

1 DNA barcodes reveal deeply neglected diversity and numerous invasions of micromoths in  
2 Madagascar

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25  
26 **Abstract**  
27 Madagascar is a prime evolutionary hotspot globally, but its unique biodiversity is under threat,  
28 essentially from anthropogenic disturbance. There is a race against time to describe and protect  
29 the Madagascan endangered biota. Here we present a first molecular characterization of the  
30 micromoth fauna of Madagascar. We collected 1572 micromoths mainly using light traps in both  
31 natural and anthropogenically disturbed habitats in 24 localities across eastern and northwest  
32 Madagascar. We also collected 1384 specimens using a Malaise trap in a primary rain forest at  
33 Andasibe. In total, we DNA barcoded 2956 specimens belonging to 1537 Barcode Index  
34 Numbers (BINs), 88.4% of which are new to BOLD. Only 1.7% of new BINs were assigned to  
35 species. Of 47 different families found, Dryadaulidae, Bucculatricidae, Bedelliidae,  
36 Batrachedridae and Blastobasidae are newly reported for Madagascar and the recently recognized  
37 Tonzidae is confirmed. For test faunas of Canada and Australia, 98.9-99.4% of Macroheterocera  
38 BINs exhibited the molecular synapomorphy of a Phenylalanine in the 177<sup>th</sup> complete DNA  
39 barcode codon. Non-macroheteroceran BINs could thus be sifted out efficiently in the Malaise  
40 sample. The Madagascar micromoth fauna shows highest affinity with the Afrotropics (146 BINs  
41 also occur in the African continent). We found 22 recognised pests or invasive species, mostly  
42 occurring in disturbed habitats. Malaise trap samples show high temporal turnover and alpha  
43 diversity with as many as 507 BINs collected; of these, astonishingly, 499 (98.4%) were novel to  
44 BOLD and 292 (57.6%) were singletons. Our results provide a baseline for future surveys across  
45 the island.

46  
47 **Key words**

48 Africa, invasive alien species, Lepidoptera, Malaise trap, plant pests

## 49 **Introduction**

50 Madagascar is one of the top priority global hotspots for biodiversity conservation with high  
51 endemism and under large anthropogenic pressure (Vences et al. 2009). There is an urgent need  
52 to describe what remains of the unique biota of Madagascar so as to locate hotspots of  
53 biodiversity and endemism and protect them. Conservation efforts in Madagascar are mainly  
54 focused on vertebrates (Herrera 2017; Jenkins et al. 2014) and plants (Royal Botanic Gardens  
55 Kew 2016). Arthropods are rarely taken into account in conservation in Madagascar, despite the  
56 fact that many species are micro-endemics at greatest risk of extinction (Danielczak et al. 2017;  
57 Wesener & Rudolf 2017; Wesener et al. 2014).

58 With up to 4900 described species currently listed from Madagascar (Viette 1990; Krüger 2007;  
59 Lees & Minet 2003; Libert 2014; Lees 2016; De Prins & De Prins 2018), the order Lepidoptera  
60 (moths and butterflies) is a significant component of the arthropod biota. Since lepidopterans  
61 have been widely used as bioindicators of habitat disturbance (Kremen 1994; Enkhtur et al. 2017,  
62 Hawes et al. 2009), they could provide a strong signal for conservation efforts and priorities.  
63 Unfortunately, Madagascan Lepidoptera are relatively poorly known, particularly the  
64 'micromoths', a polyphyletic group excluding Macroheterocera and butterflies (Lees et al. 2003)  
65 of about 1600 described species (Viette 1990; De Prins & De Prins 2018), with many species yet  
66 to be described (Lees & Minet 2003). Biodiversity assessment studies rarely take into account  
67 micromoths because of the difficulty in identifying them, for a general lack of taxonomic  
68 expertise, and the need for specialised technical skills for specimen mounting and dissecting. The  
69 use of DNA barcoding, however, has proved an efficient and affordable method to alleviate this  
70 taxonomic impediment. Operational taxonomic units derived from DNA barcodes can accurately  
71 and objectively represent species diversity and then be used to survey micromoth diversity in  
72 poorly known and hyperdiverse areas of the World (Lees et al. 2013; Miller et al. 2016).

73 The Barcode of Life Datasystem (BOLD; [www.boldsystems.org](http://www.boldsystems.org); Ratnasingham and Hebert  
74 2007) now contains over six million DNA barcodes and represents a huge resource to accelerate  
75 identification and quantify biodiversity. However, the coverage for the Madagascan lepidopteran  
76 fauna is very sparse. Nevertheless, the use of Barcode Index Numbers (BINs) (Ratnasingham and  
77 Hebert 2013) as proxies for species allows the assessment of hyperdiverse groups that are  
78 taxonomically poorly known, such as micromoths (Schmidt et al. 2017; Aagaard et al. 2016; Lees  
79 et al. 2013; Lopez-Vaamonde et al. 2012). As of 29th June 2018 (including the current study),  
80 there were 2852 DNA barcode BINs for Madagascar out of a total of 113,161 lepidopteran BINS,  
81 according to a search of the BIN Database in the public portal of BOLD. Nieuwerkerken et al. (2011)  
82 estimated 157,424 described species of Lepidoptera, and the upper bounds for true richness may  
83 be as much as half a million species (Solis and Pogue 1999). Very few of all these BINs  
84 representing Madagascan Lepidoptera are yet publicly identified on BOLD to described species.  
85 As of 30th March 2018 there were only 287 publicly released species names according to the  
86 BIN portal of BOLD, of which only 277 had correctly composed names; 173 represented  
87 Macroheterocera, 77 represented butterflies and only 27 represented micromoths, 24 of which  
88 were Tortricoidea and Pyraloidea. – Furthermore, only 201 of these species had BIN numbers  
89 allocated. Progress in DNA barcoding the described fauna of Madagascan Lepidoptera lags thus  
90 far behind most countries. The first implementation of the Global Malaise Program in  
91 Madagascar (Bio-Inventory and Collections Unit, Biodiversity Institute of Ontario, 2015)  
92 provides a local instance where identification of Lepidoptera samples below Order level is  
93 problematic by external morphology (Lepidoptera wings being poorly preserved) or very time  
94 consuming by individual sequence queries. We asked if a previously observed simple molecular  
95 synapomorphy in the DNA barcode (Lees et al. 2011) was reliable enough to filter out the clade  
96 Macroheterocera from such samples.

97 From a biogeographic point of view, Madagascar has a very unbalanced or disharmonic fauna,  
98 with some taxa overrepresented and some underrepresented relative to the mainland source area  
99 (Briggs 1987). Indeed, the Madagascan fauna is characterised by a significant number of large  
100 endemic radiations such as lemurs and tenrecs now extinct on mainlands (Poux et al. 2005) and a  
101 large number of major continental lineages that appear not to have established at all on the island  
102 (the lack of poritiine lycaenids which are highly diverse in Africa is evident: Lees et al. 2003).  
103 The lepidopteran fauna of Madagascar is, in particular, quite dissimilar to that of southern Africa,  
104 much more so than the relatively more harmonic fauna of the neighbouring island fauna of La  
105 Réunion (Krüger 2007). Southern Africa has twice as many described lepidopteran species  
106 described as Madagascar, while Noctuoidea is overrepresented in Madagascar. By contrast,  
107 “primitive” Lepidoptera (defined as consisting of the non-ditrysian grade of micromoths that  
108 includes groups from Micropterigoidea to Tischerioidea; Krüger 2007), as well as Tineoidea and  
109 Gelechioidea are, in particular, underrepresented in Madagascar. However, these general  
110 faunistic patterns are based on current checklists, which are particularly incomplete for the  
111 Madagascan lepidopteran fauna and also biased towards the best-studied families (for example,  
112 Viette specialized on the noctuid fauna of both Madagascar and La Réunion: Viette, 1963, Viette  
113 1965 and Viette 1967).

114 Finally, many microlepidopteran species are highly invasive and serious pests of agricultural and  
115 ornamental plants (Lopez-Vaamonde et al. 2010). Despite their potential economic and  
116 ecological impact, there is limited information available on invasive insects in Madagascar  
117 (Fisher et al. 1998; Kull et al. 2014; Irwin et al. 2010).

118 The main aims of our study were: 1) to carry out a survey of micromoth diversity using DNA  
119 barcodes across several sites in Madagascar from disturbed to primary rainforests using DNA  
120 barcodes; 2) to identify any molecular synapomorphy(ies) within the DNA barcode fragment that

121 would allow us to more accurately identify samples and to better evaluate sequence queries  
122 where external morphology was problematic; 3) to characterize as far as possible the  
123 biogeographic origins of the Madagascan microlepidopteran (based purely on proximity, a  
124 predominantly African mainland affinity would be expected); 4) to identify the presence of any  
125 cosmopolitan, invasive, agricultural and forestry pest species, which should be more prevalent in  
126 disturbed habitats than in well preserved ones.

127

## 128 **Material and Methods**

### 129 **Specimen collection**

130 Micromoths were collected in non-protected areas by one of us (CW) between October-  
131 November 2013 and March 2015. CW used two to three light towers with 15W ultraviolet  
132 fluorescence actinic tubes ([www.bioform.de](http://www.bioform.de)) operated with lithium batteries (Li-Ion Akku  
133 HELLPOWER 12V/10.5Ah 116.60Wh). Micromoths were sampled from nine collecting sites in  
134 disturbed habitats around the Nosy Be area (northwestern Madagascar) (Table 1). All these  
135 specimens are deposited at the Natural History Museum of Carinthia (Austria).

136 Specimens were also collected largely within protected areas across eastern Madagascar by  
137 another of us (DCL) with 160W blended tungsten/mercury-vapour lamps or 15W actinic lights  
138 (Bioquip) powered with a generator (Honda EX350) (lights suspended on a white sheet with a  
139 protective transparent tarpaulin), sampled in November-December 2011, January-February 2014,  
140 and November 2014. All specimens collected by DCL are deposited at the Natural History  
141 Museum in London.

142 One Townes-style Malaise trap (standard for the Global Malaise Trap Program, Geiger et al.  
143 2016) was set up by another of us (AA) in two sites of PN Andasibe-Mantadia, specifically the  
144 forest originally designated as the Réserve Spéciale d'Analamazaotra (for short, we refer

145 hereafter to this reserve as its current popular name “Andasibe”; it was also popularly known as  
146 Perinet). This is a c. 810-hectare fragment of the once far larger Analamazaotra rainforest (Table  
147 1). One site was sampled during 65 days at the end of the wet to beginning of dry season (from  
148 April 1st until 28th May 2014) (M1) at 1000 m elevation and a second site, 0.8 km away from the  
149 first site at 1050 m, was sampled during 67 days at the end of dry to beginning of wet season  
150 (from September 1st until 6th November 2014) (M2) (elevations adjusted for coordinates in  
151 Table 1 using Google Earth). Each sample was collected in a 500 ml plastic Nalgene bottle that  
152 was filled with 375 ml of 95% ethanol and then attached to the trap head. The catch was  
153 harvested weekly by AA and brought to the University of Antananarivo where the bulk ethanol  
154 was replaced with fresh 95% ethanol before storage at -4°C until samples were drained and sent  
155 to the Centre for Biodiversity Genomics in Canada (CBG; [www.biodiversitygenomics.net](http://www.biodiversitygenomics.net)).

156

### 157 **DNA barcoding**

158 We DNA barcoded in most cases only one specimen per morphospecies for light-trapped and  
159 day-netted specimens. Morphospecies were defined using external morphology, mainly wing  
160 pattern. DNA was extracted using hind legs of pinned specimens or entire body extracts in the  
161 case of smaller Malaise-trapped Lepidoptera. DNA barcodes (658 bp of the COI mitochondrial  
162 gene) were generated using traditional Sanger sequencing at the CBG using standard high-  
163 throughput protocols (Ivanova et al. 2006).

164 Malaise trap samples were also processed at CBG as part of the Global Malaise Program  
165 (<http://biodiversitygenomics.net/projects/gmp/>) following the protocol described in deWaard et  
166 al. (2017), which involves unidirectional sequencing, so those sequences are usually shorter than  
167 658 bp. Larger moths were pinned, smaller ones kept in their original wells. A randomly selected

168 example of each BIN was imaged at Guelph; as usual for the order Lepidoptera captured via this  
169 method, these samples tend not to be in good condition for external morphological analysis.

170 DNA sequences, along with the voucher data, images, and trace files, are deposited in the  
171 Barcode of Life Data Systems (BOLD v4) (Ratnasingham and Hebert 2007,  
172 [www.barcodinglife.org](http://www.barcodinglife.org)), and the sequences were deposited in GenBank. All data are available in  
173 BOLD through the public dataset: DS-MICROMA ([dx.doi.org/10.5883/DS-MICROMA](https://dx.doi.org/10.5883/DS-MICROMA)).

174 To aggregate barcodes of the polyphyletic group micromoths (which includes some larger moths  
175 such as thyridids) from the Malaise trap data set, we asked if a previously noticed molecular  
176 synapomorphy for the clade Macroheterocera (Lees et al. 2011) was reliable enough to partition  
177 out all non-macroheterocerans. To do this we used a test dataset of two well-identified  
178 lepidopteran faunas, namely that of Australia (n=14965 BINs analyzed) and Canada (4684 BINs  
179 analyzed).

