



**HAL**  
open science

## **DROP: Molecular voucher database for identification of Drosophila parasitoids**

Chia-Hua Lue, Matthew L Buffington, Sonja Scheffer, Matthew Lewis, Tyler A Elliott, Amelia R I Lindsey, Amy Driskell, Anna Jandova, Masahito T Kimura, Yves Carton, et al.

► **To cite this version:**

Chia-Hua Lue, Matthew L Buffington, Sonja Scheffer, Matthew Lewis, Tyler A Elliott, et al.. DROP: Molecular voucher database for identification of Drosophila parasitoids. *Molecular Ecology Resources*, 2021, 10.1111/1755-0998.13435 . hal-03275697

**HAL Id: hal-03275697**

**<https://hal.science/hal-03275697v1>**

Submitted on 1 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **DROP: Molecular voucher database for identification of *Drosophila* parasitoids**

2 Resource article

3 Word count: 7850 excluding references

4

5 **Authors**

6 Chia-Hua Lue (C-HL), (0000-0002-5245-603X), [chiachia926@gmail.com](mailto:chiachia926@gmail.com), corresponding

7 author

8 Matthew L. Buffington (MLB)

9 Sonja Scheffer (SS)

10 Matthew Lewis (ML)

11 Tyler A. Elliott (TAE)

12 Amelia R. I. Lindsey (AL)

13 Amy Driskell (AD), (0000-0001-8401-7923)

14 Anna Jandova (AJ)

15 Masahito T. Kimura (MTK)

16 Yves Carton (YC)

17 Robert R. Kula (RRK)

18 Todd A. Schlenke (TAS)

19 Mariana Mateos (MM), (0000-0001-5738-0145)

20 Shubha Govind (SG), (0000-0002-6436-639X)

21 Julien Varaldi (JV)

22 Emilio Guerrieri (EG), (0000-0002-0583-4667)

- 23 Massimo Giorgini (MG), (0000-0001-8670-0945)
- 24 Xingeng Wang (XW)
- 25 Kim Hoelmer (KH)
- 26 Kent M. Daane (KMD)
- 27 Paul K. Abram (PKA)
- 28 Nicholas A. Pardikes (NAP), (0000-0002-9175-4494)
- 29 Joel J. Brown (JJB), (0000-0002-3608-6745)
- 30 Melanie Thierry (MT)
- 31 Marylène Poirié (MP)
- 32 Paul Goldstein (PG), (0000-0002-1443-7030)
- 33 Scott E. Miller (SEM), (0000-0002-4138-1378)
- 34 W. Daniel Tracey (WDT)
- 35 Jeremy S. Davis (JSD), (0000-0002-5214-161X)
- 36 Francis M. Jiggins (FMJ)
- 37 Bregje Wertheim (BW)
- 38 Owen T. Lewis (OTL)
- 39 Jeff Leips (JL)
- 40 Phillip P. A. Staniczenko (PPAS)
- 41 Jan Hrcek (JH), (0000-0003-0711-6447), [janhrcek@gmail.com](mailto:janhrcek@gmail.com)
- 42
- 43
- 44

45 **Affiliations**

46 (C-HL, AJ, NAP, JJB, MT, JH) Biology Centre of the Czech Academy of Sciences, Institute  
47 of Entomology, Branisovska 31, 37005 Ceske Budejovice, Czech Republic

48 (C-HL, PPAS) Department of Biology, Brooklyn College, City University of New York  
49 (CUNY), 2900 Bedford Ave, Brooklyn, NY11210, USA

50 (MLB, SS, ML, RRK, PG) Systematic Entomology Laboratory, ARS/USDA c/o Smithsonian  
51 Institution, National Museum of Natural History, 10th& Constitution Ave, NW,  
52 Washington DC 20560, USA

53 (TAE) Centre for Biodiversity Genomics, 50 Stone Road East, University of Guelph,  
54 Guelph, Ontario, N1G2W1, Canada

55 (AL) Department of Entomology, University of Minnesota, Saint Paul MN 55108.

