Taxonomic identification accuracy from BOLD and GenBank databases using over a thousand insect barcodes from Colombia

Short title: Taxonomic identification accuracy from BOLD and GenBank databases using insect barcodes

Nathalie Baena-Bejarano[1]*, Catalina Reina[2], Diego Esteban Martínez-Revelo[3, 4], Claudia A. Medina[1], Eduardo Tovar[1], Sandra Uribe-Soto[4], Jhon Cesar Neita-Moreno[1], Mailyn A. Gonzalez[1]*

[1]Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia.

[2]ICA - Instituto Colombiano Agropecuario, Soledad, Colombia.

[3]Asociación GAICA, Pasto, Nariño, Colombia.

[4]Grupo de Investigación en Sistemática Molecular, Universidad Nacional de Colombia, sede Medellín, Medellín, Colombia.

* Corresponding authors

E-mails: ntbaena@gmail.com (NBB), magonzalez@humboldt.org.co (MAG)

Abstract

Recent declines of insect populations at high rates have resulted in the need to develop a quick method to determine their diversity and to process massive data for the

identification of species of highly diverse groups. A short sequence of DNA from COI is widely used for insect identification by comparing it against sequences of known species. Repositories of sequences are available online with tools that facilitate matching of the sequences of interest to a known individual. However, the performance of these tools can differ. Here we aim to assess the accuracy in identification of insect taxonomic categories from two repositories, BOLD Systems, and GenBank. This was done by comparing the sequence matches between taxonomist identification and the suggested identification from the platforms. We used 1160 sequences in eight orders of insects from Colombia. After the comparison, we reanalyzed the results from a representative subset of the data from the subfamily Scarabaeinae (Coleoptera). Overall, BOLD systems outperformed GenBank, and the performance of both engines differed by orders and other taxonomic categories (species, genus and family). Higher rates of accurate identification were obtained at family and genus level. The accuracy was higher in BOLD for the orders Coleoptera at family, for Coleoptera and Lepidoptera at genus, and species level. The other orders performed similarly in both repositories. Moreover, the Scarabaeinae subset showed that in this group species were correctly identified when BOLD match percentage was above 93.4% and a total of 85% of the samples were correctly assigned to a taxonomic category. These results accentuate the great potential of the identification engines to place insects accurately into their respective taxonomic categories based on DNA barcodes and highlight the reliable use of BOLD Systems for insect identification in the absence of a large reference database for a highly diverse country.

Key words: Antioquia; barcoding; biodiversity; BLAST; BOLD; Batch ID engine; Coleoptera; Diptera; DNA; GenBank; Hemiptera; Hymenoptera; Lepidoptera; Odonata; Psocodea; Public databases; Santander; species identification; Vichada.

Introduction

More than a million species of plants and animals are in danger of extinction according to the latest global assessment of the intergovernmental platform on biological diversity and ecosystem services [1]. Declines in biodiversity and projections of a sixth mass extinction [2] have placed the impact of such losses on the agendas of governments all over the world [3]. Although insects constitute one of the most diverse groups on the planet, many species remain undiscovered and recent evidence suggests the decline of their populations at high rates [4,5]. To date, the risk of extinction of 12,161 species of insects has been evaluated according to the criteria of the IUCN red list and 2,291 species are considered “threatened” around the world [6].

Colombia is known as a biodiversity hotspot in the world. Although insects are the most studied class of animals in the country [7], research involving their genetic diversity is still currently underrepresented. Countries around the world are implementing massive sampling of insects with barcode generation under the premise that barcodes will allow us to assess biodiversity at a higher speed [8]. Barcodes in taxonomic works are showing an incredibly hidden diversity of insects with descriptions of hundreds of new species facilitated by DNA, morphology, and natural history [9,10]. As a megadiverse country, Colombia has great challenges to complete the inventory of

its biodiversity and then the sustainable use of its resources. In particular, accelerating species identification using an integrative taxonomy is a priority.

Assessing biodiversity via sampling and DNA barcoding has been successfully applied in areas where coordinated sampling and barcoding facilitated the creation of extensive libraries for a particular region. An example of such collaboration is described in Morinière et al [8] where the Bavarian State Collection of Zoology (ZSM), the Barcoding Fauna Bavarica (BFB), and the German Barcode of Life (GBOL) project generated a library of 120,000 reliably identified species in Coleoptera, Diptera, Hymenoptera, and Lepidoptera for Germany and Central Europe. A closer example in the Neotropics is Costa Rica which has been leading the Bioalfa project and within the last 15 years has generated 45,000 insect species barcodes recorded [11]. Added to this initiative are other countries across the globe such as Canada, Ecuador, Sweden, and Singapore [9,12–16]. Moreover, the development and implementation of effective methods for species monitoring are important for assessing biodiversity, and overall health of ecosystems [8]. The species richness of insects in terrestrial ecosystems facilitates their use as bioindicators for measuring biodiversity and tracking the effects of changes in environmental conditions. The challenges faced by biodiversity declines have resulted in a need to generate new strategies for monitoring that are capable of generating vast amounts of data in a rapid and cost-effective manner. One such strategy that has been gaining traction in recent years has been the implementation of DNA barcoding.

Barcode sequences are generated from a standardized region that consists of short DNA sequences (generally between ~300 to ~700 bp). The mitochondrial

Cytochrome c oxidase subunit I (COI) is most often used for insect barcoding. A DNA barcoding repository is built from sampling the DNA of individuals that are retained as vouchers, from which a photograph is taken. In the case of insects, most often a leg is removed from the specimen and used to obtain the DNA. In some cases, whole bodies of the samples are used when the genetic material available is limited by the size of the specimen. Although non-destructive DNA extraction methods are preferred to keep the morphology for future reference, photographs are suggested for facilitating identification before this step. This approach requires the creation of a large DNA reference database where the identification of unknown specimens is achieved through the information confirmed and curated in the database. The idea behind is that unknown samples could be identified by matching their sequences to the species that are curated from the database. The Barcode of Life Data Systems BOLD (http://www.boldsystems.org) [17] is a platform specifically developed for this type of information. The BOLD platform provides the identifications match based on BINs (Barcode Index Numbers) [18] that are automatically generated through algorithms for the specimens. Although unidentified species coded in BINs can help estimating diversity, the identification of species is also possible using the Basic Local Alignment Tool (BLAST) from GenBank [19,20]. BLAST allows matching sequences to a query sequence. The search consists of comparing nucleotide sequences hosted in the database and to provide statistical significance of these matches. The main goal from BLAST is not necessarily to provide species identification because the same principle can be used for the search of gene families; uses of identification databases are vast in biology. Scientists frequently use these two platforms to identify species from barcodes. An assessment of these two main public

113 repositories from curated samples across taxa found a slightly better performance from
114 GenBank vs BOLD for insects (n=17) at species and genus level identification without
115 statistical significance, and more even results between repositories for plants (n=61)
116 and macro-fungi (n=16) [21]. Later, a revision of this insect data found the results to be
117 more similar than previously reported for this group [22]. Accurate identification of
118 species and overall composition in ecosystems based on sampling is a key factor for
119 conservation initiatives. Therefore, evaluating the performance of these platforms
120 assigning specimens to a specific taxonomic category level has a significant impact on
121 the advancement of other fields.

122 Here, we analyze a large inventory of over 1,000 insect barcodes for Colombia to
123 the database which includes the first strepsipteran barcode from Colombia. We aim to
124 compare the matches of the taxonomist identification to species, genus, and family level
125 with the search engines of BOLD and BLAST service from GenBank in order to provide
126 suggestions and next steps for the use of these tools. We assess the accuracy of the
127 species identification engine from BOLD by using a subset of insects corresponding to
128 Scarabaeinae subfamily (Coleoptera: Scarabaeidae).

129 Materials and Methods

130 Collection Locality/Geography

131 Insect samples were collected from ten departments of Colombia through different
132 projects (Colombia BIO, Santander BIO). A majority of the samples (81.0%) came from
133 three departments of Colombia (Antioquia, Vichada, and Santander), the spatial data of

the localities were processed in ArcGIS 10.2 (License E300 04/26/2013) (Fig 1). The elevation of the samples covered a wide range with the lowest sampling occurring at 56 masl and the highest at 3563 masl. Although samples were obtained with seven different entomological collecting methods (malaise, pitfall, light trap, hand-picked, entomological net, Van Someren-Rydon trap and folding net), nine malaise traps accounted for 62.6% of the total collected material, followed by pitfall 17.2%, and light trap 8.1%.

Fig 1. Map of Colombia showing collecting localities in black dots. Ocean base map from Esri, GEBCO, NOAA, National Geographic, DeLorme, HERE, Geonames.org, and other contributors. Hillshade map based on SRTM30_PLUS v8 [23].

Taxa description

This dataset includes insects from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Hemiptera, Odonata, Psocodea and Strepsiptera, from which Coleoptera is the most represented order in the dataset (35%). The specimens were identified from morphology by students working on their thesis and by specialists. As a result of varying expertise, we provide an identification that goes from order to species level (S1 Table); additionally in Scarabaeinae, identification was done to morphospecies level following González-Alvarado et al [24]. Currently, few samples (7%) have been identified to lower levels from molecular data by BOLD Data Manager. The specimens were deposited at collections with 999 samples between the Entomological and Tissue collection of the Institute Alexander von Humboldt (IAvH-E and IAvH-CT), Museo Javeriano de Historia

156 Natural (MPUJ) at Pontificia Universidad Javeriana with 63, Museo Francisco Luis
157 Gallego (MEFLG) at Universidad Nacional de Colombia, Sede Medellin with 55, and
158 Colección Entomológica Universidad de Antioquia (CEUA) with the remaining 43
159 samples. Records of some samples are also available for search in SIB Colombia.

160 DNA sampling and extraction

161 One leg was removed from each insect, when possible, but some samples required the
162 use of the full body to provide enough DNA. All samples were sent to the molecular
163 laboratory of Instituto Humboldt at CIAT, Palmira. Extraction protocols were conducted
164 following the high throughput DNA isolation from Ivanova et al [25]. The concentration of
165 selected DNA samples was estimated by quantification using the NanoDrop 1000
166 (Thermo Fisher Scientific). Amplification of cytochrome c oxidase I (COI) fragments
167 (658 bp) took place by polymerase chain reaction (PCR) with a laboratory prepared
168 master mixture which contained 2 µL of template DNA (~10-50 ng), 1X Taq buffer
169 ((NH4)2SO4), 200 µM of each deoxynucleoside triphosphate, 2 mM MgCl2, 0.2 µM of
170 each primer, 0.4 µg/µL bovine serum albumin, and 1 U of Taq DNA polymerase. A
171 cocktail of four primers was used: LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-
172 3', HCO2198 5'- TAAACTTCAGGGTGACCAAAAAATCA-3' [26], Lep-F1 5'-
173 ATTCAACCAATCATAAAGATATTGG-3' and Lep-R1 5'-
174 TAAACTTCTGGATGTCCAAAAAATCA-3' [27]. Primers were selected following
175 Canadian Centre for DNA Barcoding (CCDB) suggested primers for taxonomic groups.
176 The PCR cycling conditions consisted of a first cycle of denaturation at 94 °C for 3 min;
177 followed by 5 cycles of 94 °C for 30 s, 45 °C for 40 s, and 72 °C for 1 min; then 35

178 cycles of 94 °C for 30 s, 51 °C for 40 s, and 72 °C for 1 min; and a final extension cycle
179 at 72 °C for 5 min. PCR products were visualized by electrophoresis on 1.5% agarose
180 gels stained with SYBR Safe (Thermo Fisher Scientific) and using 1X TBE. The
181 ExoSAP-IT protocol was used to clean PCR products, after which Sanger sequencing of
182 amplicons were carried out at The Universidad Nacional de Colombia and the University
183 of the Andes. Sequences were assembled and edited in Geneious 10.0.9 2017
184 (Auckland, New Zealand) [28]. All files were uploaded in BOLD systems and are found
185 in the dataset "insects-all" (DS-CBIH2020; Dataset available at: DOI will be made
186 available after acceptance).

