator species of moth (Lepidoptera: Heterocera) assemblages in different vegetation zones in Gangotri Landscape, Western Himalaya. *ENVIS Bulletin: Arthropods and their Conservation (Insects & Spiders)* 14(1): 116–132.
- , Uniyal, V. P., Chandra, K. & Bhardwaj, M. 2013. Diversity, distribution pattern and seasonal variation in moth assemblages in altitudinal gradient in Gangotri Landscape, Western Himalaya. *Journal of Threatened Taxa* 5(2): 3646–3653.
- , Dey, P., Uniyal, V. P., Chandra, K. & Raha, A. 2017. Geometridae Stephens, 1829 from different altitudes in Western Himalayan protected areas of Uttarakhand, India (Lepidoptera: Geometridae). *SHILAP Revista de Lepidopterología* 45(177): 143–163.
- Schmidt, S., Schmid-Egger, C., Morinière, J., Haszprunar, G., Hebert, P. D. N. 2015. DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea partim). *Molecular Ecology Resources* 15: 985–1000.
- Scoble, M. J., Gaston, K. J. & Crook, A. 1995. Using taxonomic data to estimate species richness in Geometridae. Utilizando datos taxonómicos para estimar la riqueza de especies en Geometridae. *Journal of the Lepidopterists' Society* 49(2): 136–147.
- Sihvonen, P., Mutanen, M., Kaila, L., Brehm, G., Hausmann, A. & Staude, H. S. 2011. Comprehensive molecular sampling yields robust phylogeny for geometrid moths (Lepidoptera: Geometridae). *PLoS One* 6(6): e20356.
- Silva-Brandão, K. L., Lyra, M. L. & Freitas, A. V. 2009. Barcoding Lepidoptera: current situation and perspectives on the usefulness of a contentious technique. *Neotropical Entomology* 38(4): 441–451.
- Smetacek, P. 2008. Moths recorded from different elevations in Nainital District, Kumaon Himalaya, India. *Bionotes* 10: 5–15.
- Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W. & Hebert, P. D. N. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences USA* 103: 3657–3662.
- Sondhi, Y. & Sondhi, S. 2016. A partial checklist of moths (Lepidoptera) of Dehradun, Mussoorie and Devalsari in Garhwal, Uttarakhand, India. *Journal of Threatened Taxa* 8(5): 8756–8776. doi:10.11609/jott.2814.8.5.8756-8776
- Strutzenberger, P., Brehm, G. & Fiedler, K. 2012. DNA barcode sequencing from old type specimens as a tool in taxonomy: a case study in the diverse genus *Eois* (Lepidoptera: Geometridae). *PLoS One* 7(11): e49710.
- Summerville, K. S., Ritter, L. M. & Crist, T. O. 2003a. Forest moth taxa as indicators of Lepidopteran richness and habitat disturbance: preliminary assessment. *Biological Conservation* 116: 9–18.
- , Boulware, M. J., Veech, J. A. & Crist, T. O. 2003b. Spatial variation in species diversity and composition of forest Lepidoptera in eastern deciduous forests of North America. *Conservation Biology* 17: 1045–1057.
- 2004. Functional groups and species replacement: testing for the effects of habitat loss on moth communities. *Journal of the Lepidopterists' Society* 58: 114–117.
- Tautz, D., Arctander, P., Minelli, A., Thomas, R. H. & Vogler, A. P. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18(2): 70–74.

- Veldman, S., Otieno, J., Gravendeel, B., van Andel, T. & de Boer, H. 2014. Conservation of endangered wild harvested medicinal plants: use of DNA barcoding. Pp. 81–88 in: Gurib-Fakim, A. (ed.) Novel plant bioresources: applications in food, medicine and cosmetics. Chichester (Wiley-Blackwell).
- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 360(1462): 1847–1857.
- Warren, W. 1889. On Lepidoptera collected by Major Yerrbury in Western India in 1886 and 1887. *Proceeding of the Scientific Meetings of the Zoological Society, London*: 292–339.
- Webb, J. M., Jacobus, L. M., Funk, D. H., Zhou, X., Kondratieff, B., Geraci, C. J., DeWalt, R. E., Baird, D. J., Richard, B., Phillips, I. & Hebert, P. D. N. 2012. A DNA barcode library for North American Ephemeroptera: progress and prospects. *PLoS One* 7(5): e38063.
- Wiens, J. J. & Penkrot, T. A. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51(1): 69–91.
- Winfree, R., Bartomeus, I., Cariveau, D. P. 2011. Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution and Systematics* 42: 1–22. doi:10.1146/annurev-ecolsys-102710-145042
- Yamamoto, S. & Sota, T. 2007. Phylogeny of the Geometridae and the evolution of winter moths inferred from a simultaneous analysis of mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 44(2): 711–723.
- Yang, Z., Landry, J. F. & Hebert, P. D. 2016. A DNA barcode library for North American Pyraustinae (Lepidoptera: Pyraloidea: Crambidae). *PLoS One* 11(10): e0161449.
- Young, C. J. 2006. Molecular relationships of the Australian Ennominae (Lepidoptera: Geometridae) and implications for the phylogeny of Geometridae from molecular and morphological data. *Zootaxa* 1264: 1–147.
- Zahiri, R., Lafontaine, J. D., Schmidt, B. C., Zakharov, E. V. & Hebert, P. D. N. 2014. A transcontinental challenge – a test of DNA barcode performance for 1,541 species of Canadian Noctuoidea (Lepidoptera). *PLoS One* 9(3): e92797.
- , Lafontaine, J. D., Schmidt, B. C., Zakharov, E. V. & Hebert, P. D. N. 2017. Probing planetary biodiversity with DNA barcodes: The Noctuoidea of North America. *PLoS One* 12(6): e0178548.
- Zenker, M. M., Rougerie, R., Teston, J. A., Laguerre, M., Pie, M. R. & Freitas, A. V. 2016. Fast census of moth diversity in the Neotropics: a comparison of field-assigned morphospecies and DNA barcoding in tiger moths. *PLoS One* 11(2): e0148423.
- Zhang, A. B., He, L. J., Crozier, R. H., Muster, C. & Zhu, C. D. 2010. Estimation of sample sizes for DNA barcoding. *Molecular Phylogenetics and Evolution* 54(3): 1035–1039. doi:10.1016/j.ympev.2009.09.014.
- Zhou, X., Adamowicz, S. J., Jacobus, L. M., DeWalt, R. E. & Hebert, P. D. N. 2009. Towards a comprehensive barcode library for arctic life-Ephemeroptera, Plecoptera, and Trichoptera of Churchill, Manitoba, Canada. *Frontiers in Zoology* 6(1): 6–30.
- , Robinson, J. L., Geraci, C. J., Parker, C. R., Flint Jr, O. S., Etnier, D. A., Ruiter, D., Edward DeWalt, R., Jacobus, L. M. & Hebert, P. D. N. 2011. Accelerated construction of a regional DNA-barcode reference library: caddisflies (Trichoptera) in the Great Smoky Mountains National Park. *Journal of the North American Benthological Society* 30(1): 131–162. doi:10.1899/10-010.1

Appendix

A Neighbour-joining tree based on the Kimura-2-parameter model of all analysed species including the sequences from the materials of the Herbulot collection is provided in the supplementary data (Electronic Supplement S1), details of the specimens from the current study with DNA barcode specimen ID numbers and elevation details are provided as supplementary data (Electronic Supplement S2). Intraspecific variation and distances to nearest neighbours obtained from Barcode Gap Analysis in BOLD of all the 113 species analysed are provided in the supplementary data (Electronic Supplement S3).