180 In the case of the Malaise trap sample, which had been predetermined to Lepidoptera before  
181 sequencing, we first filtered out all Papilionoidea (butterflies), which could be verified by batch  
182 queries on BOLD because all genera and most species had already been DNA barcoded.

183 To determine the number of BINs novel for this study for BOLD, we derived the number of  
184 uniques and non-uniques from the dataset front page “Data Summary”. However, we subtracted  
185 36 BINs that were currently reported as private data to the CBG from the reported list of non-  
186 uniques. These data we inferred to be additional members from the Malaise trap not integral to  
187 our project as was derived from project container GMTAD (Global Malaise Programme  
188 Madagascar Malaise 2014).

189

## 190 **Data analyses**



191 Diversity analyses were carried out on both Malaise and light trap samples. Community analyses  
192 were performed only on Malaise samples from Andasibe since it is the only site for which we  
193 have abundance data.

194 Data analyses were done with R ver.3.4.3 (R Development Core Team 2004) using different  
195 packages for community and species richness analyses.

196 iNEXT (Chao et al., 2014, Hsieh et al. 2016) allowed us to calculate  $\alpha$ -diversity and generate  
197 accumulation curves using 50 resampling replicates with replacement (Chao et al 2014). We used  
198 BINs as species proxy (Ratnasingham and Hebert 2013) and plotted them against both the  
199 sampling coverage (measure of sample completeness that estimates the proportion of the total  
200 number of individuals in a community that belong to the species represented in the sample) and  
201 the total number of caught individuals taken as a measure of sampling intensity. We ran the  
202 analyses for late wet to early dry (M1) and late dry to early wet (M2) seasons, both covered by  
203 the sampling at Andasibe.

204 Abundance Coverage Estimator (ACE) (Gotelli & Colwell 2010) and Chao1 (Chao et al. 2009)  
205 are two other diversity indices that were calculated with the package *Vegan* ver. 2.4-6 (Oksanen  
206 et al. 2016) in order to estimate the potential species richness in accordance with the sampling  
207 intensity.

208 We carried out a distributional data analysis by extracting from BOLD a list of all countries for  
209 which each BIN has been barcoded. Each appearance of a BIN per country was assigned to a  
210 biogeographical region (Afrotropical, Australasian, Nearctic, Neotropical, Oriental and  
211 Palearctic) by looking at the corresponding countries associated to the records in BOLD. Each  
212 BIN was counted only once per region but might be spread over multiple ones.

213

214 **Specimen identification**

215 Specimens were identified by both external morphology (without dissection) and by using DNA  
216 barcode queries using all data present in BOLD. For each sequence we queried, we used the  
217 “Current Database” and the “Search Database” query option “All Barcode Records” on the  
218 Identification Engine of BOLD and then we built a NJ tree in BOLD (=“Tree-based  
219 Identification”) to find the nearest neighbour. Then we searched for the minimum corresponding  
220 p-distance(s) in the list of 99 top hits. We looked in particular for interspecific query tails among  
221 the hit list that seemed informative, i.e. with the hit(s) showing potential signal standing proud of  
222 the noise of background hits (often evident as the sequence Similarity value directly preceding  
223 the sharpest inflexion in the Similarity Score graph before it starts to asymptote), or otherwise  
224 stated 'Non-informative' under Taxonomy Notes. We took particular note when nearest hits  
225 derived from apparent local radiations. We also considered amino acid information, in particular  
226 ignoring Similarity values for irrelevant hits inside or outside of the Macroheterocera (see  
227 below), and also looking qualitatively at unusual codon changes shared between taxa as shown in  
228 Conservation plot mode against a reference sequence in Bioedit v7. In most cases, the sequence  
229 divergence(s), to the nearest identified BINs on BOLD, expressed as 100-Similarity, are noted  
230 under "Taxonomy notes", particularly for records from the BOLD projects MADAM and  
231 MIMAD. In that field, we were often able to specify closely related BIN numbers by building a  
232 corresponding Image database for the Tree Based Identification query. Where relevant hits  
233 existed, we assigned species-level identifications for low pairwise divergences expressed as 100-  
234 Similarity (<approx. 2%) but if not, increasing taxonomic ranks where further hits showed  
235 taxonomic consistency in the NJ tree. Since application of strict thresholds may generally be  
236 misleading particularly for supraspecific ranks, and since no support levels are specified on  
237 BOLD NJ trees, we also used independent ML analyses in PHYML 3.0 (Guindon et al. 2010) to  
238 identify barcoded specimens by examining their phylogenetic position within a clade containing

239 identified individuals, some of which were downloaded from BOLD, at the best justifiable  
240 taxonomic rank. In Phyml 3.0, we used default options except: GTR (or automatic model  
241 selection), all parameters estimated, and SPR. In general, we looked for ABayes support levels  
242  $>0.94$  to assume nestedness within a clade. Identifications from the light-trapped and day-netted  
243 samples run alongside the malaise samples in an ML analysis helped the identifications of  
244 Malaise samples. We specified the identification method(s) or combination thereof (e.g. External  
245 morphology, COI-5P (NJ), COI-5P (ML), COI-5P (codons) i.e. amino-acid based identification)  
246 under the field Identification Method.

247 We compared the 1572 light-trapped moths and day-netted moths with specimens, including  
248 where possible, accessible types, deposited in the two most important reference collections of  
249 Madagascan Lepidoptera, namely the Muséum national d'Histoire naturelle (MNHN, Paris) and  
250 the Natural History Museum (NHMUK, London), and to illustrations in reference works. We  
251 have not attempted an exhaustive type comparison with our specimens and anticipate that more  
252 matches will come to light as the collections are digitised and/or as DNA sequencing of the types  
253 is attempted.

## 255 **Results**

### 256 *DNA barcodes and identification rates*

257 We successfully barcoded 2956 micromoth specimens (1572 light-trapped and day-netted moths  
258 and 1384 micromoths collected with the one Malaise trap) belonging to 1537 BINs (six of 2956  
259 samples do not qualify as full barcodes and so lack BIN numbers). Those 1537 BINs belonged to  
260 44 families as currently classified in BOLD (see Table 2, where 47 family-level groupings are  
261 specified; these include families currently lumped on BOLD). 32.7% of BINs (503 out of 1537  
262 BINs) were identified to genus level and 6.2% of BINs (95 out of 1537 BINs) were identified to

263 species level. Many of those identified BINs correspond to well-known cosmopolitan species  
264 more likely to have been DNA barcoded elsewhere (Table 3).

265 88.4% of BINs (1358 out of 1537 BINs) obtained are new to BOLD and only 179 BINs (13.2%)  
266 were already in the BOLD database.

267  
268 By analysing two barcoded lepidoptera faunas from Canada and Australia we found that almost  
269 all Macroheterocera indeed show a Phenylalanine rather than Leucine or other character state in  
270 the 177<sup>th</sup> complete codon (5'→3') of the (up to) 658 bp nucleotide sequence.

271 For the Australian fauna 4093 BINs (99.4%) exhibiting a Phenylalanine in the 177<sup>th</sup> position  
272 pertain to sequences identified as macroheteroceran families, while 11 exceptions belong to the  
273 genus *Aristeis* (Oecophoridae) and one to another Oecophoridae genus. Five others belong to  
274 Crambidae: Acentropinae, one to Crambinae, two to Lecithoceridae, two to Gelechiidae, two to  
275 Tineidae: Harmacloninae and one to Heliozelidae. Exceptions to the reliability of this  
276 synapomorphy (total n=26, discounting an apparently contaminated Lycaenidae) are not only rare  
277 in general, but phylogenetically also very narrowly represented. Also, true conversely for this  
278 dataset, 99.5% of 9070 BINs exhibiting another state than a Phenylalanine in that position  
279 (usually Leucine) are identified as belonging to non-macroheteroceran families, including those  
280 of butterflies. Of the exceptions (n=48), 21 belong to Oenosandridae and three to Nolidae, while  
281 three of seven Geometridae, three of eight Erebiidae and three of seven to Noctuidae seem  
282 correctly identified (the rest are micromoths from images), while one imaged “Saturniidae” also  
283 represents a micromoth. For the Canadian Lepidoptera fauna (4684 BINs analysed), the presence  
284 of a Phenylalanine in this position is 98.9% reliable as a surrogate for Macroheterocera (99.75%  
285 reliable when excluding Crambidae: Acentropinae and Tineidae: Meessiinae), while presence of  
286 other character states is 99.8% reliable for non-macroheteroceran Lepidoptera (exceptions one

287 geometrid, one nolid and two noctuids). We did not detect any cases of such parallelisms in our  
288 Madagascan dataset, suggesting that the synapomorphy was fully reliable for this fauna, but in  
289 the case of filtering of the malaise sample, Macroheterocera were only represented by 170 BINs  
290 (whereas Papilionoidea by 20 BINs).

291 Within the 507 BINs collected with the Malaise trap, 50 BINs (9.9%) have been identified to  
292 genus and only three BINs (0.59%) have been identified to species level (*Angustalius malacellus*,  
293 *Bradina admixtalis* and *Lobesia aeolopa*). The only other five BINs already on BOLD  
294 were a cosmopterigid *Stilbosis* sp. (BOLD:ABY7721, Kenya), a spilomeline (BOLD:ACT8113,  
295 South Africa), two tortricids and another spilomeline from Ranomafana (*Pandemis* sp.  
296 BOLD:ACO0519; *Olethreutes* sp. BOLD:ACS0054 and *Herpetogramma* sp. BOLD:ACT6691).  
297 All remaining 499 BINs, 270 of which singletons and 109 doubletons, are at present only known  
298 as endemic to Andasibe. Two BINs (BOLD:ACS0229, an *Elachista* and BOLD:ACS1392,  
299 Tineidae: Hieroxestinae) have more than 70 individuals ( $n=75$  and  $91$ , respectively) but even  
300 these abundant taxa have as yet no species name (Fig. 3).

301 A total of 113 BINs were not identified to family level. According to NJ building on BOLD  
302 and/or external morphology of pinned specimens, these 113 BINs were overwhelmingly  
303 dominated by possible or probable Gelechioidea (>77%) which could not be reliably assigned at  
304 present to family. Around 16% may represent tineoids while superfamily was unassigned even  
305 tentatively for 6%. Maximum divergences among all of those unidentified BINs to any other BIN  
306 was no smaller than 14.3%. Over 40% of those unknown BINs were more closely related to one  
307 or more unidentified Madagascan BINs than to BINs outside Madagascar.

308 Only 23 BINs were shared between the samples collected with Malaise (507 BINs) and those  
309 collected with light trapping + netting by day (1053 BINs) (Table 2). Of the non Malaise-trapped  
310 material, approximately 92% were light-trapped and the remainder netted by day, so there was a

311 strong bias towards nocturnal activity. The low number of shared BINs is particularly striking for  
312 tineids, considering the high diversity of this family (95 BINs collected with Malaise and 65  
313 BINs collected by other methods), with only one BIN shared (Table 2).

314 Some groups were much more strongly represented in Malaise samples such as Nepticulidae,  
315 Tineidae, Immidae, Lecithoceridae and Elachistidae. Other families were much better represented  
316 in the mainly light-trapped samples than in the Malaise, notably Gracillariidae, Tortricidae,  
317 Cosmopterigidae, Gelechiidae, Pyralidae, and Crambidae (Table 2).

318  
319 *Taxonomic composition and biogeographical distribution*

320 Fig. 1 shows the difference in distribution of BINs per family between light trap and Malaise trap  
321 samples. The three families with highest number of BINs within the 507 BINs collected with  
322 Malaise traps are: Tineidae (95 BINs, 18.7%), Depressariidae s.l. ('Peleopodidae': Oditinae) (54  
323 BINs, 10.7%), and Lecithoceridae (51 BINs, 10.1%). Within the 1053 BINs collected with light-  
324 traps and netted by day the three most representative families are: Gelechiidae (145 BINs,  
325 13.8%), Crambidae (139 BINs, 13.2%) and Tortricidae (132 BINs, 12.5%).

326 Of the up to 47 different micromoth families found, Dryadaulidae, Bucculatricidae, Bedelliidae,  
327 Batrachedridae and Blastobasidae are newly reported for the island (Table 2). Other families,  
328 namely Micropterigidae, Opostegidae, Tonzidae and Eriocottidae, have been previously reported  
329 from Madagascar, but have no described species there (Krüger 1997; Lees & Minet 2003; Davis  
330 & Stonis 2007; Gibbs 2016; Kobayashi et al. 2018).

331 The analysis revealed that 55 BINs show a widespread distribution over more than one  
332 biogeographical region (Table 3). Out of the 162 BINs shared between Madagascar and other  
333 biogeographical regions, 146 BINs (90.1%) occur in Africa and 105 are found only in the  
334 Afrotropical region. More surprisingly, 49 BINs (30.3%) detected in Madagascar also occur in

335 Australasia, 29 BINs (17.9%) in the Oriental region, 27 BINs (16.7%) in the Palearctic, 18 BINs  
336 (11.1%) in the Neotropics, and 17 BINs (10.5%) in the Nearctic (Fig. 2).

337

### 338 *Invasive and pest species*

339 Of the above 55 BINs that show a widespread distribution occurring outside the Afrotropical  
340 region, at least 40% (22 out of 55) are known to be pests and/or invasive somewhere in their  
341 distribution range, while at least an additional five species occasionally feed on crops or may be  
342 minor pests. At least 50.9% (28 out of 55) are recorded for the first time in Madagascar (Table 3).