56 (AD) Laboratories of Analytical Biology, Smithsonian Institution, National Museum of  
57 Natural History, 10th & Constitution Ave, NW, Washington DC 20560, USA

58 (MTK) Hokkaido University Museum, Sapporo, Hokkaido 060-0810, Japan

59 (YC) “Évolution, Génomes, Comportement, Écologie”, CNRS et Université Paris-Saclay

60 (TAS) Department of Entomology at the University of Arizona, Forbes 410, PO BOX  
61 210036, Tucson, AZ 85721-0036.

62 (MM) Wildlife and Fisheries Sciences Department, Texas A&M University

63 (SG) The Graduate Center of the City University of New York, 365 Fifth Avenue, New  
64 York, NY10016, USA

65 (JV) Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie  
66 Evolutive UMR 5558, F-69622 Villeurbanne, France

67 (EG, MG) CNR- Institute for Sustainable Plant Protection (CNR-IPSP), National Research  
68 Council of Italy, Piazzale E. Fermi 1, 80055 Portici, Italy.

69 (XW, KH) United States Department of Agriculture, Agricultural Research Services,  
70 Beneficial Insects Introduction Research Unit. 501 S. Chapel St., Newark, DE 19713, USA

71 (KMD) Department of Environmental Science, Policy and Management. University of  
72 California, Berkeley. Mulford Hall, 130 Hilgard Way, Berkeley, CA 94720.

73 (PKA) Agriculture and Agri-Food Canada, Agassiz Research and Development Centre,  
74 6947 Hwy #7, Agassiz, V0M 1A0, British Columbia, Canada

75 (JJB, MT, JH) University of South Bohemia, Faculty of Science, Branisovska 31, 37005,  
76 Czech Republic

77 (MP) Université “Côte d’Azur”, INRAE, CNRS. and Evolution and Specificity of  
78 Multitrophic Interactions (ESIM) Sophia Agrobiotech Institute, 400 Route des Chappes,  
79 BP 167, 06903 Sophia Antipolis, France

80 (SEM) Smithsonian Institution, National Museum of Natural History, 10th & Constitution  
81 Ave, NW, Washington DC 20560, USA

82 (WDT, JSD) Department of Biology, Indiana University Bloomington. 702 N. Walnut  
83 Grove, Bloomington, IN47405

84 (WDT) Gill Center for Biomolecular Science, Indiana University Bloomington. 702 N.  
85 Walnut Grove, Bloomington, IN47405

86 (JSD) Biology Department, University of Kentucky, 101 T. H. Morgan Building, Lexington,  
87 KY, 40506

88 (FMJ) Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2

89 3EH UK

90 (BW) Groningen Institute for Evolutionary Life Sciences, University of Groningen,

91 Nijenborgh 9, 9747 AG Groningen, the Netherlands

92 (OTL) Department of Zoology, University of Oxford. 11a Mansfield Road, Oxford OX1

93 3SZ, UK.

94 (JL) Department of Biological Sciences, University of Maryland Baltimore County. 1000

95 Hilltop circle, Baltimore, MD, 21250

96

97

98 **Abstract**

99       Molecular identification is increasingly used to speed up biodiversity surveys and  
100 laboratory experiments. However, many groups of organisms cannot be reliably  
101 identified using standard databases such as GenBank or BOLD due to lack of sequenced  
102 voucher specimens identified by experts. Sometimes a large number of sequences are  
103 available, but with too many errors to allow identification. Here we address this  
104 problem for parasitoids of *Drosophila* by introducing a curated open-access molecular  
105 reference database, DROP (*Drosophila* parasitoids). Identifying *Drosophila* parasitoids is  
106 challenging and poses a major impediment to realize the full potential of this model  
107 system in studies ranging from molecular mechanisms to food webs, and in biological  
108 control of *Drosophila suzukii*. In DROP (<http://doi.org/10.5281/zenodo.4519656>),  
109 genetic data are linked to voucher specimens and, where possible, the voucher

110 specimens are identified by taxonomists and vetted through direct comparison with  
111 primary type material. To initiate DROP, we curated 154 laboratory strains, 853  
112 vouchers, 545 DNA sequences, 16 genomes, 11 transcriptomes, and 6 proteomes drawn  
113 from a total of 183 operational taxonomic units (OTUs): 113 described *Drosophila*  
114 parasitoid species and 70 provisional species. We found species richness of *Drosophila*  
115 parasitoids to be acutely underestimated and provide an updated taxonomic catalogue  
116 for the community. DROP offers accurate molecular identification and improves cross-  
117 referencing between individual studies that we hope will catalyze research on this  
118 diverse and fascinating model system. Our effort should also serve as an example for  
119 researchers facing similar molecular identification problems in other groups of  
120 organisms.