187 # Data analysis

188 ## BOLD Batch ID engine

189 To identify samples to species level, we ran BOLD's Identification Engine in
190 datasets created for insect orders in July 2020. The identification engine allows the user
191 to select the search within the COI Species Database or COI Full Database. We
192 selected COI Species Database which will match the sequence against an identified
193 species as opposed to individuals without identification to species level. We kept filters
194 as default for a minimum overlap of 300 bp and sequence length ≥ 100 bp. We ran the
195 analysis with 97% similarity, a percentage that accounts for within species variation and
196 error of the sequence [8]. Under the criteria selected of 97% numerous species ID were
197 not obtained. We decided to lower the criteria and include matches using the 80%
198 similarity filter due to the lack of matches at 97%. The 80% similarity is the minimum
199 filter allowed in BOLD. A match was accepted as correct when the identification by the

200 taxonomist was in agreement by the suggested identification by BOLD. The ID search
201 engine provides a list of maximum 100 closest specimens, and we selected the first
202 suggestion from the list that has the highest percentage match and base pair overlap.
203 We included an alternative match when the suggestion was done towards a sequence
204 from our project and ran statistical tests separately to prevent circular analysis of
205 results. Matches were checked at species, genus, and family level.

206 BLAST

207 We used BLAST from GenBank in Geneious 10.0.9 2017 by default to look for
208 matches of the sequences. The parameters included were the database search from
209 Nucleotide collection, program set as the MegaBlast, results displayed as a Hit table,
210 and retrieve Matching region with Maximum hits of 100. The search was set to provide a
211 list of 100 closest nucleotide sequences, from which we selected the first suggestion
212 that obtained the highest Bit-score or Max score (name when the BLAST search is done
213 from the GenBank website). Most of the time the E value was 0 (or closest to zero) and
214 the Query cover 100%. Matches were checked at species, genus and family level.
215 The identification analyses in both approaches (BOLD and Blast) followed the
216 assumption that species from the databases are correctly identified; however, this
217 approach might not be accurate in cases where the most similar species were wrongly
218 identified from the databases.

219 Dung beetles (Scarabaeinae) case study

220 The Scarabaeinae subfamily was selected due to the high representation of this
221 group in the samples of Coleoptera (42%) and from the total insect samples (15%). In

222 order to evaluate the accuracy of species identification suggested by BOLD, we
223 consulted taxonomists with expertise in the identification of the Scarabaeidae family.
224 They were asked to reanalyzed the results and mention if they agree with the species
225 and genus identification suggested by BOLD and to explain why, as follows: Correctly
226 Identified Genus, Correctly Identified Species, Probable Correctly Identified Species,
227 Incorrect Identification (Table 1). When available, specimens deposited at the IAVH-E
228 collection and photographs uploaded to BOLD were checked for specimens to
229 corroborate the taxonomy, as field notes from the collectors.
230

231 **Table 1. List of categories used by the taxonomist to define the identification**
232 **results suggested by BOLD with the Scarabaeinae subfamily.**

Category	Definition
Correctly identified genus	Agreement between the taxonomist and BOLD at the genus level
Correctly identified species	Agreement between the taxonomist and BOLD at the species level
Probable correctly identified species	Agreement between the taxonomist morphospecies and the species suggested by BOLD (the genus matched and probable species)
Incorrect identification	Disagreement between the taxonomist and BOLD (the genus or species did not match)

233

234 Statistical analysis

235 Chi-square analyses were performed between match results for BOLD and
236 GenBank at the studied taxonomic levels (species, genera and family) to evaluate if
237 there was a significant difference between the platforms performance. An additional

series of Chi-square analyses were performed to identify differences in the platforms by each order of insect at the species, genus, and family level. We adjusted our significance threshold at each taxonomic level based on the number of orders compared using the Bonferroni Correction. Flagged records were removed from the analysis.

The non-parametric Kruskal-Wallis test in PAST, Version 4.05 [29] was used to determine any significant differences of medians for BOLD % match of the correct Scarabaeinae categories against incorrect identification. Assumptions for an ANOVA were not fulfilled. Dunn's post hoc was carried out to identify differences between categories.

Results

Description of the data

A total of 1160 sequences of insects from Colombia were generated since 2019. From this set, 1088 sequences were barcode compliant and placed in 708 BINS (Table 2). Of these 708 BINS, 500 were identified as unique (Fig 2). The most frequent BINS were all in Coleoptera, BOLD:ADN3981 *Paulosawaya* sp. (11 specimens), BOLD:ADO6609 *Paulosawaya ursina* (Blanchard, 1850) (10 specimens), and BOLD:ADJ9394 Ptilodactylidae (9 specimens). From the specimens with barcode compliant 33 were flagged as problematic records in most instances due to suspected contamination, specimen mix-up or misidentification.

259 **Fig 2. Summary of Barcode index numbers (BINS) obtained by orders of insects.**

260 A total of 500 BINs were identified as unique and 208 BINs as non-unique in the

261 platform BOLD Systems.

262 **Table 2. Summary of specimens and barcodes data by orders after removal of flagged records.**

Order	Specimens #	Flagged record	Family count	Specimen count IDed Family	Unknown	Genera count	Specimen count IDed Genus	Species count	Specimen count IDed Species	Barcode compliant	Species count	BINS	Unique Bins	Non-Unique Bins
Coleoptera	411	14	25	397	0	57	271	63	117	369	57	218	184	34
Diptera	277	14	24	257	6	4	22	0	N/A	257	0	165	116	49
Hemiptera	82	4	11	78	0	0	N/A	0	N/A	68	0	58	56	2
Hymenoptera	201	2	10	199	0	16	47	2	3	176	2	142	112	30
Lepidoptera	166	0	8	161	5	57	154	79	128	163	73	104	18	86
Odonata	17	0	6	17	0	14	17	13	15	16	13	15	8	7
Psocodea	5	0	4	5	0	0	N/A	N/A	N/A	5	N/A	5	5	0
Strepsiptera	1	0	1	1	0	1	1	0	N/A	1	0	1	1	0
Total	**1160**	**34**	**89**	**1115**	**11**	**149**	**512**	**157**	**263**	**1055**	**145**	**708**	**500**	**208**

Coleoptera

S1 Table and S2 Table; Barcodes were obtained from 369 out of 411 coleopteran specimens and placed in 218 BINS, from which 184 (84.4%) were identified as unique in BOLD. The samples were assigned to 25 families, 57 genera and 63 species.

BOLD search engine: The search at 80% provided identifications for a total of 395 specimens. A match in agreement with the taxonomist was found for 337 (85%) specimens at the family level. For genus, the search resulted in 189 (70%) specimens of 271 and for species in 57 (49%) specimens of 117 total.

BLAST: The search provided identifications for a total of 379 specimens. A match in agreement with the taxonomist was found for 294 (78%) specimens at the family level. For genus, the search resulted in 83 (33%) specimens of 255 and for species in 20 (20%) specimens of 101 total.

Diptera

S1 Table and S2 Table; Barcodes were obtained from 257 out of 277 dipteran specimens and placed in 165 BINS, from which 116 (70.3%) were identified as unique in BOLD. The samples were assigned to 24 families and 4 genera. There were 6 specimens not assigned to any category.

BOLD search engine: The search at 80% provided identifications for a total of 245 specimens. A match in agreement with the taxonomist was found for 202 (82%) specimens at the family level, and for genus, 16 of 18 specimens (89%) matched the identification of the taxonomist. No species were identified by the taxonomist.

BLAST: The search provided identifications for a total of 241 specimens. A match in agreement with the taxonomist was found for 184 (76%) specimens at the family level, and for genus 13 of 14 specimens (93%) matched the identification of the taxonomist. No species were identified by the taxonomist.

Hemiptera

S1 Table and S2 Table; Barcodes were obtained from 68 out of 82 hemipteran specimens and placed in 58 BINS, from which 56 (97%) were identified as unique in BOLD. The samples were assigned to 11 families.

BOLD search engine: The search at 80% provided identifications for a total of 77 specimens. A match in agreement with the taxonomist was found for 66 (86%) specimens at the family level. No genera or species were identified by the taxonomist.

BLAST: The search provided identifications for a total of 67 specimens. A match in agreement with the taxonomist was found for 55 (82%) specimens at the family level. No genera or species were identified by the taxonomist.

Hymenoptera

S1 Table and S2 Table; Barcodes were obtained from 176 out of 201 hymenopteran specimens and placed in 142 BINS, from which 112 (78.9%) were identified as unique in BOLD. The samples were assigned to 10 families, 16 genera and 2 species.

BOLD search engine: The search at 80% provided identifications for a total of 199 specimens. A match in agreement with the taxonomist was found for 193 (97%)

307 specimens at the family level. At the genus level, the search resulted in 46 (98%) of 47
308 specimens and 3 of 3 (100%) specimens at the species level.
309 BLAST: The search provided identifications for a total of 182 specimens. A match
310 in agreement with the taxonomist was found for 176 (97%) specimens at the family
311 level. At the genus level, the search resulted in 38 (97%) of 39 specimens and 0 of 3
312 specimens at the species level.

313 Lepidoptera

314 S1 Table and S2 Table; Barcodes were obtained from 163 out of 166
315 lepidopteran specimens and placed in 104 BINS, from which 18 (17%) were identified
316 as unique in BOLD. The samples were assigned to 8 families, 57 genera and 79
317 species, 5 not assigned to any category.
318 BOLD search engine: The search at 80% provided identifications for a total of
319 161 specimens. A match in agreement with the taxonomist was found for 147 (91%)
320 specimens at the family level. For genus, the search resulted in 140 (91%) specimens
321 of 154 and for species in 86 (68%) specimens of 127 total.
322 BLAST: The search provided identifications for a total of 154 specimens. A match
323 in agreement with the taxonomist was found for 143 (93%) specimens at the family
324 level. For genus, the search resulted in 117 (79%) specimens of 148 and for species in
325 49 (49%) specimens of 99 total.

Odonata

S1 Table and S2 Table; Barcodes were obtained from 16 out of 17 Odonata specimens and placed in 15 BINS, from which 8 (53.3%) were identified as unique in BOLD. The samples were assigned to 6 families, 14 genera and 13 species.

BOLD search engine: The search at 80% provided identifications for a total of 17 specimens. A match in agreement with the taxonomist was found for 17 (100%) specimens at the family level. For genus, the search resulted in 13 (76%) specimens of 17 and for species in 6 (46%) specimens of 13 total.

BLAST: The search provided identifications for a total of 17 specimens. A match in agreement with the taxonomist was found for 17 (100%) specimens at the family level. For genus, the search resulted in 9 (56%) specimens of 16 and for species in 3 (20%) specimens of 15 total.

Psocodea

S1 Table and S2 Table; Barcodes were obtained from 5 out of 5 psocodean specimens and placed in 5 BINS, each identified as unique in BOLD (100%). The samples were assigned to 4 families.

BOLD search engine: The search at 80% provided identifications for a total of 5 specimens. A match in agreement with the taxonomist was found for 2 (40%) specimens at the family level. No genera or species were identified by the taxonomist.

BLAST: The search provided identifications for a total of 4 specimens. A match in agreement with the taxonomist was found for 1 (25%) specimen at the family level. No genera or species were identified by the taxonomist.

348 **Strepsiptera**

349 S1 Table and S2 Table; A barcode was obtained from one male specimen and
350 placed in 1 new BIN. The sample was identified to the genus *Caenocholax*.
351 BOLD search engine: The search at 80% provided identification for the 1
352 specimen. A match in agreement with the taxonomist was found for it (100%) at the
353 family and genus level. No species were identified by the taxonomist.
354 BLAST: The search provided identification for the 1 specimen. A match in
355 agreement with the taxonomist was found for it (100%) at the family and genus level. No
356 species were identified by the taxonomist.

357 Performance of BOLD and Blast (GenBank) identification
358 based on taxonomist match

359 The performance of BOLD vs BLAST for identification of specimens was
360 significantly greater with BOLD for the pooled comparison of all species ($x2 = 29.5$, $df = 1$, $P < 0.001$), genera ($x2 = 67.7$, $df = 1$, $P < 0.001$), and families ($x2 = 8.3$, $df = 1$, $P = 0.004$). Comparisons of the performance of BOLD and BLAST by Orders at the different
363 taxonomic levels revealed significantly more matches with BOLD (Fig 3), at the family
364 level (Fig 3A) in Coleoptera ($x2 = 7.7$, $df = 1$, $P = 0.006$), at genus level (Fig 3B) in
365 Coleoptera ($x2 = 72.8$, $df = 1$, $P < 0.001$) and Lepidoptera ($x2 = 8.4$, $df = 1$, $P = 0.004$)
366 and at the species level (Fig 3C) in Coleoptera ($x2 = 19.8$, $df = 1$, $P < 0.001$) and
367 Lepidoptera ($x2 = 7.7$, $df = 1$, $P = 0.006$). No differences were found for the other orders
368 tested at the different taxonomic categories. Same results were found when analyses
369 were performed with matches to data from our project, the only comparison that

370 changed was at the genus level where no significant differences were found for
371 Lepidoptera (S3 Table).
372
373 **Fig 3. Comparison by order of performance of BOLD Systems and BLAST for**
374 **identification of specimens in percentages.** Correct matches for BOLD Batch ID
375 engine at 80% are shown in dark gray. Correct matches from MegaBlast search by
376 default (GenBank) are shown in light gray. *Is next to orders where Chi-square analyzed
377 with Bonferroni correction was significant. The sample size is displayed on top of each
378 column. A) by families. B) by genera C) by species.