343 All widespread BINs are identified to species level except nine. These included a tineid  
344 (BOLD:ACS7592); a glyphipterigid (BOLD:AAY2216) previously barcoded from Australia but  
345 1.7% divergent; a tortricid unidentified to genus (BOLD:ACS7628), one cosmopterigid of the  
346 genus *Gisilia* (BOLD:ACS6187), one *Ascalenia* (BOLD:AAG0134), two crambids of the genus  
347 *Herpetogramma* (BOLD:ACD5135 and BOLD:AAB6841), a gracillariid of the genus  
348 *Stomphastis* (BOLD:AAM6667) with barcodes from Australia (also currently without associated  
349 species name) and a pterophorid of the genus *Stenoptilia* (BOLD:AAD0716) with barcodes from  
350 Africa, Asia and Australia (Table 3).

351 38.2% (21 out of 55 BINs) of widespread BINs belong to the family Crambidae, a family known  
352 for many highly dispersive species (Lopez -Vaamonde et al. 2010).

353

### 354 *Species richness and turnover*

355 The analysis of 1384 microlepidopteran (three of which are without BIN allocations) collected  
356 over 16 weeks of Malaise trapping revealed a total of 507 BINs. Astonishingly, nearly all (499  
357 BINs or 98.4%) were novel to BOLD given an also surprisingly small overlap (4.5% of the  
358 Malaise sample, 2.2% of others and 1.5% of the total sample) with the principally light-trapped

359 samples in this study. Moreover, 57.6% (292 out of the 507 Malaise BINs) are represented by  
360 singletons (i.e. by single individual) in our data set (Fig. 3). The high number of singletons  
361 demonstrates that even 16 weeks and two seasons are entirely inadequate to sample with Malaise  
362 traps most of the species that must be present in the studied area.

363 The Malaise trap automatically collected a total of 335 micromoths (representing 165 BINs) at  
364 the end of wet to beginning of dry season (M1), whereas 1046 individuals (representing 404  
365 BINs) were collected at the end of dry to beginning of wet season (M2).

366 The BINs shared between Malaise trap samples collected at M1 and M2 are only 12.2% (62 BINs  
367 out of 507 BINs) but with many individuals (528 specimens out of 1384 individuals collected,  
368 38.1%). Therefore many of the species collected during the two periods belong to relatively  
369 common species.

370 Fig. 4 shows a clear temporal turnover with a strong relationship between temporal distance of  
371 samples and amount of species overlap. For each site (M1 and M2) taken separately and  
372 compared, a clear decline in sample overlap with temporal distance is evident. The intercept for  
373 M2 is higher than M1, but slopes, p-values, and R-squared values are very similar.

374 Rarefaction curves show that both species diversity and sampling coverage indices were perhaps  
375 surprisingly higher at end of wet towards early dry season (M1) than at the end of the dry towards  
376 early wet season (M2) (Figs. 5a and 5b). They also show that 16 weeks of Malaise sampling is  
377 not nearly enough to capture all the Lepidoptera diversity in the studied area (Fig. 5c). We  
378 collected with one Malaise trap 507 BINs at Andasibe, whereas both non parametric indices,  
379 Chao 1 index and ACE suggest that at least twice as many species could occur in the studied area  
380 (Fig 6).

381

382 **Discussion**



383 *Massive 'Linnean shortfall' of micromoths in Madagascar*

384 The majority of BINs (1358 of 1537) found in our study are new to BOLD and most of them  
385 remain unidentified to species level, as we only recovered 1.7% (23 out of 1358 BINs) of  
386 corresponding taxonomic assignation (and several of these were assigned using external  
387 morphology only since new to BOLD). Remarkably, despite the impressive lepidopteran  
388 coverage on BOLD, we do not know the names of some of the most abundant species in  
389 Madagascan ecosystems, and without comprehensive barcoding of museum collections, it is  
390 difficult to be sure what names may already be available for them.

391 115/507 BINs (22.7%) in the Malaise trap were identified to five families within Tineoidea, one  
392 of which is newly reported for Madagascar (Fig. 1). Among the non-tineoid Ditrysia, exceptional  
393 diversity was found among Gelechioidea identified to families, which with 181 BINs (208  
394 including Gelechioidea *incertae sedis*) form 35.7% (41%) of the entire Malaise sample. Of these,  
395 Lecithoceridae (with 51 BINs by Malaise) was the richest family, while elsewhere in the  
396 Depressariidae assemblage (Depressariinae s.l. on BOLD), the Malaise trap sampled a large  
397 diversity of the local radiation of "Oditinae" (54 BINs) and Oecophoridae only had nine BINs,  
398 most of these in *Metachanda*. This depressariid assemblage (Sohn et al. 2015), not yet adequately  
399 sorted at family level but probably including numerous Peleopodidae, comprise a high proportion  
400 of leaf litter detritivores (this provisional classification, including Gelechioidea *incertae sedis*, as  
401 well as 'Stenomatidae' and Lecithoceridae, is included in Table 2). In the Malaise trap, we also  
402 found eight BINs of Elachistinae (Elachistidae), a leaf mining group reported by Lees & Minet  
403 (2003) but with only one reported (Parenti, 2006) and one undescribed (Lees and Minet 2003:  
404 751) Madagascan species (De Prins and De Prins 2018 duplicate *Pauroptila* in Parametrioninae,  
405 but it is here placed in Cosmopterigidae; see also Koster and van Nieukerken, 2018). In the  
406 Malaise trapped Gelechiidae, Dichomeridinae with 27 BINs clearly form another significant local

407 radiation. The Malaise trap evidently captures diurnal as well as nocturnal species, and the use of  
408 such a passive and stationary sampling method allowed us to recover three families not detected  
409 by light trapping and a much better diversity of some local radiations. The Malaise sampling,  
410 however, was clearly limited in finding 27 rather than 45 families (using the expanded  
411 Depressariidae classification in Table 2), but the light trapping and day netting encompassed a  
412 greater geographic range and number of sampling sites.

413 The large differences in taxonomic composition between the two main collecting methods could  
414 be explained by differing geography, sampling times and human vs malaise collecting bias.  
415 Indeed, each method, of course, has its own inherent strong taxonomic biases, but the Malaise  
416 trap was largely free of human bias and its proportions reflect its passively sampled abundances.  
417 Indeed, Malaise trapping is likely to be the most unbiased method, since we did not control for all  
418 the possible biases (location, time of day, weather conditions, local flora, wind, etc.) that may  
419 have affected our acquired sample composition, in particular for light trapping.

420 The low number of “primitive” Lepidoptera in Madagascar reported by Krüger (2007) is  
421 probably an artefact of insufficient sampling. The only primitive non-ditrysians found in the  
422 Malaise trap samples were Nepticulidae (21 BINs with an additional seven BINs, all at light), a  
423 family with only one described species (*Fomoria scobleella* (Minet, 1990)) in Madagascar. Other  
424 non-ditrysians found by other methods (such as adelids) were hand netted by day (apart from one  
425 *Nemophora* sampled at light). The micropterigids (two BINs), a group also known to enter  
426 Malaise traps, were also hand netted by day by one of us (DCL), but it is likely that the Malaise  
427 trap did not intersect with their particularly narrow flight phenology. It may be that such lineages  
428 as adeloids actually need special sampling techniques and habitat surveys for their detection.  
429 Interestingly, we also noticed the absence of heliozelids, which does not fit field observations of  
430 their leaf-mines in Madagascar, and the fact that one species, *Antispila merinaella* Viette, [1956],

431 has been already described. The diverse primitive moth group Exoporia, that includes the  
432 Hepialidae, has not yet been detected in Madagascar, but are exceptionally depauperate in  
433 tropical Africa (in fact unknown there in tropical rainforests) and so might be really absent in  
434 Madagascar or present in poorly sampled habitats. Targeted samplings, such as plant internal  
435 feeder rearings, leaf mine and gall collections, soil and periphyton layer analysis, as well as  
436 vigorous netting for moths by daytime during peak emergence months synchronized to rains may  
437 increase the probability of discovering such primitive groups on the island.

438 These observations, reinforced by the apparent high seasonal turnover found in our study,  
439 demonstrate the paucity of knowledge regarding Madagascan lepidopteran diversity, notably for  
440 micromoths, and its true taxonomic makeup. They also highlight the lack of progress in  
441 sequencing identified and unidentified museum collections, as well as the extreme shortfall in  
442 documentation of true diversity of the fauna resulting from what remains to be found in the wild  
443 and then described. The actual number of public BINs on BOLD for Madagascan Lepidoptera  
444 (2852 BINs) relative to all described Madagascan species (~4900 species) is about 58.2%. This  
445 may seem like substantial progress, but a high percentage of those BINs are unlikely to  
446 correspond to checklists of Madagascan Lepidoptera. Most micromoths in Madagascar must be  
447 undescribed: the ratio of micromoths to Macroheterocera in the French checklist, for example, is  
448 about 2.1 and if such a ratio were to hold for Madagascar too, where ~ 3000 described  
449 Macroheteroceran species are currently known, one would expect to find at least 6300  
450 micromoths. The paucity of well identified moth DNA barcode clusters highlights the gap in  
451 reference barcode libraries for Madagascar and the urgency of this task in regard to conservation  
452 of this biodiversity hotspot. Indeed, a comprehensive DNA barcoding library of Madagascan  
453 moths is needed, and that could be achieved by rapid digitization and sequencing of specimens  
454 deposited at the two main collections in the Natural History Museums of Paris and London. A

455 good example of how that could be achieved is the DNA barcoding of the Australian National  
456 Insect Collection (Hebert et al. 2013).

457 For micromoths, the numbers of BINs from our study (1537 BINs) equals approximately the  
458 number of non-macroheterocerans in the current list as updated in Table 2 (based on a resolution  
459 of Viette 1990 and De Prins and De Prins 2018). There are around 1510 described species for the  
460 micromoth families detected here [1539 including *Gelechioidea incertae sedis*] out of a tally  
461 today for all micromoth families of 1598 species (Table 2). Ultimately, we would anticipate a  
462 very low overlap between the current checklist of described species and the list of BINs in our  
463 samples, in the hypothetical case that the types in museums were successfully DNA barcoded.  
464 We were able to identify relatively few (162) specimens down to species level (94 species  
465 representing 95 BINs), either using DNA barcode searches on BOLD (for the Malaise trap, just  
466 three species) or for pinned material, by two weeks working in the Paris Museum (MNHN).  
467 Comparative analysis of the external morphology of our pinned material with museum reference  
468 collections suggests that a large percentage of our barcoded material are likely to represent  
469 undescribed species. Moreover, some higher taxa have so few described species that we can be  
470 almost certain (given a likely very high species endemism rate in these groups) that the  
471 undescribed rate is also very high in those groups (for example, only one nepticulid and eight  
472 hieroxestine tineids are described). For the 27 families that show more BINs than described  
473 species (notable among which are Gelechiidae and Tineidae), the number of BINs (889) exceeds  
474 the number of described species (270) by 619; only 15 of these BINs are identified to species. For  
475 the remaining 34 families (with 1298 described species), a minor proportion of their 482 BINs  
476 are likely to intersect greatly with their described species, considering only 79 of those BINs  
477 could be identified, 70 of which represent just four families (Crambidae, Pyralidae, Tortricidae  
478 and Limacodidae). These figures alone allow a range of 45-93% undescribed species, with a

479 tendency towards the upper figure, among the 1371 BINs identified to family (or family-level  
480 grouping). An additional 166 BINs were not even identified to family. It is of paramount  
481 importance to DNA barcode the reference collections deposited in both Paris and London in  
482 particular, using new barcoding technologies (Zuccon et al. 2012) and with a particular focus on  
483 types (Hausmann et al. 2016) in order to more precisely estimate the Linnean shortfall (Cardoso  
484 et al 2011) in Madagascan moths. Micromoths will be more challenging in this respect due to the  
485 need to minimise tissue removal on holotypes, but as an alternative, morphologically linkable  
486 non-primary type material is frequently available. This need for reference libraries from  
487 collection types also echoes the call for the barcoding effort to be extended to local  
488 metabarcoding studies, that all need to be linked into the BOLD system in order to improve  
489 database comprehensiveness (Porter and Hajibabaei 2017).

490

#### 491 *Molecular synapomorphy to identify Macroheterocera*

492 We found that the presence of a Phenylalanine in the 177<sup>th</sup> position of the barcode  
493 fragment is shared by almost all Macroheterocera analysed from the two test data sets. We have  
494 determined that the Phenylalanine state is a shared character state of the clade Macroheterocera  
495 which is very seldom reversed or paralleled within the Lepidoptera. Its precise reliability is hard  
496 to gauge due to the possibility of false positive and false negative identifications, but appears to  
497 be of the order of 99.5%. This molecular synapomorphy allowed us to filter out reliably non-  
498 macroheteroceran barcodes from data sets where external morphology of voucher specimens is  
499 poorly preserved (e.g. Malaise trap samples), although butterflies, which share a Leucine with  
500 most micromoths in the homologous position, need to be independently removed. Use of this  
501 character should be very useful to barcoders of Lepidoptera and also provides a means when

502 using the identification engine to evaluate nearest neighbours (e.g. from irrelevant families) that  
503 are spuriously close to the sequence being queried.

504

505 *Higher taxonomic diversity of micromoths in Madagascar*

506 Our survey detected 38 micromoth families previously recognized for Madagascar (including  
507 confirmation of the newly recognized Tonzidae) and added five new ones (Dryadaulidae,  
508 Bucculatricidae, Bedellidae, Batrachedridae, Blastobasidae). It also added four higher taxa which  
509 may be valid at family level but are currently included on BOLD as subfamilies, respectively, of  
510 Lyonetiidae (s.l.) (Cemiostomidae) and Depressariidae (s.l.) (Ethmiidae, Peleopodidae,  
511 Stenomatidae; *Orygocera* is *incertae sedis* and needs to be excluded from the latter). Seventeen  
512 micromoth families previously listed for Madagascar (Viette, 1990; Lees and Minet, 2003; Sohn,  
513 2015; De Prins and de Prins, 2018) that we did not detect in this survey were Heliozelidae,  
514 Tischeriidae, Lyonetiidae (s. str.), Ypsolophidae, Plutellidae, Dudgeoneidae, Metarbelidae,  
515 Sesiidae, Zygaenidae, Somabrachyidae, Xyloryctidae, Autostichidae, Momphidae,  
516 Coleophoridae, Hyblaeidae, Callidulidae, and Whalleyanidae. Whalleyanidae is included in  
517 Thyrididae and *Boisduvalodes tamatavana* in Limacodinae in De Prins and de Prins, 2018 but  
518 here we follow Lees and Minet 2003 (see p. 758, note 22) in treating the former as a valid family  
519 and the latter as a representative of Somabrachyidae. That brings the Madagascan micromoth  
520 fauna to as much as 64 families. It is not at all surprising that we did not find the other undetected  
521 families since they are essentially diurnal (Heliozelidae, Ethmiidae, Sesiidae, Zygaenidae,  
522 Hyblaeidae, Callidulidae), or are also rare and represented by only one or two described species  
523 (Tischeriidae, Lyonetiidae s.s., Ypsolophidae, Dudgeoneidae, Metarbelidae, Somabrachyidae,  
524 Autostichidae, Momphidae) or occur outside the sampled region (Whalleyanidae). The main

525 surprise is the absence of Sesiidae and Zygaenidae, known to occur in Malaise traps, and  
526 Heliozelidae, mentioned earlier.

527 It is always an exciting possibility that one or more new families could be represented in our  
528 dataset, considering that the 113 unknowns to family level include a number of local still  
529 unidentified radiations and exhibit sometimes striking divergences to any other BIN from  
530 Madagascar or outside it.

531

### 532 *Invasive and pest species*

533 We found 55 species with a widespread distribution range mostly outside the Afrotropical region.  
534 Of those, 28 species appear to be new to Madagascar as they have not been recorded previously  
535 by three works: Viette (1990) checklist, Martiré and Rochat, (2008), and the T@RTS database  
536 (Gilligan et al. 2014). Therefore, it is unknown whether these newly recorded species are  
537 established in the island or represent interceptions of new arrivals.

538 Most of the widespread and the 22 pest species recorded in Table 3 were detected in disturbed  
539 habitats of the Nosy Be area. However, we found some pest species even in primary habitats. For  
540 example, in primary forest in Andringitra we found both *Prays citri* and *Diasemiopsis*  
541 *ramburialis* for the first time although the former is not clearly distinguishable from the  
542 sympatric *P. oleaeoides* Gibeaux, 1985 (B. Heckford, pers. comm.), raising the possibility *P. citri*  
543 has been present there for decades.

544 These results show the impoverishment and homogenization of the micromoth fauna in disturbed  
545 areas and the importance of preserving intact primary forests (Watson et al. 2018). In addition,  
546 they highlight the importance of DNA barcoding as a bio-surveillance tool to facilitate the  
547 identification and detection of plant pests (Frewin et al. 2013).

548

549 *Biodiversity assessments*

550 DNA barcoding has facilitated the use of hyperdiverse groups such as micromoths in biodiversity  
551 studies (Miller et al. 2016). Traditionally, especially in the tropics, micromoths have been largely  
552 ignored in biodiversity assessment. This study adds much motivation to this type of effort,  
553 considering also that they are so straightforward to distinguish with DNA barcodes (e.g., to  
554 separate from very small erebids, or within types of sampling like Malaise trapping, where wing  
555 pattern identification is rendered for the most part impractical). Identifying micromoths to family  
556 level or below, however, still requires a large effort and integrated morphological and molecular  
557 analysis. Here, building a comprehensive DNA barcode reference library on BOLD with as  
558 complete taxonomic information as possible alongside lists of BINs will prove indispensable for  
559 assisting future identification, surveys and comparisons of poorly known faunas such as that of  
560 Madagascar. Hopefully such efforts will stimulate a new wave of species description while time  
561 is left to highlight disappearing forest regions, with slash and burn for hill rice cultivation now  
562 exacerbated by downwards spiralling poverty and the rosewood logging crisis. They will also  
563 assist agriculturalists and horticulturalists to identify threats to plants via documentation of plant  
564 pests and invasive species.

565

566

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592

593 **References**

594 Aagaard, K., Berggren, K., Hebert, P.D.N., Sones, J., McClenaghan, B., and Ekrem, T. 2017.

595 Investigating suburban micromoth diversity using DNA barcoding of Malaise trap samples.

596 *Urban Ecosystems*, **20**(2): 353–361. doi:10.1007/s11252-016-0597-2.

597

598 Baker R.H.A., Eyre D., and Brunel, S. 2013. Matching methods to produce maps for pest risk

599 analysis to resources. *NeoBiota*, **18**: 25–40.

600

601 Barrion, A., Litsinger, J., Medina, E.B., Aguda, R.M., Bandong, J.P., Pantua P.C., et al. 1991.

602 The rice *Cnaphalocrocis* and *Marasmia* (Lepidoptera: Pyralidae) leaf folder complex in the603 Philippines: taxonomy, bionomics and control. *Philippine Entomologist*, **8**: 987–1074.

604

605 Bio-Inventory and Collections Unit, Biodiversity Institute of Ontario. 2015. Global Malaise Trap

606 Program Progress Report 2015. [http://biodiversitygenomics.net/site/wp-](http://biodiversitygenomics.net/site/wp-content/uploads/2015/12/GMP-Progress-Report-2015.pdf)607 [content/uploads/2015/12/GMP-Progress-Report-2015.pdf](http://biodiversitygenomics.net/site/wp-content/uploads/2015/12/GMP-Progress-Report-2015.pdf). Accessed 16 July 2018.

608

609 Briggs, J.C. 1987. *Biogeography and plate tectonics*. Elsevier, Amsterdam 203 pp.

610

611 Brown, J., Copeland, R., Aarvik, L., Miller, S., Rosati, M.E., and Luke, Q. 2014. Host Records

612 for fruit-feeding Afrotropical Tortricidae (Lepidoptera). *African Entomology*, **22**: 343–376.

613

- 614 Buthelezi, N.M., Conlong, D.E., and Zharare, G.E. 2013. A comparison of the infestation of  
615 *Aproaerema simplexella* (Walker) on groundnut and other known hosts for *Aproaerema*  
616 *modicella* (Deventer) (Lepidoptera: Gelechiidae). African Entomology, **21**(2): 183–195 .  
617
- 618 Cardoso, P., Erwin, T.L., Borges, P.A.V., and New, T.R. 2011. The seven impediments in  
619 invertebrate conservation and how to overcome them. Biological Conservation, **144**(11): 2647–  
620 2655. doi: 10.1016/j.biocon.2011.07.024.  
621
- 622 Chao A., Colwell R.K., Lin C.W., and Gotelli N.J. 2009. Sufficient sampling for asymptotic  
623 minimum species richness estimators. Ecology, **90**: 1125–1133.  
624
- 625 Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., et al. 2014.  
626 Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in  
627 species diversity studies. Ecological Monographs, **84**: 45–67. doi: [https://doi.org/10.1890/13-](https://doi.org/10.1890/13-0133.1)  
628 0133.1.  
629
- 630 Danielczak, A., Devriese, H., and Hochkirch, A. 2017. *Agkistropleuron simplex*.  
631 <http://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T103896223A103900575.en> Accessed  
632 31/03/2018.  
633
- 634 Davis, D.R., and Stonis, J.R. 2007. A revision of the new world plant-mining moths of the family  
635 Opostegidae. Smithsonian Contributions to Zoology, 625: 1–212.  
636 doi:<https://doi.org/10.5479/si.00810282.625>.  
637

638 De Prins, J., and De Prins, W. 2018. Afromoths, online database of Afrotropical moth species  
639 (Lepidoptera). World Wide Web electronic publication  
640 ([www.afromoths.net](http://www.afromoths.net)<<http://www.afromoths.net>>) [7-14 May, 2018].

641  
642 deWaard, J.R., Levesque-Beaudin, V., deWaard, S.L., Ivanova, N.V., McKeown, J.T.A., Miskie,  
643 R., et al. 2017. Expedited assessment of terrestrial arthropod diversity by coupling malaise traps  
644 with DNA barcoding. bioRxiv doi:<http://dx.doi.org/10.1101/192732>.

645  
646 Evans, D.E. 1970. The parasites of three Kenya coffee tortricids (Lepidoptera: Tortricidae).  
647 Journal of the Entomological Society of South Africa, **33**(2): 349–350.

648  
649 Enkhtur, K., Pfeiffer, M., Lkhagva, A., and Boldgiv, B. 2017. Response of moths (Lepidoptera:  
650 Heterocera) to livestock grazing in Mongolian rangelands. Ecological Indicators, 72 : 667–674.  
651 doi:<https://doi.org/10.1016/j.ecolind.2016.08.053>.

652  
653 Favetti, B.M., Catoia, B., Thais G. G., and Bueno, C.O.F. 2018. Population dynamics of *Omiodes*  
654 *indicata* (Fabricius) (Lepidoptera: Pyralidae) on Soybean in Brazil. Journal of Agricultural  
655 Science, **10**(1). DOI: 10.5539/jas.v10n1p245

656  
657 Fisher, B.L., Ratsirarson, H., and Razafimandimby, S. 1998. Inventaire biologique de la forêt  
658 littorale de Tampolo (Fenoarivo Atsinanana). In Les Fourmis (Hymenoptera: Formicidae). Edited  
659 by J.Ratsirarson, and S.M. Goodman. CIDST, Antananarivo. Ppp. 107–131.

660

- 661 Frewin, A. Scott-Dupree, C., and Hanner, R. 2013. DNA barcoding for plant protection:  
662 applications and summary of available data for arthropod pests. *CAB Reviews*, **8**(18) : 1–13.  
663 doi:10.1079/PAVSNNR20138018.
- 664
- 665 Geiger, M. F., Moriniere, J., Hausmann, A., Haszprunar, G., Wagele, W., Hebert, P. D., et al.  
666 2016. Testing the Global Malaise Trap Program - how well does the current barcode reference  
667 library identify flying insects in Germany? *Biodiversity Data Journal*, **4**: e10671.
- 668
- 669 Gibbs, G.W. 2016 *Ghosts of Gondwana*. Revised Edition. Potton and Burton, New Zealand. 367  
670 pp.
- 671
- 672 Gilligan, T.M., Baixeras, J., Brown, J.W., and Tuck, K.R. 2014. T@RTS: Online World  
673 Catalogue of the Tortricidae (Ver. 3.0). <<http://www.tortricid.net/catalogue.asp>>. Accessed  
674 31/03/2018.
- 675
- 676 Gotelli, N.J., and Colwell, R.K. 2010. Estimating species richness. *In* *Frontiers in Measuring*  
677 *Biodiversity*. Edited by A.E. Magurran and B.J. McGill., Oxford University Press, New York. Pp.  
678 39–54.
- 679
- 680 Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., and Gascuel O. 2010. New  
681 algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the  
682 performance of PhyML 3.0. *Systematic Biology*, **59**(3): 307–321.
- 683

- 684 Hausmann, A., Miller, S.E., Holloway, J.D., deWaard, J., Pollock, D., Prosser, S., et al. 2016.  
685 Calibrating the taxonomy of a megadiverse insect family: 3000 DNA barcodes from geometrid  
686 type specimens (Lepidoptera, Geometridae). *Genome*, **59**(9): 671–684.  
687 doi:<http://dx.doi.org/10.1139/gen-2015-0197>.  
688
- 689 Hayden, J.E., and Buss, L.J. 2013. Olive Shootworm, *Palpita persimilis* Munroe (Insecta:  
690 Lepidoptera: Crambidae). Document EENY556, Department of Entomology and Nematology  
691 series. <https://edis.ifas.ufl.edu/pdffiles/IN/IN99500.pdf>  
692
- 693 Hawes, J., da Silva Motta, C., Overal, W.L., Barlow, J., Gardner, T.A., and Peres, C.A. 2009.  
694 *Journal of Tropical Ecology*, **25**(3) : 281–300. doi:<https://doi.org/10.1017/S0266467409006038>.  
695
- 696 Hebert, P.D.N., deWaard, J.R., Zakharov, E.V., Prosser, S.W.J., Sones, J.E., McKeown, J.T.A.,  
697 et al. 2013. A DNA ‘Barcode Blitz’: rapid digitization and sequencing of a natural history  
698 collection. *PLoS ONE*, **8**(7): e68535. doi:10.1371/journal.pone.0068535  
699
- 700 Heckford, R.J. 2004. *Anatrachyntis simplex* (Walsingham, 1891) (Lepidoptera:  
701 Cosmopterigidae), an adventive species new to the British Isles and a larval description.  
702 *Entomologist’s Gazette*, **55**: 95–101.  
703
- 704 Herrera, J.P. 2017. Prioritizing protected areas in Madagascar for lemur diversity using a  
705 multidimensional perspective. *Biological Conservation*, **207**: 1–8. doi:  
706 <https://doi.org/10.1016/j.biocon.2016.12.028>.  
707