379 Case of study Scarabaeinae subfamily (Coleoptera:
380 Scarabaeidae)

381 A total of 172 specimens were analyzed. The samples in the subfamily
382 Scarabaeinae were assigned to 16 genera and 27 species (S1 Table).
383 BOLD search engine: The search at 80% provided identifications for the 172
384 specimens (Fig 4A, S2 Table). A match in agreement with the taxonomist was found for
385 82 (80%) specimens of 103 at the genus level. For species, the search resulted in 55
386 (78%) specimens of 69 total. Based on this information, these records were classified
387 as 137 (80%) in agreement with the taxonomist and 35 (20%) in disagreement (Table 3,
388 Fig 4A).

389 **Table 3. Summary of matches between the taxonomist and BOLD identifications for the subfamily of dung**

390 **beetles Scarabaeinae.** The first section (I) corresponds to the initial matches performed in agreement with the taxonomist

391 after the search engine in BOLD at 80%. The second section (II) in grey includes the categories used by the taxonomist to

392 reevaluate the identifications provided by BOLD in (I) and the final matches in agreement.

				Matches BOLD & Taxonomist	**Count**	**Total**	**%**	**Difference % (disagreement with taxonomist)**
(I) BOLD search engine identifications				Genus	82	103	80	
				Species	55	69	78	
				Sum agreement	**137**	172	**80**	20
	Categories	**Count**	**%**					
(II) Taxonomist corroborated identifications – *de novo*	Correctly identified genus	52	30	Genus	52	73	71	
	Correctly identified species	77	45	Species	95	99	96	
	Probable correctly identified species	18	10					
	Incorrect identification	25	15	**Sum agreement**	**147**	172	**85**	15

Fig 4. Results of the reanalysis performed by the taxonomist in the subfamily of dung beetles Scarabaeinae (Coleoptera: Scarabaeidae). A) Comparison between the identification (ID) matches before (I, BOLD IDs) and after (II, taxonomist – *de novo* IDs) reanalyzing the data. B) Percentage of the categories assigned by the taxonomist to the identifications suggested with BOLD's search engine at 80%.

Then, we corroborated *de novo* the identification for these 172 records of dung beetle with a taxonomist (Fig 4B). The *de novo* specimens were assigned to the same 16 genera and 27 species (70 specimens), in addition to 31 morphospecies (genus and code "H", 97 specimens) (Table 4 and S4 Table). The new review resulted in 95 records (55%) correctly matched the taxonomist species identification or was deemed likely to be the species (Correctly identified species and Probable correctly identified species categories). At the genus level, 52 records (30%) correctly matched the taxonomist genus identification but either were not accurately matched with the species or the species identification was not provided (Correctly identified genus category). There were 25 records (15%) at the genus level which did not match the identification of the taxonomist and arguments against BOLD identification were provided by the taxonomist (Incorrect identification category) (S4 Table). Therefore, the records previously classified as 137 (80%) increased to 147 (85%) in agreement with the taxonomist and were reduced from 35 (20%) to 25 (15%) in disagreement based on the BOLD search engine at 80% (Table 3, Fig 4A).

415 **Table 4. List of Scarabaeinae species identified by BOLD in agreement with the taxonomist's *de novo***

416 **identification.** Morphospecies with genus and code "H" are included [24]. Only categories for correct species and

417 probably correct species are presented here.

Process ID	BIN	Current ID	% Match	Overlap (bp)	Taxonomist's *de novo* ID	Match Species BOLD	Match BIN	Match notes
CBIHM686-18	BOLD:ACW4694	*Ateuchus*	93.4	568	*Ateuchus aeneomicans*	*Ateuchus aeneomicans*	BOLD:AAW8107	Correct species
CBIHI151-18	BOLD:ADL3091	*Canthon juvencus*	99.85	567	*Canthon juvencus*	*Canthon juvencus*	BOLD:ADL3091	Correct species
CBIHM706-18	BOLD:ADL3091	*Canthon juvencus*	99.85	567	*Canthon juvencus*	*Canthon juvencus*	BOLD:ADL3091	Correct species
CBIHM705-18	BOLD:ADL3091	*Canthon juvencus*	99.69	566	*Canthon juvencus*	*Canthon juvencus*	BOLD:ADL3091	Correct species
CBIHM633-18		*Canthon*	98.2	563	*Sylvicanthon aequinoctialis* [30]	*Canthon aequinoctialis*	BOLD:ABA2649	Correct species
CBIHM627-18	BOLD:ABA2649	*Canthon*	98.01	561	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialis*	BOLD:ABA2649	Correct species
CBIHM635-18	BOLD:ABA2649	*Canthon*	97.55	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialis*	BOLD:ABA2649	Correct species
CBIHM636-18	BOLD:ABA2649	*Canthon*	97.55	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialis*	BOLD:ABA2649	Correct species
CBIHM632-18	BOLD:ABA2649	*Canthon*	97.25	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialis*	BOLD:ABA2649	Correct species
CBIHM631-18	BOLD:ABA2647	*Canthon*	100	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis1*	BOLD:ABA2647	Correct species
CBIHM637-18	BOLD:ABA2647	*Canthon*	100	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis1*	BOLD:ABA2647	Correct species
CBIHM638-18	BOLD:ABA2647	*Canthon*	100	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis1*	BOLD:ABA2647	Correct species
CBIHM634-18	BOLD:ABA2647	*Canthon*	99.85	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis1*	BOLD:ABA2647	Correct species
CBIHM629-18	BOLD:ABA2649	*Canthon*	98.17	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis3*	BOLD:ABA2649	Correct species
CBIHM628-18	BOLD:ABA2649	*Canthon*	98.08	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis3*	BOLD:ABA2649	Correct species
CBIHM639-18	BOLD:ABA2649	*Canthon*	97.86	570	*Sylvicanthon aequinoctialis*	*Canthon aequinoctialisASolis3*	BOLD:ABA2649	Correct species
CBIHM747-18	BOLD:ACW3356	*Canthon subhyalinus*	100	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM673-18	BOLD:ACW3356	*Canthon subhyalinus*	100	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM674-18	BOLD:ACW3356	*Canthon subhyalinus*	100	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species

CBIHM727-18	BOLD:ACW3356	*Canthon subhyalinus*	100	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM744-18	BOLD:ACW3356	*Canthon subhyalinus*	100	567	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM718-18	BOLD:ACW3356	*Canthon subhyalinus*	99.24	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM716-18	BOLD:ACW3356	*Canthon subhyalinus*	98.93	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM717-18	BOLD:ACW3356	*Canthon subhyalinus*	98.93	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHM741-18	BOLD:ACW3356	*Canthon*	100	570	*Canthon subhyalinus*	*Canthon subhyalinus*	BOLD:ACW3356	Correct species
CBIHI156-18	BOLD:ADO2303	*Canthon triangularis*	100	570	*Canthon triangularis*	*Canthon triangularis*	BOLD:ADO2303	Correct species
CBIHI157-18	BOLD:ADO2303	*Canthon triangularis*	100	570	*Canthon triangularis*	*Canthon triangularis*	BOLD:ADO2303	Correct species
CBIHM659-18	BOLD:ADL4806	*Coprophanaeus corythus*	97.24	540	*Coprophanaeus corythus*	*Coprophanaeus corythus*	BOLD:ACW3300	Correct species
CBIHM687-18	BOLD:ADL4607	*Coprophanaeus gamezi*	94.65	570	*Coprophanaeus gamezi*	*Coprophanaeus gamezi*	BOLD:ACW3623	Correct species
CBIHI019-17	BOLD:ADJ7028	*Deltochilum mexicanum*	95.26	570	*Deltochilum mexicanum*	*Deltochilum mexicanum*	BOLD:AAP9113	Correct species
CBIHI159-18	BOLD:ACW3574	*Dichotomius*	95.61	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM648-18	BOLD:ACW3574	*Dichotomius*	95.61	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM667-18	BOLD:ACW3574	*Dichotomius*	95.36	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM666-18	BOLD:ACW3574	*Dichotomius*	95.28	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM646-18	BOLD:ACW3574	*Dichotomius*	95.23	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM647-18	BOLD:ACW3574	*Dichotomius*	95.21	567	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM645-18	BOLD:ACW3574	*Dichotomius*	95.12	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHM665-18	BOLD:ACW3574	*Dichotomius*	95.12	570	*Dichotomius agenor*	*Dichotomius agenor*	BOLD:ABA2814	Correct species
CBIHI144-18	BOLD:AAC9021	*Digitonthophagus gazella*	100	570	*Digitonthophagus gazella*	*Digitonthophagus gazella*	BOLD:AAC9021	Correct species
CBIHM640-18	BOLD:ADL2649	*Eurysternus caribaeus*	96.48	570	*Eurysternus caribaeus*	*Eurysternus caribaeus*	BOLD:ABA7207	Correct species
CBIHI145-18	BOLD:ACW4232	*Eurysternus caribaeus*	100	570	*Eurysternus caribaeus*	*Eurysternus caribaeus*	BOLD:ACW4232	Correct species
CBIHI146-18	BOLD:ACW4232	*Eurysternus caribaeus*	100	570	*Eurysternus caribaeus*	*Eurysternus caribaeus*	BOLD:ACW4232	Correct species
CBIHI147-18	BOLD:ACW4232	*Eurysternus caribaeus*	100	570	*Eurysternus caribaeus*	*Eurysternus caribaeus*	BOLD:ACW4232	Correct species
CBIHI023-17	BOLD:ADJ7677	*Eurysternus foedus*	95.57	570	*Eurysternus foedus*	*Eurysternus foedus*	BOLD:ABU6799	Correct species
CBIHI030-17	BOLD:AAX0271	*Onthophagus acuminatus*	100	570	*Onthophagus acuminatus*	*Onthophagus acuminatusAS1*	BOLD:AAX0271	Correct species
CBIHM653-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species

CBIHM654-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM655-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM656-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM660-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM661-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM662-18	BOLD:ADL2546	*Eurysternus mexicanus*	100	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM657-18	BOLD:ADL2546	*Eurysternus mexicanus*	99.83	570	*Eurysternus mexicanus*	*Eurysternus mexicanus*	BOLD:ADL2546	Correct species
CBIHM698-18	BOLD:ADL4924	*Malagoniella astyanax*	94.34	570	*Malagoniella astyanax*	*Malagoniella astyanax*	BOLD:ABA3615	Correct species
CBIHM701-18	BOLD:ACW3925	*Ontherus appendiculatus*	99	558	*Ontherus appendiculatus*	*Ontherus appendiculatus*	BOLD:ACW3925	Correct species
CBIHM703-18	BOLD:ACW3925	*Ontherus appendiculatus*	99	570	*Ontherus appendiculatus*	*Ontherus appendiculatus*	BOLD:ACW3925	Correct species
CBIHM699-18	BOLD:ACW3925	*Ontherus appendiculatus*	98.95	570	*Ontherus appendiculatus*	*Ontherus appendiculatus*	BOLD:ACW3925	Correct species
CBIHM702-18	BOLD:ACW3925	*Ontherus appendiculatus*	98.5	558	*Ontherus appendiculatus*	*Ontherus appendiculatus*	BOLD:ACW3925	Correct species
CBIHI160-18		*Onthophagus curvicornis*	99.82	552	*Onthophagus curvicornis*	*Onthophagus curvicornis*	BOLD:ADM9496	Correct species
CBIHI142-18	BOLD:ADM9497	*Onthophagus curvicornis*	98.17	570	*Onthophagus curvicornis*	*Onthophagus curvicornis*	BOLD:ADM9497	Correct species
CBIHI161-18	BOLD:ADM9497	*Onthophagus curvicornis*	98.17	570	*Onthophagus curvicornis*	*Onthophagus curvicornis*	BOLD:ADM9497	Correct species
CBIHI162-18	BOLD:ADM9496	*Onthophagus curvicornis*	99.82	567	*Onthophagus curvicornis*	*Onthophagus curvicornis*		Correct species
CBIHM719-18	BOLD:ADL5334	*Onthophagus lebasi*	99.84	569	*Onthophagus lebasi*	*Onthophagus lebasi*	BOLD:ADL5334	Correct species
CBIHM735-18	BOLD:ADL5334	*Onthophagus lebasi*	99.84	567	*Onthophagus lebasi*	*Onthophagus lebasi*	BOLD:ADL5334	Correct species
CBIHM726-18	BOLD:ADL5334	*Onthophagus lebasi*	99.35	567	*Onthophagus lebasi*	*Onthophagus lebasi*	BOLD:ADL5334	Correct species
CBIHM721-18	BOLD:ADL5334	*Onthophagus lebasi*	98.61	566	*Onthophagus lebasi*	*Onthophagus lebasi*	BOLD:ADL5334	Correct species
CBIHI152-18	BOLD:ADM9873	*Phanaeus bispinus*	96.94	570	*Phanaeus bispinus*	*Phanaeus bispinus*	BOLD:ACW4591	Correct species
CBIHI153-18	BOLD:ADN3451	*Phanaeus haroldi*	98.62	570	*Phanaeus haroldi*	*Phanaeus haroldi*	BOLD:ADN3451	Correct species
CBIHI154-18	BOLD:ADN3451	*Phanaeus haroldi*	98.62	570	*Phanaeus haroldi*	*Phanaeus haroldi*	BOLD:ADN3451	Correct species
CBIHM712-18	BOLD:ACW3786	*Sulcophanaeus leander*	99.83	570	*Sulcophanaeus leander*	*Sulcophanaeus leander*	BOLD:ACW3786	Correct species
CBIHM713-18	BOLD:ACW3786	*Sulcophanaeus leander*	99.68	570	*Sulcophanaeus leander*	*Sulcophanaeus leander*	BOLD:ACW3786	Correct species
CBIHM711-18	BOLD:ACW3786	*Sulcophanaeus leander*	99.66	570	*Sulcophanaeus leander*	*Sulcophanaeus leander*	BOLD:ACW3786	Correct species

CBIHM729-18	BOLD:ADL4592	*Uroxys micros*	100	504	*Uroxys micros*	*Uroxys micros*	BOLD:ADL4592	Correct species
CBIHM731-18	BOLD:ADL4592	*Uroxys micros*	100	501	*Uroxys micros*	*Uroxys micros*	BOLD:ADL4592	Correct species
CBIHM740-18	BOLD:ADL4592	*Uroxys micros*	100	504	*Uroxys micros*	*Uroxys micros*	BOLD:ADL4592	Correct species
CBIHM746-18	BOLD:ADL4592	*Uroxys micros*	99.83	513	*Uroxys micros*	*Uroxys micros*	BOLD:ADL4592	Correct species
CBIHM739-18	BOLD:ADL4592	*Uroxys micros*	99.16	494	*Uroxys micros*	*Uroxys micros*	BOLD:ADL4592	Correct species
CBIHI025-17	BOLD:ADK7115	*Canthidium*	95.39	567	*Canthidium* sp. 21H	*Canthidium centrale*	BOLD:ABU9359	Probably correct species
CBIHM650-18	BOLD:ACW2842	*Canthon*	94.97	567	*Canthon* sp. 06H	*Canthon cyanellus*	BOLD:ADN2169	Probably correct species
CBIHM651-18	BOLD:ACW2842	*Canthon*	94.97	567	*Canthon* sp. 06H	*Canthon cyanellus*	BOLD:ADN2169	Probably correct species
CBIHM663-18	BOLD:ACW2842	*Canthon*	94.9	570	*Canthon* sp. 06H	*Canthon cyanellus*	BOLD:ADN2169	Probably correct species
CBIHM664-18	BOLD:ACW2842	*Canthon*	94.9	570	*Canthon* sp. 06H	*Canthon cyanellus*	BOLD:ADN2169	Probably correct species
CBIHM649-18	BOLD:ACW2842	*Canthon*	94.85	567	*Canthon* sp. 06H	*Canthon cyanellus*	BOLD:ADN2169	Probably correct species
CBIHM742-18	BOLD:ACW4541	*Canthon*	96.83	570	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM668-18	BOLD:ACW4541	*Canthon*	96.62	567	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM669-18	BOLD:ACW4541	*Canthon*	96.62	567	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM670-18	BOLD:ACW4541	*Canthon*	96.62	567	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM730-18	BOLD:ACW4541	*Canthon*	96.62	567	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM732-18	BOLD:ACW4541	*Canthon*	96.6	570	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM820-20		*Canthon*	95.11	408	*Canthon* sp. 09H	*Canthon caelius*	BOLD:ABU7368	Probably correct species
CBIHM683-18	BOLD:ADL3950	*Canthon*	95.01	570	*Canthon* sp. 23H	*Canthon bispinus*	BOLD:ADR8659	Probably correct species
CBIHM684-18	BOLD:ADL3950	*Canthon*	95.01	570	*Canthon* sp. 23H	*Canthon bispinus*	BOLD:ADR8659	Probably correct species
CBIHI148-18	BOLD:AAX0252	*Onthophagus*	98.04	570	*Onthophagus* sp. 07H	*Onthophagus haematopus*	BOLD:AAX0252	Probably correct species
CBIHI150-18	BOLD:AAX0252	*Onthophagus*	98.04	570	*Onthophagus* sp. 07H	*Onthophagus haematopus*	BOLD:AAX0252	Probably correct species
CBIHI149-18	BOLD:AAX0252	*Onthophagus*	97.88	570	*Onthophagus* sp. 07H	*Onthophagus haematopus*	BOLD:AAX0252	Probably correct species

418

419 Correctly identified species had a percentage match range in BOLD from 93.4 to
420 100 (Fig 5). Probable correctly identified species ranged from 94.85 to 98.04. Correctly
421 identified genus range was between 87.7 and 100%. Incorrect identification
422 percentages range from 83.59 to 99.83. The categories were significantly different
423 (Kruskal-Wallis $H = 73.4$, $P < 0.001$). The correctly identified species ($P < 0.001$),
424 probable correctly identified species ($P < 0.05$) and correctly identified genus ($P < 0.05$)
425 categories were different from the incorrect identifications.
426
427 **Fig 5. Boxplot of Scarabaeinae case of study showing the range of BOLD**
428 **percentage matches for the categories: Correctly Identified Species, Probable**
429 **Correctly Identified Species, Correctly Identified Genus, and Incorrect**
430 **Identification.** The median is represented by a line, the mean is represented by an X
431 markers and outliers are the white dots.

432 Discussion

433 BOLD systems outperformed BLAST providing more accurate insect identifications.
434 This overall pattern was found for all taxonomic levels evaluated (species, genus and
435 family). Although differences were found between taxa, BOLD consistently provided
436 higher identifications for the order Coleoptera at all taxonomic levels and for
437 Lepidoptera at genus and species level. For instance, Hymenoptera and Odonata
438 performed similarly for both repositories at all taxonomic levels, alike most of the other
439 orders at the family level when analyzed separately. These results are partially in
440 disagreement with Meiklejohn et al [21] which reported no significant differences

441 between BOLD and GenBank for insect samples identification at genus and species
442 level. A further review of their data showed that correct identifications from both
443 platforms were closer than estimated previously [22]. Although we report different
444 results in the performance of BOLD compared to GenBank in this study, a key factor to
445 consider is the magnitude of insect data involved. Meiklejohn et al [21], who studied 17
446 specimens in 12 orders at genus and species level using COI whereas we included
447 1160 specimens in eight orders at family, genus and species level. BOLD Systems is a
448 platform developed with the intention of providing species identification and in that
449 sense does not perform equally to GenBank, but both are the main public repositories
450 often used to provide identifications. Often BOLD is considered to contain data more
451 curated, but GenBank data quality is good overall [22,31]. Differences in identifications
452 of taxa can be considered due to differential taxonomic curation of the databases and
453 the lack of barcodes of reference from Colombia. However, we could not identify a clear
454 pattern with the lack of references. The most represented groups were Coleoptera and
455 Lepidoptera. The group that performed the best with identifications was Coleoptera and
456 it was also the order that provided the most unique BINS, in contrast with Lepidoptera
457 that provided the least unique BINS also outperformed GenBank at the species and
458 genus level, but no differences were found at family.

459 A great potential of Colombian insect biodiversity was found from BINs counting
460 with 70% of the sequences assigned to a unique BIN. Each BIN represents a cluster of
461 species or an operational taxonomic unit (OTU) that is compared against the database
462 assigning the barcodes to known clusters (BIN) or creating a new cluster (unique BIN)
463 [18]. Unique BINs were found across all the orders of insects and Coleoptera provided

464 the highest count of new BINs followed by Diptera and Hymenoptera, some of the
465 hyperdiverse orders of insects. However, Lepidoptera was the order with highest non-
466 unique BINs. This finding is likely attributable to a great effort of DNA sampling directed
467 to Lepidoptera that displays 4848 barcodes from the country with 1474 BINs [32]; in
468 comparison to Coleoptera with 318 sequences in 82 BINs, Diptera 2646 with 339 BINs
469 and Hymenoptera 2179 with 240 BINs [32].

470 Furthermore, our discovery of 500 unique BINs from our dataset of 1088
471 sequences showcases the need for further sampling and identification in order to add
472 more species into these databases and increase the accuracy of matches. However,
473 this would require the implementation of a systematic method of collecting samples
474 across Colombia, a process that could take a considerable length of time and amount of
475 labor. Of our seven collecting methods used in this study, the malaise traps accounted
476 for 62.6%, and we would suggest a targeted approach by placing these traps in
477 strategic areas across Colombia. We expect that a larger sample will continue providing
478 a higher number of unique BINs, but also, we see a necessary taxonomic effort to
479 match all of this genetic diversity with the insect species already described from
480 Colombia. Nevertheless, the implementation of DNA barcodes in the country will bring
481 an opportunity to recognize any hidden diversity. As it has been suggested that BINs
482 underestimate by a 10% the species due to lack of power within close species
483 [11,33,34]. Although Meier et al [35] suggested awareness on the use of BINS as
484 identification tool specially when use for species descriptions. Therefore, an integrative
485 approach with morphology and genetic diversity can boost the recognition of species.

486 A closer look to the subfamily Scarabaeinae displayed a great performance of
487 identification at different levels. The percentage of similarity for a match (BOLD match
488 percentage) to identify species correctly in Scarabaeinae was in a wide range between
489 93 to a 100%. Although correct matches were statistically significant the range was not
490 perfect, as shown at least three specimens were incorrectly identified with values higher
491 than 94%. These outliers do not reduce the potential of genetic identification tools. The
492 high percentages could be due to specimen mix-up. Overall, the identification success
493 rate of Scarabaeinae was 85% (30% towards genera and 55% towards species) when
494 combining the barcode results with a taxonomist effort (Table 3, Fig 4A). The species
495 identification improved from 78% to 96% (or in the overall from 30% to 55%) with this
496 taxonomist *de novo* identifications (Table 3, Fig 4A). Other studies of identification of
497 beetles using barcodes found species matches as high as 92.1% of their samples when
498 considered traditionally identified species and BINS and as high as 98.3% of the
499 samples when haplotypes were considered [36]. In comparison, our identification
500 percentage was lower with previous studies. These can be due to the lack of sequence
501 records of the species in the database. Pentisaari et al [36] suggested identification
502 failures due to difficulty with morphological characters and controversial taxonomic
503 status of species. Taxonomic input is crucial for the function of this tool, also if the
504 species is not represented in the database there is no possible match to occur. In fact, a
505 few suggestions provided by BOLD for incorrect species identifications were for close
506 related species or species within their group of species. For example, *Dichotomius*
507 *andresi* and *Dichotomius protectus* were matched with *Dichotomius satanas*, a
508 morphologically related species in the "*Dichotomius satanas* species group". Similarly,

Eurysternus hypocrita was identified as *Eurysternus olivaceus*, a species in the "*Eurysternus velutinus* species group". The specimen identified as *Phanaeus pyrois* was matched as *Phanaeus malyi*, a species that was long considered synonymous with *P. pyrois*. Currently, *P. malyi* has been revalidated and registered in Colombia without a specific location [37].