- 708 Hsieh, T.C., Ma, K.H., and Chao, A. 2016. iNEXT: An R package for interpolation and  
709 extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, **7**(12) :  
710 1451–1456. doi:<https://doi.org/10.1111/2041-210X.12613>.  
711
- 712 Irwin, M.T., Wright, P.C., Birkinshaw, C., Fisher, B.L., Gardner, C.J., Glos, J., et al. 2010.  
713 Patterns of species change in anthropogenically disturbed forests of Madagascar. *Biological*  
714 *Conservation*, **143**(10) : 2351–2362. doi:[10.1016/j.biocon.2010.01.023](https://doi.org/10.1016/j.biocon.2010.01.023).  
715
- 716 Ivanova, N.V., deWaard, J.R., and Hebert, P.D.N. 2006. An inexpensive, automation- friendly  
717 protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**(4) : 998–1002. doi :  
718 <https://doi.org/10.1111/j.1471-8286.2006.01428.x>.  
719
- 720 Jamieson, L.E., Suckling, D.M., and Ramankutty, P. 2008. Mass Trapping of *Prays nephelomima*  
721 (Lepidoptera: Yponomeutidae) in *Citrus* orchards: optimizing trap design and density. *Journal of*  
722 *Economic Entomology*, **101**(4): 1295–1301. <https://doi.org/10.1093>.  
723
- 724 Jenkins, R.K.B., Tognelli, M.F., Bowles, P., Cox, N., Brown, J.L., Chan, L., et al. 2014.  
725 Extinction risks and the conservation of Madagascar’s reptiles. *PLoS ONE*, **9**(8). doi:e100173.  
726 doi:[10.1371/journal.pone.0100173](https://doi.org/10.1371/journal.pone.0100173).  
727
- 728 Kremen, C. 1994. Biological inventory using target taxa: a case study of the butterflies of  
729 Madagascar. *Ecological Applications*, **4**(3) : 407–422. <https://doi.org/10.2307/1941946>.  
730

- 731 Kobayashi, S., Matsuoka, H., Kimura, M., Sohn, J-C., Yoshiyasu, Y., and Lees, D.C. 2018.  
 732 Designation of a new family group name, Tonzidae fam. nov., for the genus *Tonza* (Lepidoptera,  
 733 Yponomeutoidea), based on immature stages of *Tonza citrorrhoa*. European Journal of  
 734 Taxonomy, **443** : 1–32. <https://doi.org/10.5852/ejt.2018.443>  
 735
- 736 Koster, J.C.(S.), and van Nieukerken, E.J. 2018. *Gielisella* gen. n., a new genus and two new  
 737 species from southern Spain (Lepidoptera: Elachistidae: Parametriotinae) with a catalogue of  
 738 parametriotine genera. Nota Lepidopterologica, 40 (20): 163–202.  
 739 <https://doi.org/10.3897/nl.40.14528>  
 740
- 741 Krüger, M. 2007. Composition and origin of the Lepidoptera faunas of southern Africa,  
 742 Madagascar and Réunion (Insecta: Lepidoptera). Annals of the Transvaal Museum, **44**(1): 123–  
 743 178.  
 744
- 745 Kull, C., Tassin, J., and Carrière, S.M., 2014. Approaching invasive species in Madagascar.  
 746 Madagascar Conservation and Development, **9**(2) : 60–70.  
 747 doi:<http://dx.doi.org/10.4314/mcd.v9i2.2>.  
 748
- 749 Kwadha, C.A, Ong'amo, G.O., Ndegwa, P.N., Raina, S.K., and Fombong, A.T. 2017. The  
 750 biology and control of the Greater Wax Moth, *Galleria mellonella*. Insects, **8**(2): E61. doi:  
 751 [10.3390/insects8020061](https://doi.org/10.3390/insects8020061).  
 752
- 753 Lees, D.C., Kremen, C., and Raharitsimba, H., 2003. Classification, diversity and endemism of  
 754 the butterflies (Papilionoidea and Hesperioidea): a revised species checklist. *In* The Natural



- 755 History of Madagascar. *Edited by* S.M. Goodman, S.M. and J.P. Benstead), , University of  
756 Chicago Press, Chicago. pp. 762–793.
- 757
- 758 Lees, D.C., and Minet, J., 2003. Lepidoptera: systematics and diversity. *In* The Natural History of  
759 Madagascar. *Edited by* S.M. Goodman, S.M. and J.P. Benstead). University of Chicago Press,  
760 Chicago. pp. 748–761.and
- 761
- 762 Lees, D.C., Ohshima, I., Kawakita, A., Kawahara A., Rougerie, R., Adamski, D., et al. 2011.  
763 Rapid inventory via DNA barcoding: cross-lepidopteran diversity survey of Nouragues  
764 inselberg, French Guiana. Published abstract, XVIIth European Congress of Lepidopterology,  
765 Luxembourg, 9-14.V.2011.
- 766
- 767 Lees, D.C., Kawahara, A. Y., Bouteleux, O., Ohshima, I., Kawakita, A., Rougerie, R., et al. 2013.  
768 DNA barcoding reveals a largely unknown fauna of Gracillariidae leaf-mining moths in the  
769 Neotropics. *Molecular Ecology Resources*, **14**(2): 286–296. doi: 10.1111/1755-0998.12178
- 770
- 771 Lees, D.C. 2016. *Heteropsis* (Nymphalidae: Satyrinae: Satyrini: Mycalesina): 19 new species  
772 from Madagascar and interim revision. *Zootaxa*, **4118** : 1–97.
- 773
- 774 Libert, M. 2014. Sur la taxonomie du genre *Celaenorrhinus* Hübner en Afrique. Rouen : M.  
775 Libert. 272 pp. 26 col plates, 54 text figs, 53 maps.
- 776
- 777 Lopez-Vaamonde, C., Agassiz, D.V.L., Augustin, S., De Prins, J., De Prins, W., Gomboc, S., et  
778 al. 2010. Lepidoptera. Chapter 11. *In* Alien terrestrial arthropods of Europe. (*Edited by* A.

- 779 Roques, M. Kenis, D. Lees, C. Lopez-Vaamonde, W. Rabitsch, J.Y. Rasplus and D.B. Roy. :  
780 Pensoft, Sofia. pp. 603–668.  
781
- 782 Lopez-Vaamonde, C., Bremn, F., Lees, D.C., van Houdt, J., and de Prins, J. 2012. Analysis of  
783 tissue dependent DNA yield for optimal sampling of micro-moths in large-scale biodiversity  
784 surveys. *European Journal of Entomology*, **109**(1): 1–6. <https://doi.org/10.14411/eje.2012.001>.  
785
- 786 Martiré, D., and Rochat, J. 2008. Les papillons de la Réunion et leurs chenilles. Mèze, France,  
787 Biotope, 496 pp.  
788
- 789 Miller, S.E., Hausmann, A., Hallwachs, W., and Janzen, D.H. 2016. Advancing taxonomy  
790 and bioinventories with DNA barcodes. *Philosophical Transactions of the Royal Society B*,  
791 **371**(1702) : 20150339. doi:<https://doi.org/10.1098/rstb.2015.0339>.  
792
- 793 Morland, G. 2015. The morphology and ecology of the Carob moth (*Ectomyelois ceratoniae*)  
794 (Zeller) in citrus orchards of the Western Cape, South Africa. MSc. Thesis, Stellenbosch  
795 University. 122 pp.  
796
- 797 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., et al. 2016.  
798 Package ‘vegan’. Available at <https://cran.r-project.org>.  
799
- 800 Parenti, U. 2006. *Elachista bassii* Parenti, a new species of Elachistidae from Nepal and  
801 *Elachista crocogastra* Meyrick in Madagascar (Lepidoptera: Elachistidae). *SHILAP Revista de*  
802 *Lepidopterologia*, **34**: 141-144.

- 803
- 804 Paulian, R., and Viette, P. 1955. Essai d'un catalogue biologique des Lepidoptères  
805 Heterocères de Tananarive. Mémoires de l'Institut Scientifique de Madagascar, **6**(E): 141–281.  
806
- 807 Porter, T.M., and Hajibabaei M. 2017. Automated High Throughput Animal DNA Metabarcoding  
808 Classification. bioRxiv. Springer US, 219675. doi :<https://doi.org/10.1101/219675>.  
809
- 810 Poux, C., Madsen, O., Marquard, E., Vieites, D.R., de Jong, W.W., and Vences, M. 2005.  
811 Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs,  
812 carnivores, and rodents as inferred from nuclear genes. *Systematic Biology*, **54**(5) : 719–730.  
813 doi:[10.1080/10635150500234534](https://doi.org/10.1080/10635150500234534).  
814
- 815 R Development Core Team. 2004. R: a language and environment for statistical computing.  
816 Foundation for Statistical Computing, Vienna. Available at: <http://www.r-project.org>.  
817
- 818 Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: the barcode of life data system  
819 (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, **7**:355–364.  
820
- 821 Ratnasingham, S., and Hebert, P.D.N. 2013. A DNA-based registry for all animal species: the  
822 Barcode Index Number (BIN) system. *PLoS ONE*, **8**(7).  
823 doi:<https://doi.org/10.1371/journal.pone.0066213>.  
824
- 825 Regier, J.C., Mitter, C., Davis, D.R., Harrison, T.L., Sohn J.-C., Cummings, M.P., et al. 2014. A  
826 molecular phylogeny and revised classification for the oldest ditrysian moth lineages

- 827 (Lepidoptera: Tineoidea), with implications for ancestral feeding habits of the mega-diverse  
 828 Ditrysia. *Systematic Entomology*, **40**(2) : 409–432.  
 829
- 830 Shankara Murthy, M., and Nagaraj, S. K. 2014. Outbreak of maize leaf roller, *Cnaphalocrocis*  
 831 *trapezalis* Guenee (= *Marasmia trapezalis*) (Crambidae: Lepidoptera) on sorghum in Karnataka,  
 832 south India. *Insect Environment*, **19**(4): 250–252  
 833
- 834 Schmidt, O., Hausmann, A., Cancian de Araujo, B., Sutrisno, H., Peggie, D., and Schmidt, S.  
 835 2017. A streamlined collecting and preparation protocol for DNA barcoding of Lepidoptera as  
 836 part of large-scale rapid biodiversity assessment projects, exemplified by the Indonesian  
 837 Biodiversity Discovery and Information System (IndoBioSys). *Biodiversity Data Journal*, **5**:  
 838 e20006. doi:<https://doi.org/10.3897/BDJ.5.e2000>.  
 839
- 840 Sharma, H.C., Saxena, K.B., and Bhagwat, V.R. 1999. The legume pod borer, *Maruca vitrata*:  
 841 bionomics and management. Information Bulletin no. 55 Patancheru 502 324, Andhra Pradesh,  
 842 India: International Crops Research Institute for the Semi-Arid Tropics. 42 pp.  
 843
- 844 Shimizu K. 2000. The biology of the cotton caterpillar (*Diaphania indica*) and the resistance to  
 845 insecticides. *Plant Protection*, **54**: 97–103.  
 846
- 847 Silva, E.B., and Mexia, A. 1999. The pest complex *Cryptoblabes gnidiella* (Millière)  
 848 (Lepidoptera: Pyralidae) and *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) on sweet  
 849 orange groves (*Citrus sinensis* (L.) Osbeck) in Portugal: interspecific association. *Boletín de*  
 850 *sanidad vegetal Plagas*, **25**: 89–98.

- 851
- 852 Solis, M.A., and Pogue, M.G. 1999. Lepidopteran biodiversity: patterns and estimators.  
853 American Entomologist, **45**(4) : 206–212. doi:<https://doi.org/10.1093/ae/45.4.206>.
- 854
- 855 Sohn J.-C., Regier, J.C., Mitter, C., Davis, D., Landry, J.-F., Zwick, A., et al. 2013. A molecular  
856 phylogeny for Yponomeutoidea (Insecta, Lepidoptera, Ditrysia) and its implications for  
857 classification, biogeography and the evolution of host plant use. PLoS ONE, **8**(1): e55066.  
858 <https://doi.org/10.1371/journal.pone.0055066>
- 859
- 860 Sohn, J.-C. 2015. A taxonomic review of the genus *Phrealcia* Chrétien, 1900 with description of  
861 a new species from Korea (Lepidoptera: Ypsolophidae). SHILAP Revista de Lepidopterología,  
862 **43**: 385–423.
- 863
- 864 Sohn, J.-C., Regier, T.C., Mitter, C., Adamski, D., Landry, J.-F., Heikkilä, M., et al. 2015.  
865 Phylogeny and feeding trait evolution of the mega-diverse Gelechioidea (Lepidoptera:  
866 Obtectomera): new insight from 19 nuclear genes. Systematic Entomology, **41**(1) : 112–132.  
867 doi:<https://doi.org/10.1111/syen.12143>.
- 868
- 869 van Nieukerken, E.J., Kaila, L., Kitching, I.J., Kristensen, N.P., Lees, D.C., Minet, J., et al. 2011.  
870 Order Lepidoptera Linnaeus, 1758. Zootaxa **3148**: 212–221.
- 871
- 872 Vences, M., Wollenberg, K. C., Vieites, D.R., and Lees, D.C. 2009. Madagascar as a model  
873 region of species diversification. Trends in Ecology and Evolution, **24**(8): 456–465.  
874 doi:[10.1016/j.tree.2009.03.011](https://doi.org/10.1016/j.tree.2009.03.011).

- 875
- 876 Van Den Berg, H. Shepard, B.M., and Nasikin, 2010. Damage incidence by *Etiella zinckenella* in  
877 soybean in East Java, Indonesia. *International Journal of Pest Management*, **44**(3): 153–159.
- 878
- 879 Viette, P. 1963. Noctuelles trifides de Madagascar, écologie, biogéographie, morphologie et  
880 taxonomie (lep.). *Annales de la Société entomologique de France*, **131** (1): i–vi, 1–294 + pls 1–  
881 10.
- 882
- 883 Viette, P. 1965. Insectes. Lépidoptères Noctuidae Amphipyriinae (part.). *Faune de Madagascar*,  
884 **20**(1): i–vi, 295–490 + pls 11–12.
- 885
- 886 Viette, P. 1967. Insectes. Lépidoptères Noctuidae Amphipyriinae (part.) et Melicleptriinae. *Faune*  
887 *de Madagascar*, **20** (2): i–iv, 491–825 + pls 13–14.
- 888
- 889 Viette, P. 1990. Liste récapitulative des Lépidoptères Hétérocères de Madagascar (A provisional  
890 check-list of the Lepidoptera Heterocera of Madagascar). *Faune de Madagascar Suppl. 1*: 1–  
891 264.
- 892
- 893 Watson, J.E.M., Evans, T., Venter, O., Williams, B., Tulloch, A., Stewart, C., et al. 2018. The  
894 exceptional value of intact forest ecosystems. *Nature Ecology and Evolution*, **2**: 599–610.  
895 doi:<https://doi.org/10.1038/s41559-018-0490-x>.
- 896

- 897 Wesener, T., and Rudolf, E. 2017. *Sphaeromimus splendidus*.  
898 <<http://dx.doi.org/10.2305/IUCN.UK.2017-1.RLTS.T65526540A65527790.en>> 2017. Accessed  
899 31/03/2018.  
900
- 901 Wesener, T., Le, D.M.T., and Loria, S.F. 2014. Integrative revision of the giant pill-millipede  
902 genus *Sphaeromimus* from Madagascar, with the description of seven new species (Diplopoda,  
903 Sphaerotheriida, Arthrosphaeridae). *ZooKeys*, **414**: 67–107.  
904 doi:<https://doi.org/10.3897/zookeys.414.7730>.  
905
- 906 Yamada, H. 1979. Bionomics of the perilla leaf roller, *Pyrausta phoenicealis* Hübner. Annual  
907 Report of The Kansai Plant Protection Society, **21**: 8–11. DOI: 10.4165/kapps1958.21.0\_8  
908
- 909 Yen, S.H. 2014. Insecta: Lepidoptera, Crambidae, Acentropinae. *In* Freshwater Invertebrates of  
910 the Malaysian Region. *Edited by* C.M. Yule, C.M and H.S. Yong Academy of Sciences,  
911 Malaysia. pp. 545–554.  
912
- 913 Zharare, G.W. 2013. A Comparison of the infestation of *Aproaerema simplexella* (Walker) on  
914 Groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera:  
915 Gelechiidae). *African Entomology*, **21**(2):183–195.  
916
- 917 Zuccon D., Brisset J., Corbari L., Puillandre N., Utge J., and Samadi S. 2012. An optimised  
918 protocol for barcoding museum collections of decapod crustaceans: a case-study for a 10-40-  
919 years-old collection. *Invertebrate Systematics*, **26**(6):592–600.  
920 doi:<https://doi.org/10.1071/IS12027>.

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923

924 **Figure Legends**

925

926 **Figure 1.** Distribution of BINs over 47 different families collected with light traps or day-netted  
927 at 24 sites across Madagascar (light grey) and Malaise trap at Andasibe (dark grey). Unknown  
928 refers to individuals that could not be identified at family level. See text regarding *Depressariidae*  
929 s.l. (*Gelechioidea* i.s. are only those currently under *Depressariidae* in BOLD that do not fit in the  
930 expanded categories).

931

932 **Figure 2.** Distribution of BINs over biogeographic regions. Notice one BIN can appear in several  
933 biogeographic regions.

934

935 **Figure 3.** Abundance data for the 507 BINs detected in the Malaise trap samples. Notice that  
936 57.6% (292 out of 507) BINs are singletons. Two BINs (BOLD:ACS0229, *Elachista* and  
937 BOLD:ACS1392, *Tineidae: Hieroxestinae*, exemplars illustrated left to right) have more than 70  
938 individuals but no species name.

939

940 **Figure 4.** Shared species decay plot for Malaise trap samples collected at two sites in Andasibe:  
941 Malaise trap 1 (sampled from April 1st until 28th May 2014) in black; Malaise trap 2 (sampled  
942 from September 1st until 6th November 2014) in red.

943

944 **Figure 5.** Accumulation curves for Malaise trap samples over the two periods corresponding to  
945 end of the wet to beginning of dry season (M1) and end of dry to beginning of wet season (M2),

946 with: **A.** Species diversity (BINs) per number of individuals. **B.** Sample coverage per number of  
947 individuals. **C.** Species diversity (BINs) per sample coverage.

948

949 **Figure 6.** Species richness observed and estimated, based both on Chao1 and ACE analyses for  
950 the sites sampled with a Malaise trap at Andasibe.

951

953 Table 1. Study Sites.

<b>Locality</b>	<b>Region</b>	<b>Habitat</b>	<b>Latitude &amp; Longitude (decimal degrees)</b>	<b>Elevation (m)</b>
Ambilobe	Mainland	Scrubland	-13.108 to -13.163, 49.097	25-40
Mont Passot	Nosy Be	Degraded forest	-13.282, 48.259	25
Ambaro	Nosy Be	Degraded forest	-13.31, 48.187	20
Dzamandzar	Nosy Be	open fields	-13.333, 48.196	25
Fascene	Nosy Be	Open field surrounded by degraded forest	-13.344, 48.299	75
Hell-Ville	Nosy Be	Degraded forest	-13.367, 48.283	15
Lac Ampobilava	Nosy Be	Degraded forest	-13.395, 48.241	40
Lac Djabala	Nosy Be	Degraded forest	-13.386, 48.244	40
Ambanoro	Nosy Be	Degraded forest	-13.389, 48.3	75
Ambondro	Nosy Be	Gardens	-13.382, 48.197	10
Ambanja	Mainland	Scrubland	-13.701, 48.464	40

Manongarivo Réserve Spéciale, Antsatrotro Mt	Mainland	Protected forest	-14.082, 48.366	1235
Marojejy National Park	Mainland	Protected forest	-14.433, 49.761 -14.44, 49.74	700 1540
Anjanaharibe Sud Réserve Spéciale, below Anjividibe summit	Mainland	Protected forest above dry stream bed	-14.739, 49.462	1540
Anjanaharibe Sud Réserve Spéciale, Indri Camp	Mainland	protected forest	-14.741, 49.497	960
Anjanaharibe Sud Réserve Spéciale	Mainland	Protected forest	-14.743, 49.464	1450
Anjozorobe Mananara Lodge	Mainland	Degraded forest	18.436, 47.942	1300
Feo-ny-ala, Andasibe	Mainland	Hotel near protected forest	-18.947, 48.419	945
Mantadia National Park, Belakato trail	Mainland	Protected forest	-18.82, 48.436	1000
Andasibe, Malaise trap M1	Mainland	Protected forest	-18.9484, 48.4256	1000
Andasibe, Malaise trap M2	Mainland	Protected forest	-18.9438, 48.4316	1050
Ankazomivady	Mainland	Degraded high	-20.778, 47.178	1710

		plateau forest	-20.7948, 47.1773	1830
Andringitra National Park, camp	Mainland	Protected forest	-22.147, 46.946	1570
Andringitra National Park	Mainland	Protected forest	-22.1504, 46.9487	1625
Sahavondronina, 7 km W Vohiparara, Community forest at Ranomafana National Park	Mainland	Open field surrounded by protected forest	-21.278, 47.331	1230

954

956 Table 2. Number of BINs per family and method of collection (Malaise, light trapping and  
 957 netting by day). Families are ordered systematically and those recorded for the first time for  
 958 Madagascar are highlighted in bold. All micromoth families are shown for completeness of the  
 959 total microlepidopteran count (species included in Viette 1990 that are not synonyms of other  
 960 species, although not listed for Madagascar in Afromoths, have been included in the count).  
 961 Some other amendments have been made such as there is only one Elachistidae described from  
 962 Madagascar (Parenti, 2006; Koster & van Nieukerken, 2017). See text regarding Depressariidae  
 963 s.l. Families largely ordered following Nieukerken et al. 2011, Regier et al. (2014) for Tineoidea  
 964 and Sohn et al. (2013, 2015) for Yponomeutoidea and Gelechioidea. \*Placement in this family  
 965 based entirely on COI data, specimens in poor condition. \*\* An additional six barcodes are too  
 966 short to be allocated BINs so a total of 2950 barcodes have BINS and are subject to analysis.  
 967 \*\*\* "*Iridostoma*" *catatella* Viette, 1956 is misplaced in *Iridostoma* Meyrick, 1909; this species is  
 968 here transferred from Plutellidae to the Glyphipterigidae: Acrolepiinae (provisionally  
 969 as *Acrolepia catatella* comb. nov.).  
 970 \*\*\*\*Currently included in Lecithoceridae is an *Epichostis*-like species, *Lecithocera ojejyella*  
 971 Viette, 1958. In Gelechioidea i.s. we include 18 sequences representing 9 BINs of *Epichostis*-like  
 972 moths as indicated in the field Extra info. There are currently no *Epichostis* barcodes on BOLD  
 973 and we are currently uncertain if *L. ojejyella* together with these BINs might represent true  
 974 Xyloryctidae.

975

976

Family	Records	BINS	BINS Malaise	BINS Light trap + day-netted	shared BINs	BINS identified to Species	#Described species

Micropterigidae	2	2	0	2	0	0	0
Opostegidae	6	5	0	5	0	0	0
Nepticulidae	50	28	21	7	0	0	1
Heliozelidae	0	0	0	0	0	0	1
Adelidae	7	7	0	7	0	0	1
Tischeriidae	0	0	0	0	0	0	1
Psychidae	41	23	15	8	0	0	19
Eriocottidae	45	12	2	10	0	0	0
<b>Dryadulidae*</b>	6	3	3	0	0	0	0
Tineidae	450	159	95	65	1	1	40
Lyonetiidae s.auct. ('Cemiostomidae')	2	2	1	1	0	0	2
<b>Bucculatricidae</b>	2	2	1	1	0	0	0
Gracillariidae	73	55	17	40	2	2	22
<b>Bedelliidae</b>	8	4	1	3	0	0	0
Praydidae	2	2	0	2	0	2	2
Lyonetiidae s.str.	0	0	0	0	0	0	1
Argyresthiidae	29	5	0	5	0	0	9
Yponomeutidae	5	5	2	3	0	0	6
Ypsolophidae	0	0	0	0	0	0	1
Plutellidae***	0	0	0	1	0	0	1
Tonzidae	1	1	0	1	0	0	0
Glyhipterigidae***	29	20	11	10	1	0	7
Alucitidae	3	3	0	3	0	0	4
Pterophoridae	31	19	1	19	1	4	65
Copromorphidae	9	5	0	5	0	0	1
Carposinidae	8	3	0	3	0	0	2
Epermeniidae	30	12	0	12	0	0	7
Immidae	34	21	17	5	1	0	1
Choreutidae	4	3	0	3	0	1	1
Galacticidae	3	1	0	1	0	0	1
Tortricidae	260	150	19	132	1	19	342
Brachodidae	1	1	0	1	0	1	8
Cossidae	1	1	0	1	0	0	26
Dudgeonidae	0	0	0	0	0	0	1
Metarbelidae	0	0	0	0	0	0	2
Sesiidae	0	0	0	0	0	0	32
Epipyropidae	2	2	1	1	0	0	3
Lacturidae	2	1	0	1	0	0	9
Limacodidae incl. Chrysopolominae	11	10	1	9	0	7	70
Somabrachyidae	0	0	0	0	0	0	1
Zygaenidae	0	0	0	0	0	0	5

Gelechioidea i.s., includes ( <i>Orygocera</i> , <i>Prothamnodes</i> , " <i>Trichocirca</i> " <i>decaryanum</i> )	87	53	27	26	0	0	27
Depressariidae s.l. ('Stenomatidae': <i>Herbulotiana</i> , <i>Amontes</i> )	6	4	2	3	1	0	18
Depressariidae s.l. ('Peleopodidae': Oditinae)	230	131	54	81	4	0	61
Depressariidae s.l. ('Ethmiidae')	1	1	0	1	0	0	19
Depressariidae s.s. (Depressariidae: Depressariinae, Cryptolechiinae)	2	2	0	2	0	0	6
Oecophoridae	62	28	9	20	1	1	23
Lecithoceridae	219	79	51	31	3	0	28
Xyloryctidae****	0	0	0	0	0	0	1
Autostichidae	0	0	0	0	0	0	2
Elachistidae s.s.	85	8	8	0	0	0	1
Momphidae	0	0	0	0	0	0	1
<b>Batrachedridae</b>	4	4	0	4	0	0	0
Coleophoridae	0	0	0	0	0	0	1
<b>Blastobasidae</b>	13	7	1	6	0	1	0
Scythrididae	21	14	7	7	0	1	5
Stathmopodidae	27	17	0	17	0	0	4
Cosmopterigidae	105	59	13	47	1	2	14
Gelechiidae	313	178	36	145	3	6	32
Whalleyanidae	0	0	0	0	0	0	2
Thyrididae	4	3	0	3	0	2	32
Hyblaeidae	0	0	0	0	0	0	4
Callidulidae	0	0	0	0	0	0	4
Pyralidae	171	107	14	93	0	10	271
Crambidae	253	162	25	139	2	34	346
Unknown/i.s. Lepidoptera	190	113	52	62	1	0	1
<b>TOTAL</b>	<b>2950**</b>	<b>1537</b>	<b>507</b>	<b>1053</b>	<b>23</b>	<b>94</b>	<b>1598</b>



978 Table 3. Species detected during this study in Madagascar that are known to occur outside the  
 979 Afrotropical region. We also indicate those species that are known to be pests and/or invasive.  
 980 Those known to feed on crops but not widely acknowledged pests are indicated with an asterisk.