The distribution range of species could be an additional factor as observed with the dung beetles data where BOLD identify all species correctly in six genera (*Coprophanaeus, Digitonthophagus, Malagoniella, Onthophagus, Phanaeus* and *Sulcophanaeus*), eight genera partially correct with some of the species identified correctly but not all (*Ateuchus, Canthidium, Canthon, Deltochilum, Dichotomius, Eurysternus, Ontherus* and *Uroxys*) and failed completely for the genera *Pseudocanthon* (1 specimen) and *Scybalocanthon* (6 specimens). The correctly identified species were obtained for species with a wide distribution and shared between South America and Central America, such as *Canthon juvencus*, *Canthon subhyalinus*, *Eurysternus caribaeus*, *Eurysternus foedus*, *Eurysternus mexicanus* *Onthophagus acuminatus, Onthophagus lebasi*, and *Uroxys micros*, most likely due to representativeness of COI sequences of these species from Costa Rica [38]. These species are found across countries or ecosystems (e. g. dry and humid forests, natural and disturbed ecosystems). In contrast, the identification was incorrect for some species with restricted distributions in Colombia such as *Canthon arcabuquensis, Dichotomius andresi*, *Uroxys cuprescens*, and *Deltochilum susanae,* the latest was first identified as the morphospecies *Deltochilum* sp. 12H [39] and later described as a new species [40] or species with Andean distributions as in *Dichotomius protectus*, *Eurysternus*

marmoreus and *Ontherus brevicollis* [41–46]. In the case of Scarabaeinae dung beetles, the barcode results helped to confirm at least five species that were assigned as morphospecies in the dung beetles of Colombia “Reference Collection” hosted at of IAVH Entomological Collection. The diversity of dung beetle’s subfamily Scarabaeinae in Colombia exceeds by a large number of species that of neighboring countries such as Panama, Ecuador or Brazil. In general, a defined and well-identified species in Colombia has other species, morphologically similar and taxonomically closer, that have been assigned as morphospecies. This is why the Reference Collection was created, which so far houses about 85% percent of species of Colombia as morphospecies. We see the barcode as a fundamental integrative taxonomy tool, which will help to outline these morphospecies and define which of those are new species for science, and to improve the final taxonomic list of this important group of beetles. Overall, there are still challenges to overcome and more research is recommended in this topic. The reference databases keep improving and updating their resources and tools for users [20,31]. BOLD provided reliable identifications for the tested groups of insects overcoming challenges such as a small genetic reference of insects for a highly diverse country.

Acknowledgements

We are grateful to our funding sources: A Minciencias postdoctoral fellowship Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación "Francisco José de Caldas" call No. 848-2019 and Humboldt Institute (awarded to NBB), Colombia BIO Agreement # FP 44842-109-2016 (IAvH No. 16-062) between Colciencias (now Minciencias) and Humboldt Institute, and Santander BIO inter-agency Agreement #2243

(IAvH No. 17-199) with the General System of Royalties of the Santander government, Colombia's National Planning Department (BPIN 2017000100046), Humboldt Institute and Industrial University of Santander (BPIN 2017000100046). We would like to thank the following researchers for their fieldwork efforts collecting the samples and/or for identifications of samples: H Arenas, C Beltran, C Bota, J Cardenas, C Castro, A Clavijo, Y Correa, A Diaz, D Espitia, G Fagua, A González, D Hincapie, A Lopera, J Mendivil, R Morales, EE Palacio, A Parrales, P Pulido, JD Suaza, EA Tenorio, E Torres, X. Villalba. We want to thank to the valuable Entomological collections where these samples were deposited: the Entomological and Tissue collection of the Institute Alexander von Humboldt (IAvH-E and IAvH-CT), Museo Francisco Luis Gallego (MEFLG) at Universidad Nacional de Colombia, sede Medellín, Museo Javeriano de Historia Natural (MPUJ) at Pontificia Universidad Javeriana and Colección Entomológica Universidad de Antioquia (CEUA). Thank you to the IAvH Conservation Genetic lab members: P Montoya, P Pulido, N Franco, V Sandoval, C Quiroga, and Y Castañeda for their valuable comments and improvements to the manuscript.

Author Contributions

Conceptualization: Nathalie Baena-Bejarano, Mailyn Gonzalez.

Data curation: Nathalie Baena-Bejarano, Catalina Reina, Diego E. Martínez-Revelo.

Formal analysis: Nathalie Baena-Bejarano.

Investigation: Nathalie Baena-Bejarano, Diego E. Martínez-Revelo, Claudia A. Medina, Jhon Cesar Neita, Catalina Reina, Eduardo Tovar, Sandra Uribe, Mailyn Gonzalez.

Methodology: Nathalie Baena-Bejarano, Catalina Reina, Diego E. Martínez-Revelo, Claudia A. Medina, Eduardo Tovar, Mailyn Gonzalez.

Project administration: Nathalie Baena-Bejarano, Mailyn Gonzalez, Jhon Cesar Neita.

Supervision: Mailyn Gonzalez, Jhon Cesar Neita, Nathalie Baena-Bejarano.

Resources: Mailyn Gonzalez, Claudia A. Medina, Jhon Cesar Neita, Eduardo Tovar, Diego E. Martínez-Revelo.

Writing – original draft Preparation: Nathalie Baena-Bejarano.

Writing – review & editing: Nathalie Baena-Bejarano, Diego E. Martínez-Revelo, Claudia A. Medina, Catalina Reina, Eduardo Tovar, Jhon Cesar Neita, Mailyn Gonzalez, Sandra Uribe.

References

1. Díaz S, Settele J, Brondízio E, Ngo H, Guèze M, Agard J, et al. IPBES (2019): Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Bonn, Germany: IPBES secretariat; 2019 p. 56. Available: https://www.ipbes.net/

2. Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, et al. Has the Earth's sixth mass extinction already arrived? Nature. 2011;471: 51–57. doi:10.1038/nature09678

3. CBD. Convention on Biological Diversity. 2019 [cited 2 Sep 2019]. Available: https://www.cbd.int/

596 4. Janzen DH, Hallwachs W. Perspective: Where might be many tropical insects? Biol
597 Conserv. 2019;233: 102–108. doi:10.1016/j.biocon.2019.02.030

598 5. Sánchez-Bayo F, Wyckhuys KAG. Worldwide decline of the entomofauna: A review
599 of its drivers. Biol Conserv. 2019;232: 8–27. doi:10.1016/j.biocon.2019.01.020

600 6. IUCN. The IUCN Red List of Threatened Species. 2022 [cited 4 Sep 2022].
601 Available: https://www.iucnredlist.org

602 7. Arbeláez-Cortés E. Knowledge of Colombian biodiversity: published and indexed.
603 Biodivers Conserv. 2013;22: 2875–2906. doi:10.1007/s10531-013-0560-y

604 8. Morinière J, Cancian de Araujo B, Lam AW, Hausmann A, Balke M, Schmidt S, et
605 al. Species Identification in Malaise Trap Samples by DNA Barcoding Based on
606 NGS Technologies and a Scoring Matrix. Fontaneto D, editor. PLOS ONE.
607 2016;11: e0155497. doi:10.1371/journal.pone.0155497

608 9. Arias-Penna DC, Whitfield JB, Janzen DH, Hallwachs W, Dyer LA, Smith MA, et al.
609 A species-level taxonomic review and host associations of Glyptapanteles
610 (Hymenoptera, Braconidae, Microgastrinae) with an emphasis on 136 new reared
611 species from Costa Rica and Ecuador. ZooKeys. 2019;890: 1–685.
612 doi:10.3897/zookeys.890.35786

613 10. Sharkey MJ, Janzen DH, Hallwachs W, Chapman EG, Smith MA, Dapkey T, et al.
614 Minimalist revision and description of 403 new species in 11 subfamilies of Costa
615 Rican braconid parasitoid wasps, including host records for 219 species. ZooKeys.
616 2021;1013: 1–665. doi:10.3897/zookeys.1013.55600

617 11. Janzen DH, Hallwachs W, Pereira G, Blanco R, Masis A, Chavarria MM, et al.
618 Using DNA-barcoded Malaise trap samples to measure impact of a geothermal
619 energy project on the biodiversity of a Costa Rican old-growth rain forest. Genome.
620 2020;63: 407–436. doi:10.1139/gen-2020-0002

621 12. Geiger M, Moriniere J, Hausmann A, Haszprunar G, Wägele W, Hebert P, et al.
622 Testing the Global Malaise Trap Program – How well does the current barcode
623 reference library identify flying insects in Germany? Biodivers Data J. 2016;4:
624 e10671. doi:10.3897/BDJ.4.e10671

625 13. Steinke D, Breton V, Berzitis E, Hebert PDN. The School Malaise Trap Program:
626 Coupling educational outreach with scientific discovery. PLOS Biol. 2017;15:
627 e2001829. doi:10.1371/journal.pbio.2001829

628 14. Kutty SN, Wang W, Ang Y, Tay YC, Ho JKI, Meier R. Next-Generation identification
629 tools for Nee Soon freshwater swamp forest, Singapore. Gard Bull Singap.
630 2018;70: 155–173. doi:10.26492/gbs70(suppl.1).2018-08

631 15. Karlsson D, Hartop E, Forshage M, Jaschhof M, Ronquist F. The Swedish Malaise
632 Trap Project: A 15 Year Retrospective on a Countrywide Insect Inventory.
633 Biodivers Data J. 2020;8: e47255. doi:10.3897/BDJ.8.e47255

634 16. Srivathsan A, Lee L, Katoh K, Hartop E, Kutty SN, Wong J, et al. ONTbarcoder and
635 MinION barcodes aid biodiversity discovery and identification by everyone, for
636 everyone. BMC Biol. 2021;19: 217. doi:10.1186/s12915-021-01141-x

637 17. Ratnasingham S, Hebert PDN. bold: The Barcode of Life Data System
638 (http://www.barcodinglife.org). Mol Ecol Notes. 2007;7: 355–364.
639 doi:10.1111/j.1471-8286.2007.01678.x

640 18. Ratnasingham S, Hebert PDN. A DNA-Based Registry for All Animal Species: The
641 Barcode Index Number (BIN) System. Fontaneto D, editor. PLoS ONE. 2013;8:
642 e66213. doi:10.1371/journal.pone.0066213

643 19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search
644 tool. J Mol Biol. 1990;215: 403–410. doi:10.1016/S0022-2836(05)80360-2

645 20. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, et al.
646 NCBI Taxonomy: a comprehensive update on curation, resources and tools.
647 Database. 2020;2020: baaa062. doi:10.1093/database/baaa062

648 21. Meiklejohn KA, Damaso N, Robertson JM. Assessment of BOLD and GenBank –
649 Their accuracy and reliability for the identification of biological materials. Fugmann
650 SD, editor. PLOS ONE. 2019;14: e0217084. doi:10.1371/journal.pone.0217084

651 22. Pentinsaari M, Ratnasingham S, Miller SE, Hebert PDN. BOLD and GenBank
652 revisited – Do identification errors arise in the lab or in the sequence libraries?
653 Kuntner M, editor. PLOS ONE. 2020;15: e0231814.
654 doi:10.1371/journal.pone.0231814

655 23. Becker JJ, Sandwell DT, Smith WHF, Braud J, Binder B, Depner J, et al. Global
656 Bathymetry and Elevation Data at 30 Arc Seconds Resolution: SRTM30_PLUS.
657 Mar Geod. 2009;32: 355–371. doi:10.1080/01490410903297766

24. González-Alvarado AF, Torres E, Medina CA. Escarabajos coprófagos (Coleoptera: Scarabaeidae: Scarabaeinae) de bosques secos colombianos en la Colección Entomológica del Instituto Alexander von Humboldt. Biota Colomb. 2015;16: 88–95.

25. Ivanova NV, Dewaard JR, Hebert PDN. An inexpensive, automation-friendly protocol for recovering high-quality DNA: Technical Note. Mol Ecol Notes. 2006;6: 998–1002. doi:10.1111/j.1471-8286.2006.01428.x

26. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3: 294–299.

27. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci. 2004;101: 14812–14817. doi:10.1073/pnas.0406166101

28. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28: 1647–1649. doi:10.1093/bioinformatics/bts199

29. Hammer O, Harper DAT, Ryan PD. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electron. 4: 9.

30. Cupello M, Vaz-de-Mello FZ. A monographic revision of the Neotropical dung beetle genus Sylvicanthon Halffter & Martínez, 1977 (Coleoptera: Scarabaeidae: Scarabaeinae: Deltochilini), including a reappraisal of the taxonomic history of 'Canthon sensu lato.' Eur J Taxon. 2018. doi:10.5852/ejt.2018.467

31. Madden MJL, Young RG, Brown JW, Miller SE, Frewin AJ, Hanner RH. Using DNA barcoding to improve invasive pest identification at U.S. ports-of-entry. Labra M, editor. PLOS ONE. 2019;14: e0222291. doi:10.1371/journal.pone.0222291

32. BOLD systems public data portal - record list Database. 2022 [cited 21 Jul 2022]. Available: https://www.boldsystems.org/

33. Janzen DH, Hallwachs W. DNA barcoding the Lepidoptera inventory of a large complex tropical conserved wildland, Area de Conservacion Guanacaste, northwestern Costa Rica. Genome. 2016;59: 641–660. doi:10.1139/gen-2016-0005

34. Janzen DH, Hallwachs W, Blandin P, Burns JM, Cadiou J-M, Chacon I, et al. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. Mol Ecol Resour. 2009;9: 1–26. doi:10.1111/j.1755-0998.2009.02628.x

35. Meier R, Blaimer BB, Buenaventura E, Hartop E, Rintelen T, Srivathsan A, et al. A re-analysis of the data in Sharkey et al.'s (2021) minimalist revision reveals that BINs do not deserve names, but BOLD Systems needs a stronger commitment to open science. Cladistics. 2022;38: 264–275. doi:10.1111/cla.12489

36. Pentinsaari M, Hebert PDN, Mutanen M. Barcoding Beetles: A Regional Survey of 1872 Species Reveals High Identification Success and Unusually Deep Interspecific Divergences. Fontaneto D, editor. PLoS ONE. 2014;9: e108651. doi:10.1371/journal.pone.0108651

37. Moctezuma V, Halffter G. Taxonomic revision of the Phanaeus endymion species group (Coleoptera: Scarabaeidae), with the descriptions of five new species. Eur J Taxon. 2021;747: 1–71. doi:10.5852/ejt.2021.747.1333

38. Solís Á, Kohlmann B. Checklist and distribution atlas of the Scarabaeinae (Coleoptera: Scarabaeidae) of Costa Rica. Zootaxa. 3482: 1–32. doi:10.11646/zootaxa.3482.1.1

39. Martínez-Revelo DE, Castro-Moreno C, Medina CA. Escarabajos coprófagos de la cuenca alta y media del río Bita, Vichada, Colombia. Biota Colomb. 2018;19: 226–235. doi:10.21068/c2018.v19n01a15

40. González-Alvarado A, Vaz-de-Mello FZ. Towards a comprehensive taxonomic revision of the Neotropical dung beetle subgenus Deltochilum (Deltohyboma) Lane, 1946 (Coleoptera: Scarabaeidae: Scarabaeinae): Division into species-groups. Zhang F, editor. PLOS ONE. 2021;16: e0244657. doi:10.1371/journal.pone.0244657

41. Génier F. A revision of the Neotropical genus Ontherus Erichson (Coleoptera: Scarabaeidae, Scarabaeinae). Mem Entomol Soc Can. 1996;128: 3–170. doi:10.4039/entm128170fv

42. Camero Rubio E, Lobo JM. The Distribution of the Species of *Eurysternus* Dalman, 1824 (Coleoptera: Scarabaeidae) in America: Potential Distributions and the Locations of Areas to be Surveyed. Trop Conserv Sci. 2012;5: 225–244. doi:10.1177/194008291200500210

43. Camero Rubio E, Lobo JM. Distribución conocida y potencial de las especies del género Eurysternus Dalman, 1824 (Coleoptera: Scarabaeidae) de Colombia. Bol SEA. 2010; 257–264.

44. Sarmiento-Garcés R, Amat-García G. Escarabajos del género Dichotomius Hope 1838 (Scarabaeidae: Scarabaeinae) en Colombia. Fauna de Colombia. Monografía 4. Bogotá, Colombia: Universidad Nacional de Colombia; 2014. doi:10.13140/2.1.4692.9926

45. Medina C, González A. Escarabajos coprófagos de la subfamilia Scarabaeinae. In: Pizano C, García H, editors. El Bosque Seco Tropical en Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH); pp. 195–213.

46. Chamorro W, Marin-Armijos D, Asenjo A, Vaz-De-Mello FZ. Scarabaeinae dung beetles from Ecuador: a catalog, nomenclatural acts, and distribution records. ZooKeys. 2019;826: 1–343. doi:10.3897/zookeys.826.26488

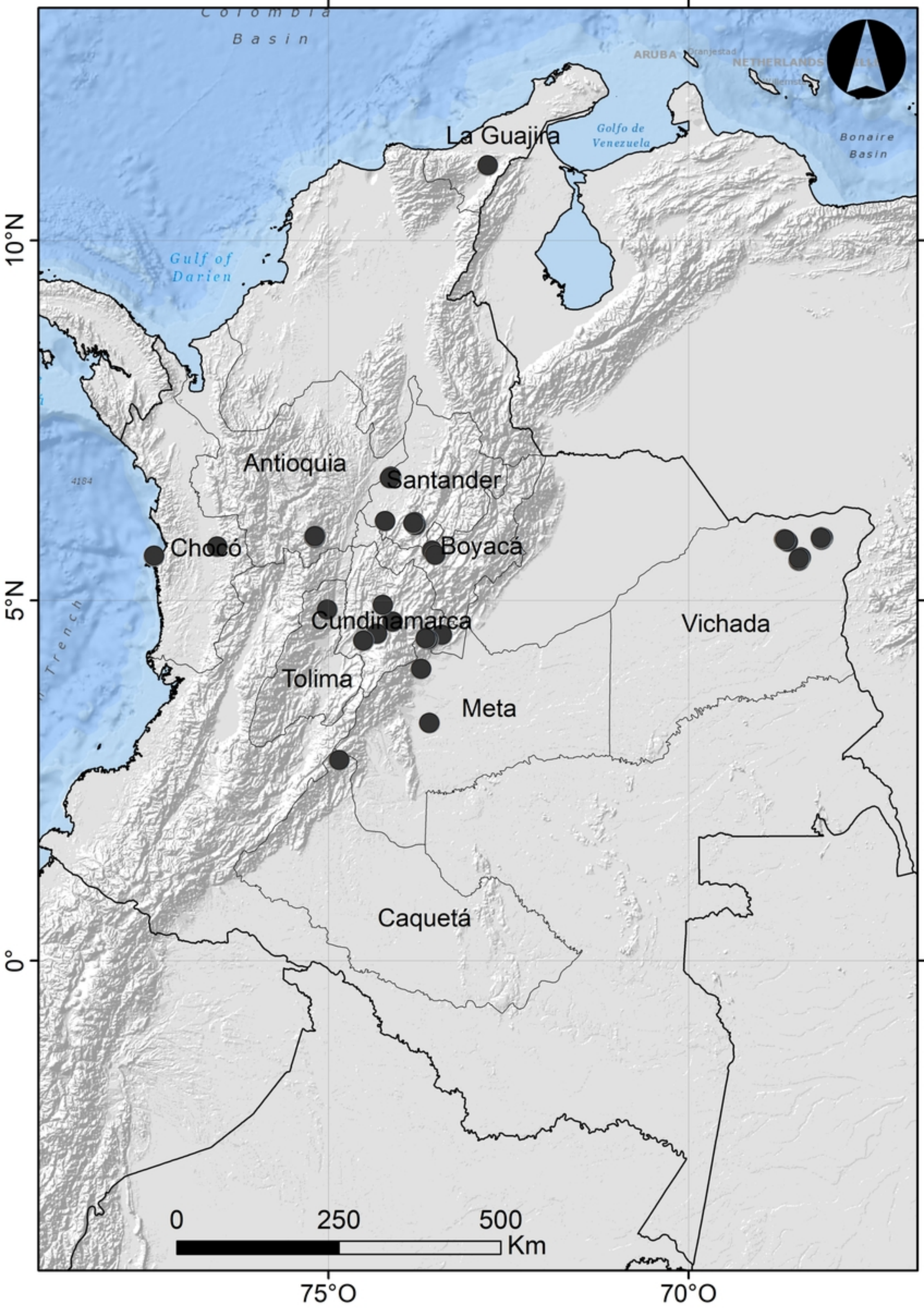
Supporting information captions

S1 Table. Project dataset with the insect records.

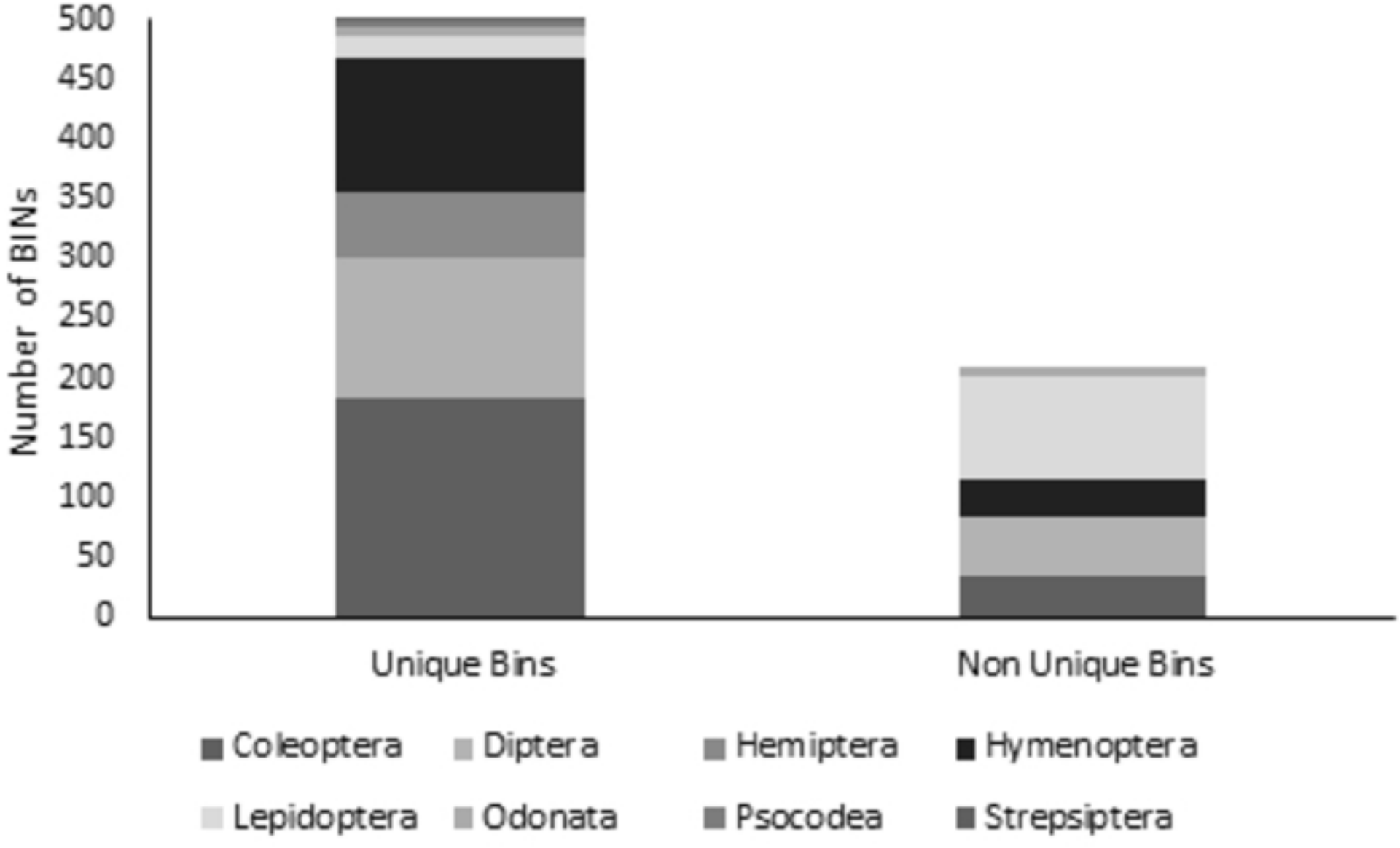
S2 Table. Records of insects by orders with matches from BOLD Batch ID engine and BLAST (GenBank).