Family/subfamily	Species name	BIN	Distribution	New record for Madagascar	Pest	Ref.
Tineidae: Erechthiinae	<i>Erechthias minutalis</i>	BOLD:ABW6327	Cosmopolitan	Yes	No	
Tineidae: Hieroxestinae	<i>Opogona</i> sp.	BOLD:ACS7592	Madagascar & Oriental	Yes	No	
Gracillariidae: Acrocercopinae	<i>Dialectica scalariella</i>	BOLD:AAL3278	Palaearctic, Afrotropics and Australia	Yes	No	
Gracillariidae: Ornixolinae	<i>Stomphastis</i> sp.	BOLD:AAM6667	Madagascar & Australia	Yes	No	
Praydidae	<i>Prays nephelomima</i>	BOLD:AAM9790	Madagascar & Australia	Yes	Yes	Jamieson et al. 2008
Praydidae	<i>Prays citri</i>	BOLD:AAW5122	Palaearctic, Afrotropics and Australia	?see text	Yes	Lopez-Vaamonde et al. 2010
Glyphipterigidae	<i>Glyphipterix</i> sp.	BOLD:AAY2216	Australia & Madagascar	Yes	No	
Pterophoridae: Platyptiliinae	<i>Hepalastis pumilio</i>	BOLD:AAD4253	Cosmopolitan	No	No	
Pterophoridae: Platyptiliinae	<i>Stenoptilia</i> sp.	BOLD:AAD0716	Cosmopolitan	Yes	No	
Pterophoridae: Platyptiliinae	<i>Sphenarches anisodactylus</i>	BOLD:AAD0725	Cosmopolitan	No	No	
Tortricidae: Olethreutinae	Genus sp.	BOLD:ACS7628	Palaearctic, Afrotropics, Oriental	Yes	No	
Tortricidae: Olethreutinae	<i>Bactra venosana</i>	BOLD:ABZ1079	Afrotropics, Oriental, Australia	Yes	No	
Tortricidae: Olethreutinae	<i>Crociosema lantana</i>	BOLD:AAH5763	Nearctic, Neotropics, Afrotropics and Australia.	Yes	No	
Tortricidae: Olethreutinae	<i>Cydia choleropea</i>	BOLD:ABW2540	Afrotropics and Oriental	Yes	No	
Tortricidae: Olethreutinae	<i>Dudua aprobola</i>	BOLD:AAT9574	Cosmopolitan	Yes	No*	
Tortricidae: Olethreutinae	<i>Lobesia aeolopa</i>	BOLD:AAJ2244	Afrotropics and Oriental	No	Yes	Evans 1970
Tortricidae: Olethreutinae	<i>Lobesia vanillana</i>	BOLD:ABV8007	Réunion, Madagascar	No	Yes	Brown et al. 2014
Tortricidae: Olethreutinae	<i>Thaumatotibia leucotreta</i>	BOLD:AAE7729	Afrotropics, intercepted in Palaearctic and Nearctic	No	Yes	Baker et al. 2013
Cosmopterigidae: Cosmopteriginae	<i>Anatrachyntis simplex</i>	BOLD:ABX3349	Cosmopolitan	Yes	Yes	Heckford, 2004
Cosmopterigidae: Cosmopteriginae	<i>Cosmopterix athesiae</i>	BOLD:AAE4001	Palaearctic and Afrotropics	Yes	No	
Cosmopterigidae: Cosmopteriginae	<i>Cosmopterix</i> sp. cf. <i>attenuatella</i>	BOLD:AAC1744	Cosmopolitan	Yes	No	

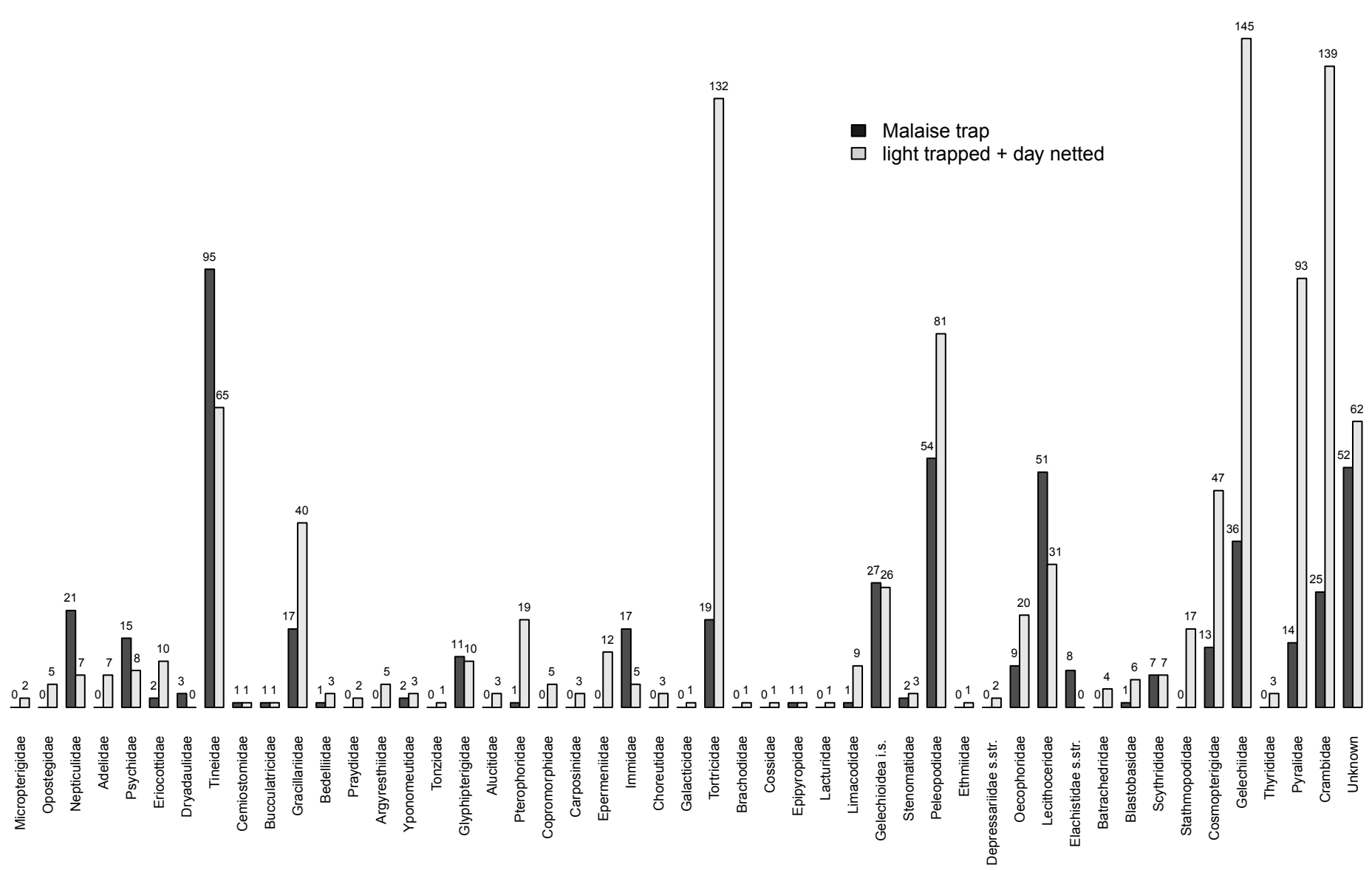
Cosmopterigidae: Chrysopeliinae	<i>Ascalenia</i> sp.	BOLD:AAG0134	Cosmopolitan	Yes	No	
Cosmopterigidae: Chrysopeliinae	<i>Gisilia</i> sp.	BOLD:ACS6187	Bangladesh & Madagascar	Yes	No	
Gelechiidae: Anacampsinae	<i>Aproaerema simplexella</i>	BOLD:ACK6985	Cosmopolitan, invasive in Afrotropics	Yes	Yes	Zharare, 2013.
Gelechiidae: Dichomeridinae	<i>Dichomeris acuminatus</i>	BOLD:AAB6409	Cosmopolitan	Yes	No	
Choreutidae	<i>Tebenna micalis</i>	BOLD:AAH9855	Cosmopolitan	Yes	No	
Pyralidae: Galleriinae	<i>Achroia grisella</i>	BOLD:ACO9701	Cosmopolitan	No	No	
Pyralidae: Galleriinae	<i>Galleria mellonella</i>	BOLD:AAA0965	Cosmopolitan	No	Yes	Kwadha et al. 2017
Pyralidae: Pyralinae	<i>Hypsopygia nostralis</i>	BOLD:AAI3521	Nearctic, Neotropics and Afrotropics	Yes	No	
Pyralidae: Phycitinae	<i>Cadra cautella</i>	BOLD:AAB9605	Cosmopolitan	Yes	Yes	Paulian & Viette 1955
Pyralidae: Phycitinae	<i>Cryptoblabes gnidiella</i>	BOLD:AAW5129	Cosmopolitan	Yes	Yes	da Silva & Mexia 1999.
Pyralidae: Phycitinae	<i>Ectomyelois ceratoniae</i>	BOLD:AAU4812	Cosmopolitan	No	Yes	Morland, 2015
Pyralidae: Phycitinae	<i>Etiella zinckenella</i>	BOLD:AAB7420	Cosmopolitan	No	Yes	Van Den Berg et al. 2010
Pyralidae: Phycitinae	<i>Thylacoptila paurosema</i>	BOLD:AAV8326	Afrotropical, Oriental and Australia	No	No*	
Crambidae: Acentropinae	<i>Paraponyx fluctuosalis</i>	BOLD:AAA0473	Cosmopolitan	No	Yes	Yen, 2014
Crambidae: Crambinae	<i>Angustalius malacellus</i>	BOLD:AAV9127	Palaearctic & Afrotropics	No	No*	
Crambidae: Pyraustinae	<i>Isocentris filalis (=Hyalobathra retinalis)</i>	BOLD:AAL8896	Afrotropical, Oriental	No	No	
Crambidae: Spilomelinae	<i>Bocchoris inspersalis</i>	BOLD:AAC5466	Cosmopolitan	No	No	
Crambidae: Spilomelinae	<i>Cnaphalocrocis trapezalis</i>	BOLD:AAC0297	Cosmopolitan	No	Yes	Shankara Murthy & Nagaraj 2014
Crambidae: Spilomelinae	<i>Cnaphalocrocis exigua</i>	BOLD:AAO9362	Afrotropics and Oriental and Oceania	Yes	Yes	Barrión et al. 1991
Crambidae: Spilomelinae	<i>Diasemiopsis ramburialis</i>	BOLD:AAD0296	Old World (Africa, Oriental Australia)	No	No*	
Crambidae: Spilomelinae	<i>Diaphania indica</i>	BOLD:AAB1719	Cosmopolitan	No	Yes	Paulian & Viette 1955 ; Shimizu 2000
Crambidae: Spilomelinae	<i>Eurrhyarodes bracteolalis</i>	BOLD:AAD1173	Cosmopolitan	No	No*	
Crambidae: Spilomelinae	<i>Herpetogramma licarsisalis</i>	BOLD:AAA3965	Palaeotropics, Australasia, Hawaii, Canaries	No	Yes	Lopez- Vaamonde et al. 2010
Crambidae: Spilomelinae	<i>Herpetogramma</i> sp.	BOLD:AAB6841	Afrotropics and Oriental	No	No	
Crambidae: Spilomelinae	<i>Herpetogramma</i> sp.	BOLD:ACD5135	Oriental	Yes	No	
Crambidae: Spilomelinae	<i>Hymenoptychis sordida</i>	BOLD:AAF8520	Old World (Africa, Oriental Australia)	No	No	