S3 Table. Chi-squared tests. A) Dataset analyses including BOLD's first suggestion with the highest percentage match and base pair overlap. This dataset includes matches to sequences on our project. B) Alternative dataset analyses including next higher percentage match and base pair overlap. This excludes matches to sequences from our project. *Is next to orders where Chi-square was significant. **Is next to orders where Chi-square analyzed with Bonferroni correction was significant.

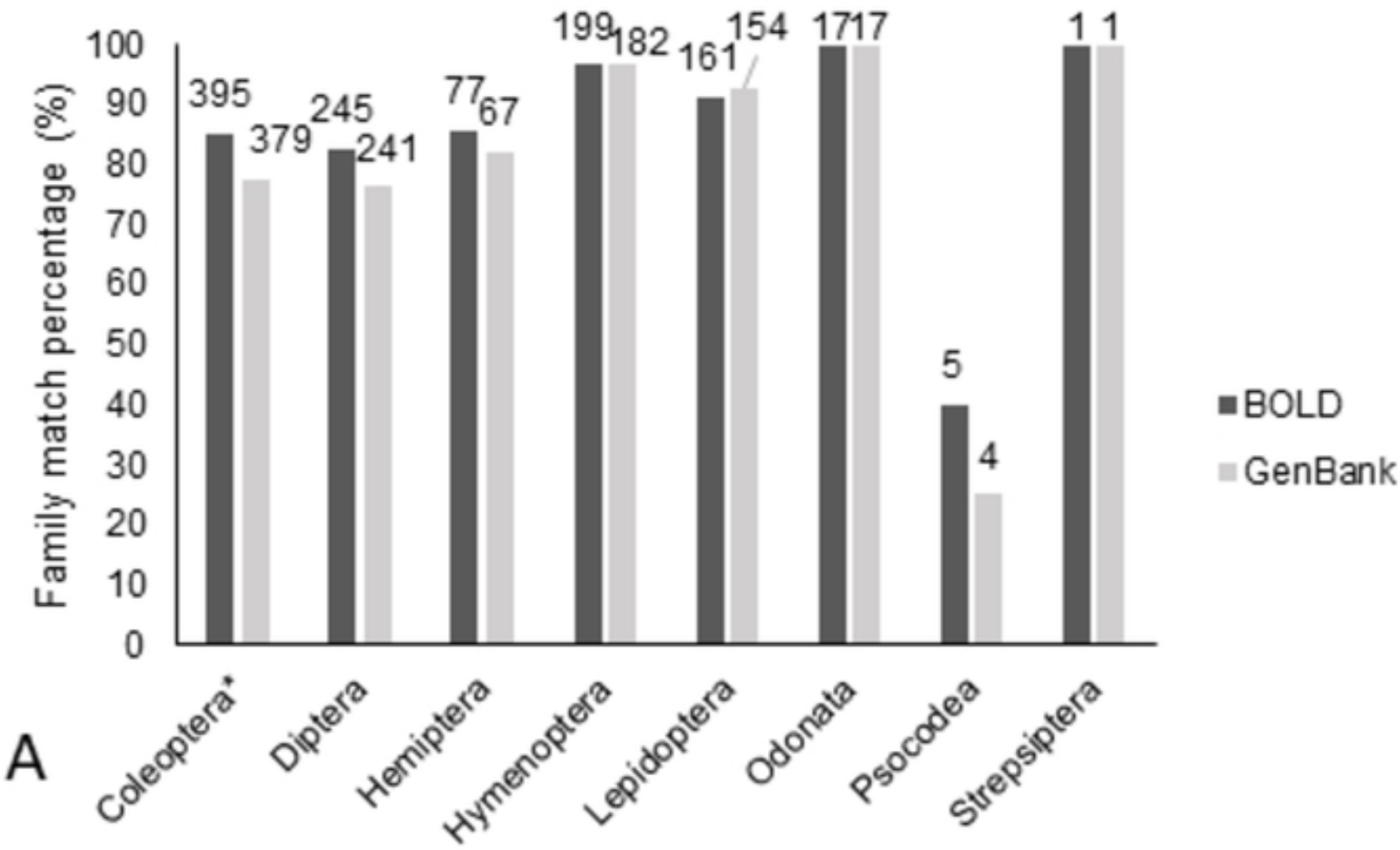
S4 Table. List of Scarabaeinae in other categories as determined by the taxonomist.

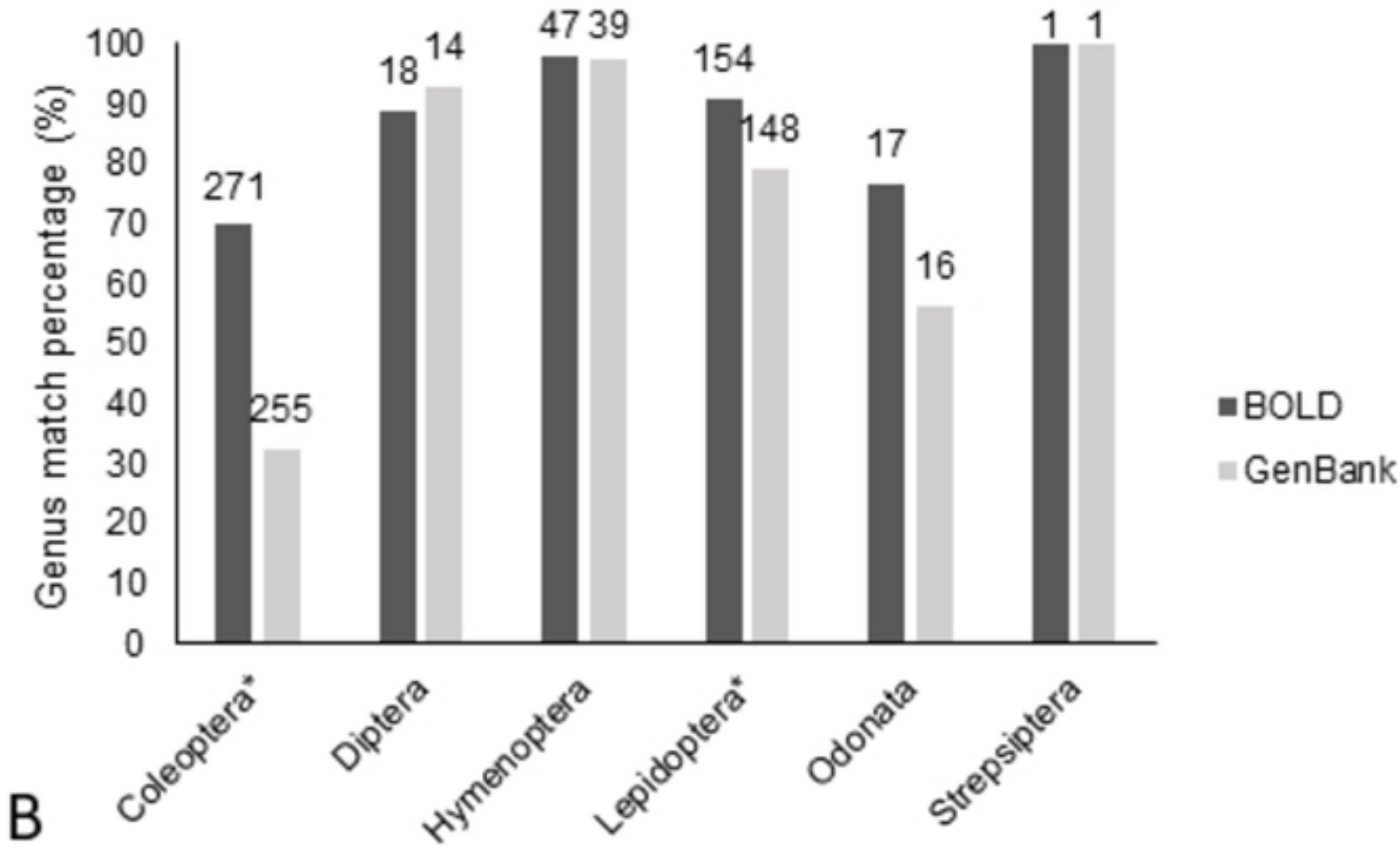

Colombia
Basin
ARUBA
Oranjestad
NETHERLANDS
La Guajira
Golfo de
Venezuela
Bonaire
Basin
10°N
Gulf of
Darien
Antioquia
Santander
Chocó
Boyacá
5°N
Cundinamarca
Vichada
Tolima
Meta
Caquetá
0°
0
250
500
Km
75°O
70°O

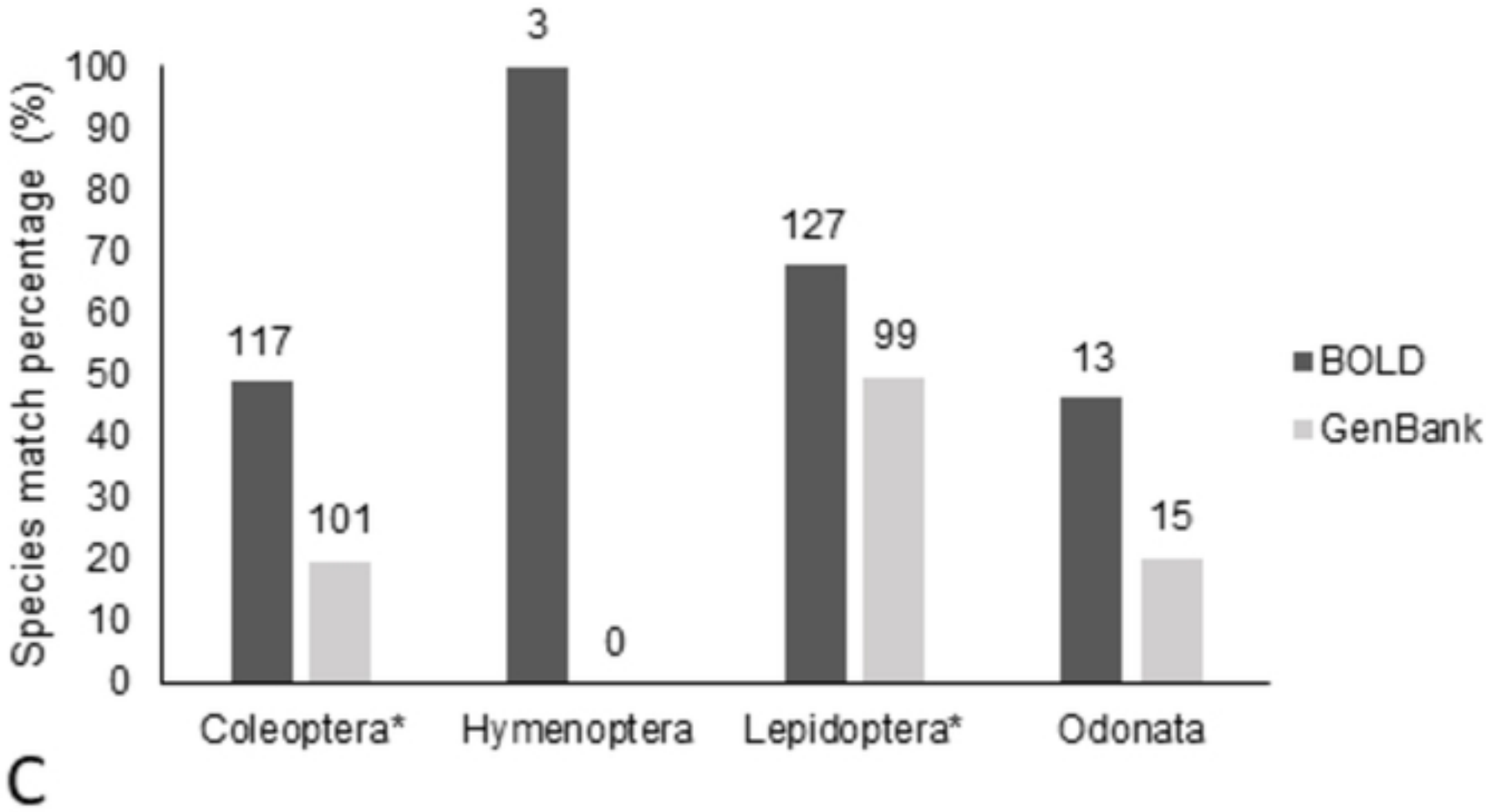
Figure


500
450
400
350
300
250
200
150
100
50
0
Number of BINs
Unique Bins
Non Unique Bins
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Odonata
Psocodea
Strepsiptera

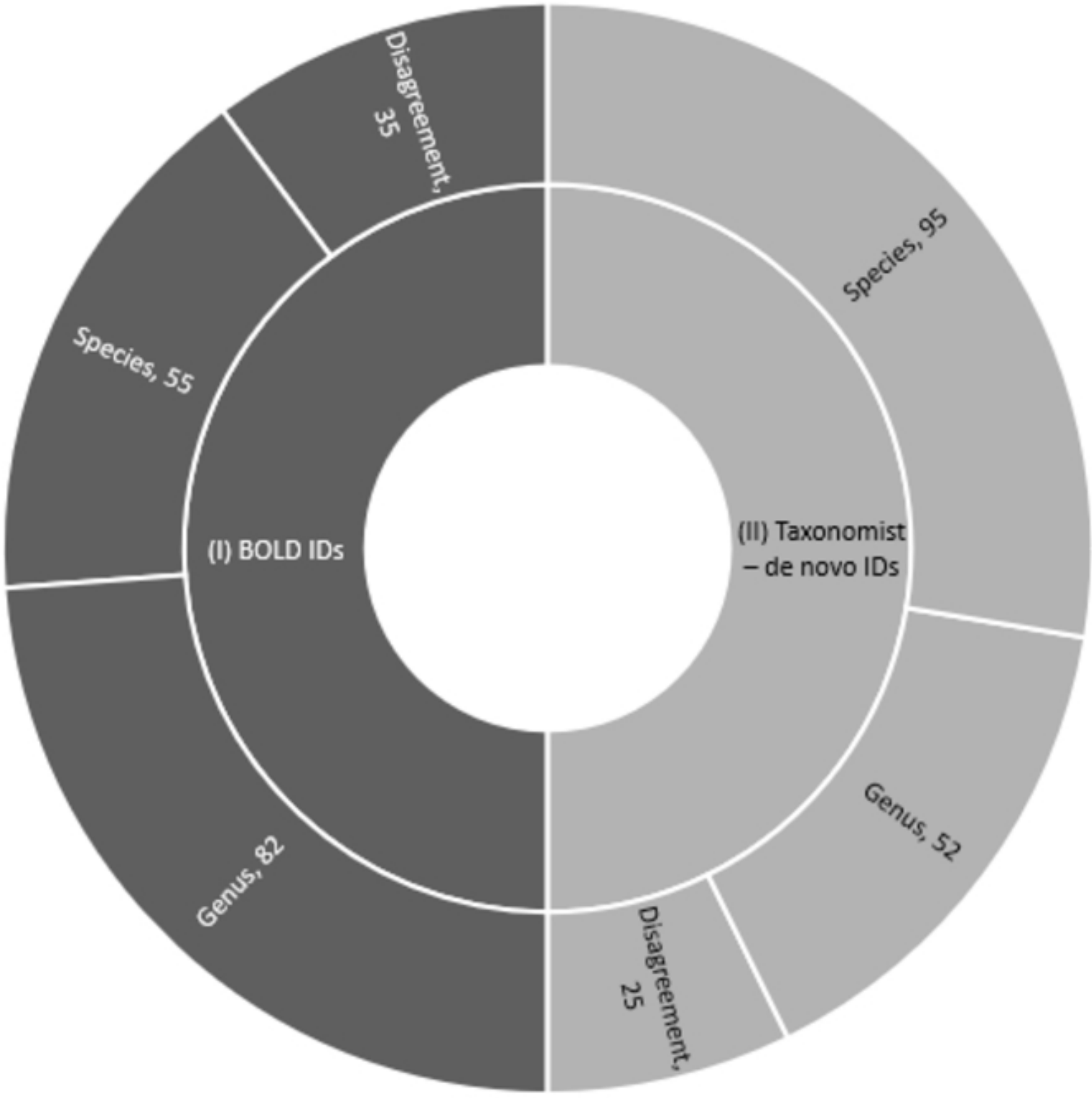
Figure


Family match percentage (%)
100
90
80
70
60
50
40
30
20
10
0
395
379
245
241
77
67
199
182
161
154
171
17
5
4
1
1
BOLD
GenBank
Coleoptera*
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Odonata
Psocodea
Strepsiptera
A

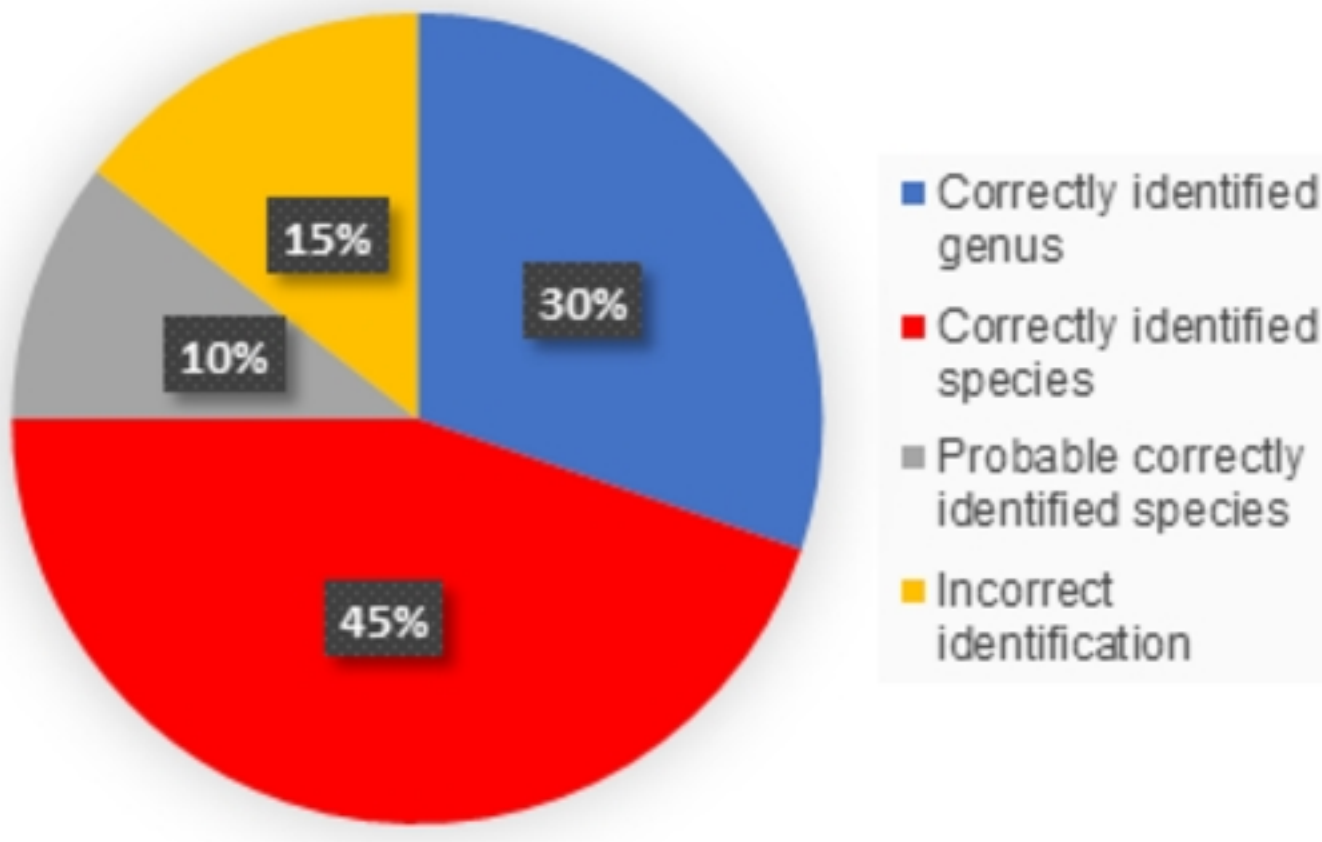

Genus match percentage (%)
100
90
80
70
60
50
40
30
20
10
0
271
255
18
14
47
39
154
148
17
16
1
1
BOLD
GenBank
Coleoptera*
Diptera
Hymenoptera
Lepidoptera*
Odonata
Strepsiptera
B


Species match percentage (%)
100
90
80
70
60
50
40
30
20
10
0
117
101
3
0
127
99
13
15
BOLD
GenBank
Coleoptera*
Hymenoptera
Lepidoptera*
Odonata
C

Figure

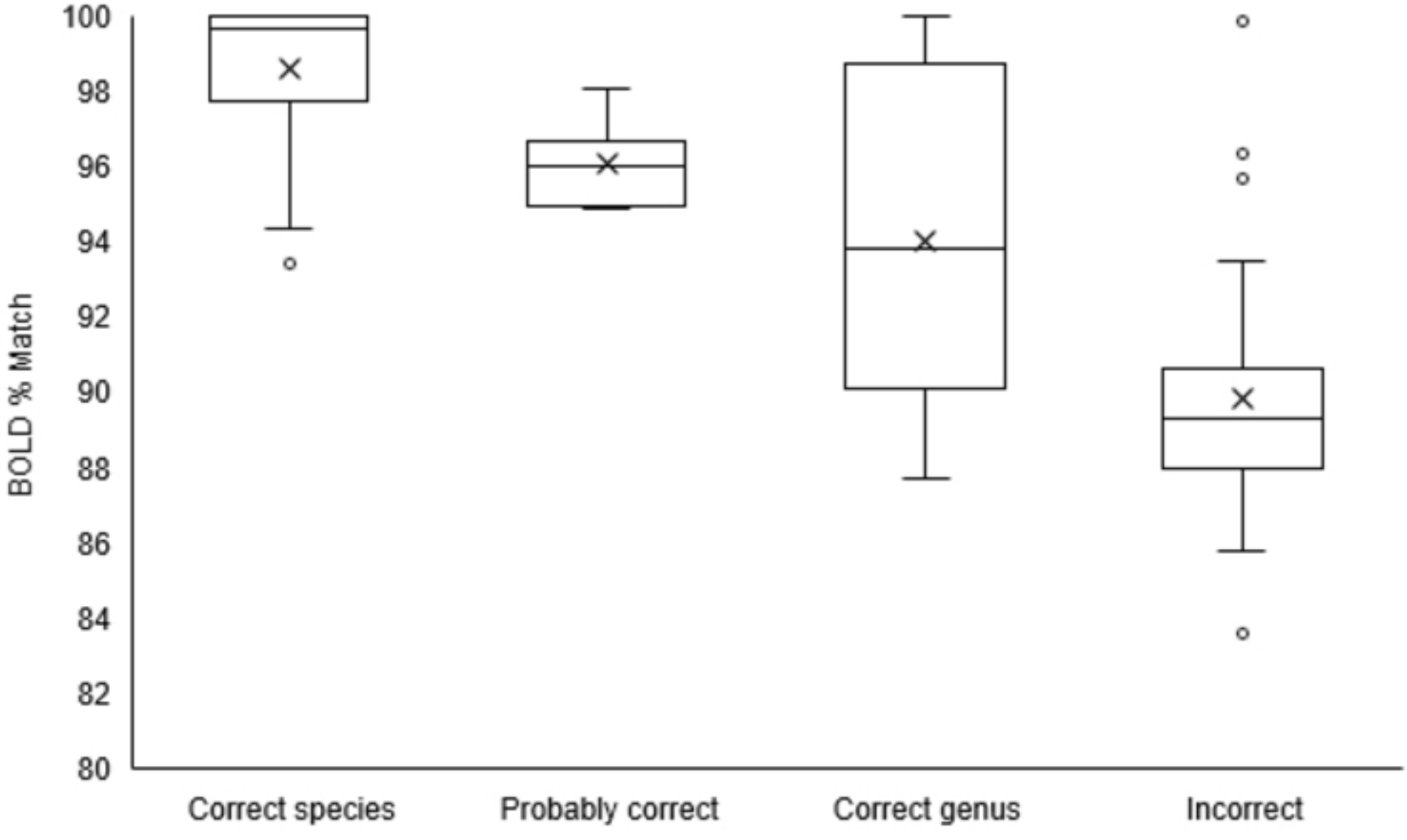

Disagreement, 35
Species, 55
Genus, 82
(I) BOLD IDs
Species, 95
(II) Taxonomist – de novo IDs
Genus, 52
Disagreement, 25

A


15%
30%
10%
45%
Correctly identified genus
Correctly identified species
Probable correctly identified species
Incorrect identification

B

Figure


100
98
96
94
92
90
88
86
84
82
80
BOLD % Match
Correct species
Probably correct
Correct genus
Incorrect

Figure