Crambidae: Spilomelinae	<i>Hyalobathra olesialis</i>	BOLD:ACN7820	Aftrotropical, India and Australia	Yes	No	
Crambidae: Spilomelinae	<i>Maruca fuscalis</i>	BOLD:AAD9057	Australia	Yes	No	
Crambidae: Spilomelinae	<i>Maruca vitrata</i>	BOLD:AAB2756	Pantropical	No	Yes	Sharma et al. 1999
Crambidae: Spilomelinae	<i>Omiodes indicata</i>	BOLD:AAB5389	Cosmopolitan	No	Yes	Favetti et al., 2018
Crambidae: Spilomelinae	<i>Palpita vitrealis</i>	BOLD:AAC1043	Cosmopolitan	No	Yes	Hayden & Buss, 2013
Crambidae: Spilomelinae	<i>Pyrausta phoenicealis</i>	BOLD:AAF5760	Cosmopolitan	No	Yes	Yamada 1979
Crambidae: Spilomelinae	<i>Salbia haemorrhoidalis</i>	BOLD:AAD3428	Cosmopolitan	Yes	No	
Crambidae: Spilomelinae	<i>Spoladea recurvalis</i>	BOLD:AAA3666	Cosmopolitan	No	Yes	Paulian & Viette 1955

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# Number of BINs recovered per Family with Malaise and light traps + nets



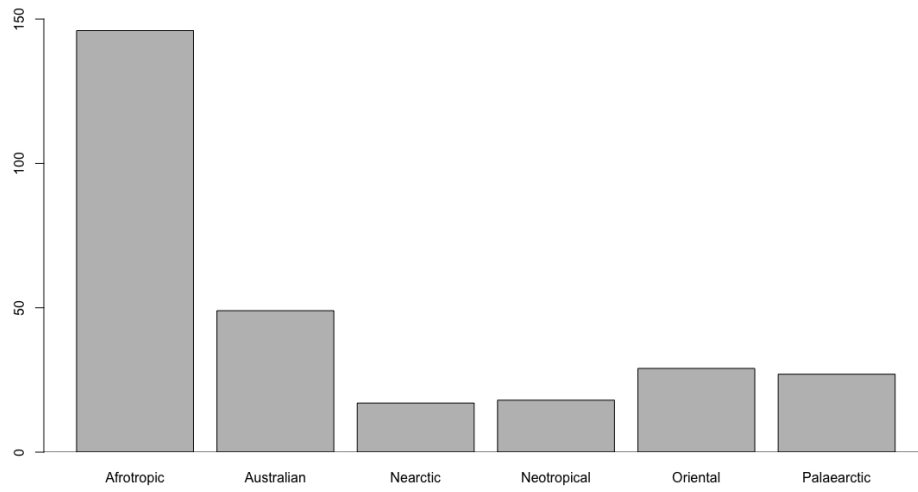
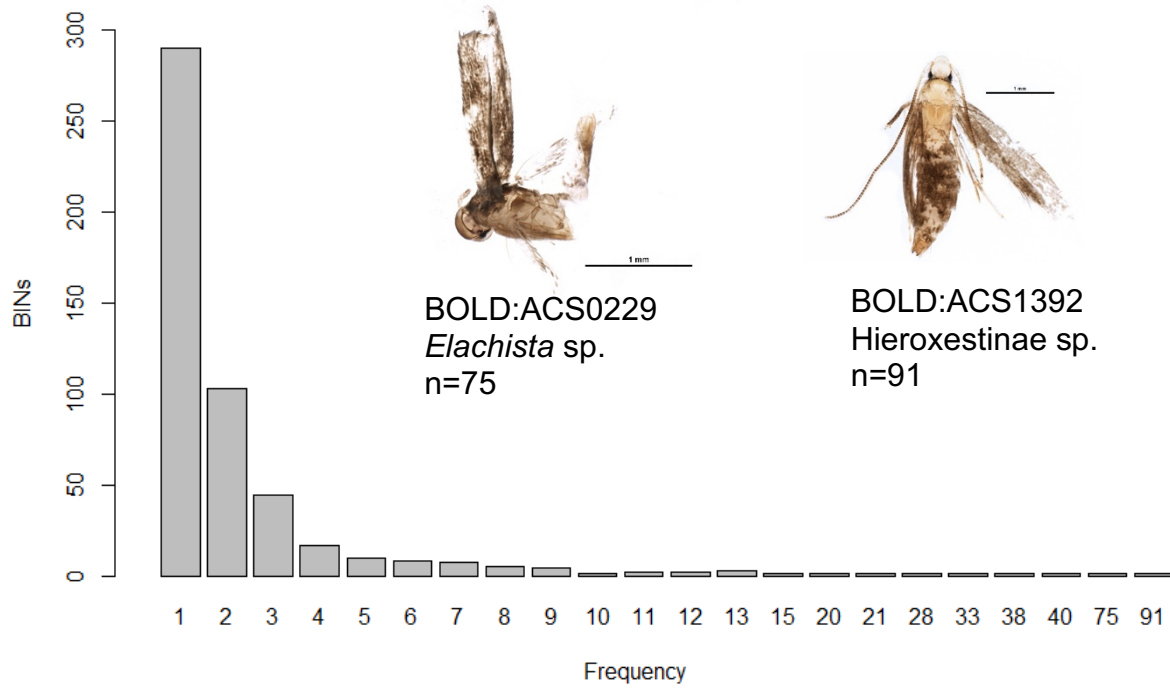
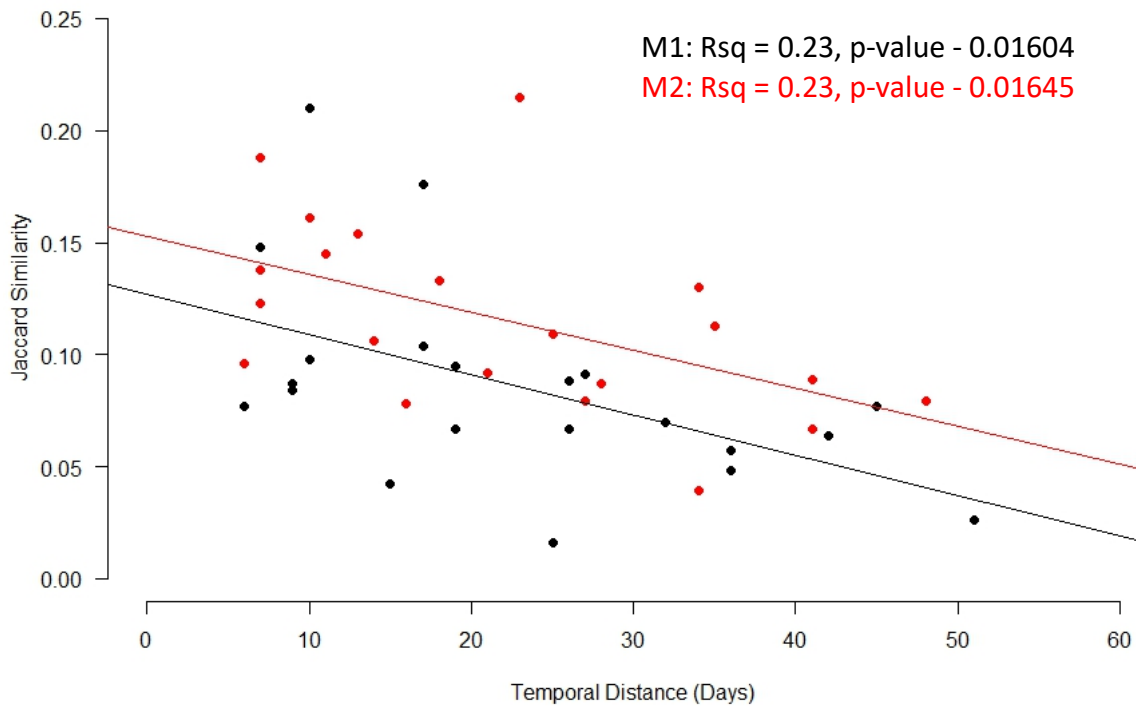


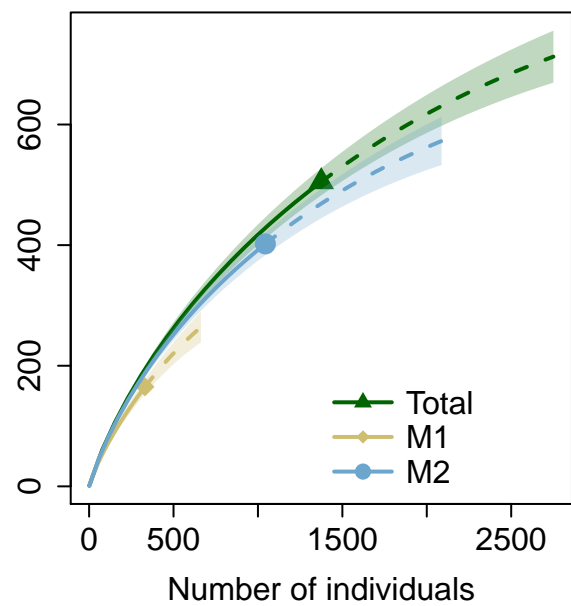
Figure 2. Distribution of BINs over biogeographic regions. Notice one BIN can appear in several biogeographic regions.

408x267mm (72 x 72 DPI)

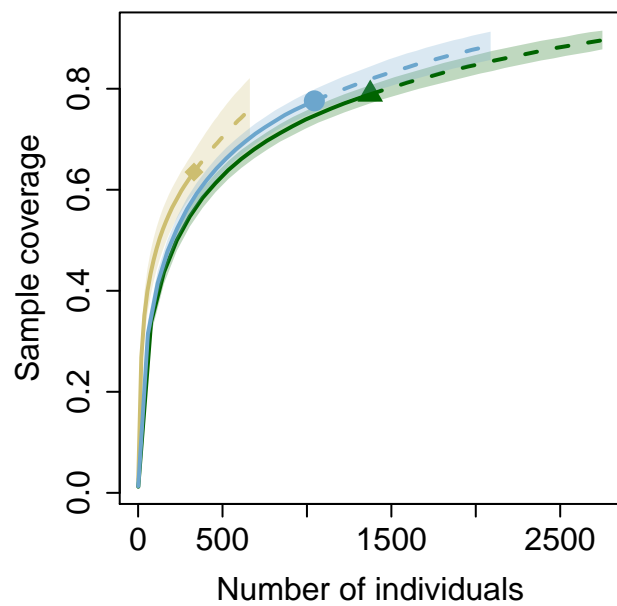




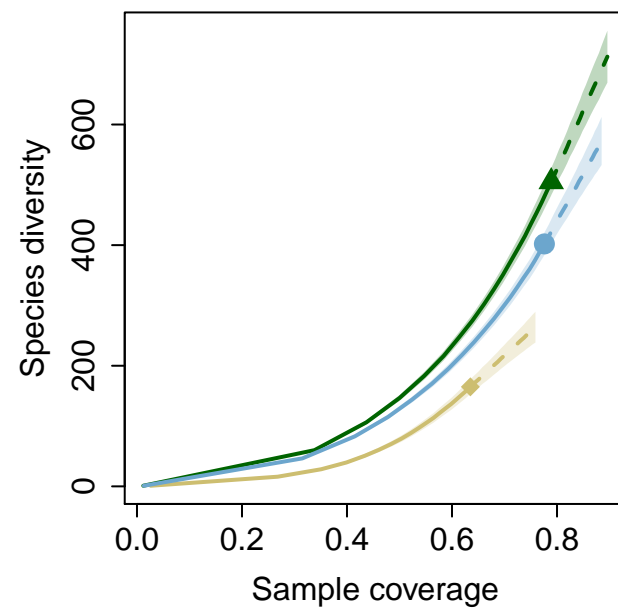
**A**



**B**



**C**





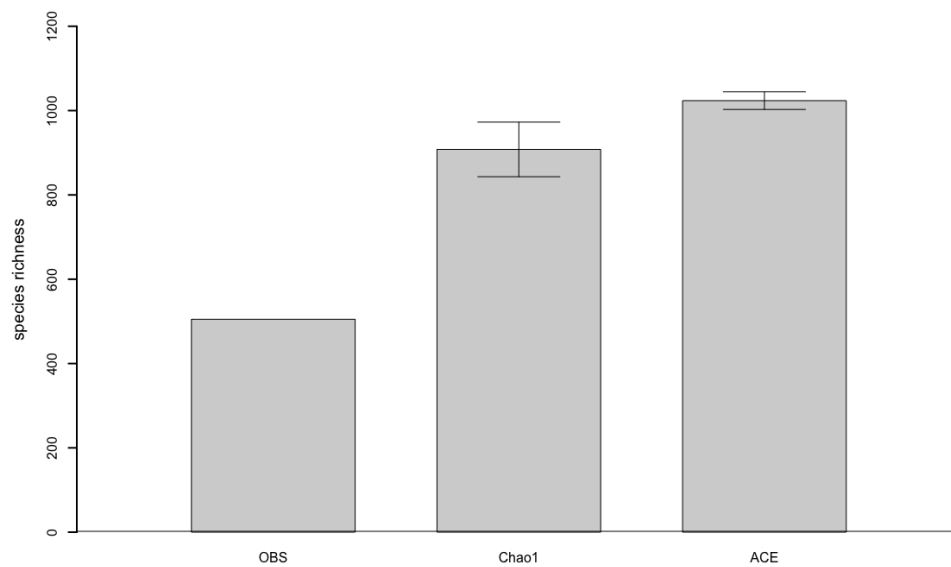


Figure 6. Species richness observed and estimates based both on Chao1 and ACE analyses for the site sampled with Malaise trap at Andasibe

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