121

122 **Key Words**

123 Biodiversity, DNA sequences, Genomes, Integrative taxonomy, Molecular diagnostics,

124 Biological control

125

126

127

128

129

130

131

## 132 **Introduction**

133 Building a knowledge base that encompasses ecology, evolution, genetics, and  
134 biological control is contingent on reliable taxonomic identifications. Molecular  
135 identification is commonly used in groups of organisms with cryptic species that are  
136 difficult to identify morphologically (Fagan-Jeffries et al., 2018; Miller et al., 2016;  
137 Novotny & Miller, 2014), for the molecular detection of species interactions (Baker et  
138 al., 2016; Condon et al., 2014; Garipey et al., 2019; Hrček & Godfray, 2015; Hrcek et al.,  
139 2011), and for identification of species from environmental DNA samples (Shokralla et  
140 al., 2012). The accuracy of molecular identification, however, depends on the accuracy  
141 of identifications associated with sequences databased in existing online depositories.  
142 The foundations of that accuracy are the voucher specimens which were sequenced and  
143 the collaboration of a taxonomic authority in the deposition of the sequence data.

144 GenBank serves as the most widely used sequence depository; however,  
145 deposition of sequences in GenBank, which is required by most peer-reviewed journals,  
146 does not require deposition of associated vouchers. The Barcode of Life Data System  
147 database (BOLD) (Ratnasingham & Hebert, 2007) explicitly aims to provide a framework  
148 for identifying specimens using single-locus DNA sequences (Hebert et al., 2003; Smith  
149 et al., 2005), and while these are associated with vouchers and metadata, the curation  
150 of these data is not consistently maintained by those submitting material. A recent  
151 study by Pentinsaari et al. (2020) showed misidentification in both databases caused by  
152 missteps in the protocols from query sequences to final determination.



153           Although the BOLD database function “BOLD-IDS” allows considerable database  
154   curation (e.g., sequences are used for identification and/or flagging of  
155   misidentified/contaminated records), it also automatically includes sequences from  
156   GenBank, and may perpetuate the shortcomings previously mentioned since these  
157   cannot be curated from within BOLD. As such, the quality of sequences and the  
158   reliability of identifications obtained from BOLD-IDS can vary, and depends on the  
159   curation by systematists focusing on individual taxa (Meiklejohn et al., 2019). BOLD-IDS  
160   works well for taxa where qualified taxonomists have been involved with assuring data  
161   quality; some insect examples include beetles (Hendrich et al., 2015), butterflies  
162   (Escalante et al., 2010), geometrid moths (Hausmann et al., 2011, 2016; Miller et al.,  
163   2016), true bugs (Raupach et al., 2014), and microgastrine wasps (Smith et al., 2013).

164           Unfortunately, this is not the case of parasitoids (Insecta: Hymenoptera) of  
165   *Drosophila* flies (Insecta: Drosophilidae). There are vast numbers of *Drosophila*  
166   parasitoid sequences readily available in GenBank and BOLD, as these parasitoids and  
167   their hosts are important model organisms in biology. As of this writing, there are  
168   88,666 nucleotide sequences deposited in GenBank for *Leptopilina heterotoma*  
169   (Thomson) and *L. boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae)  
170   alone. However, less than 1 % of the identifications associated with these sequences  
171   have been confirmed by taxonomists or are associated with voucher specimens  
172   deposited in museum collections. With sequencing shifting from individual genes to  
173   genomes we risk that the identification problems will soon apply to whole genomes.

174        There are around 4000 described species of Drosophilidae, and *Drosophila* contains  
175        more than a third of the family's described species (O'Grady & DeSalle, 2018). By  
176        contrast, although parasitic wasps are generally a species-rich group (Dolphin & Quicke,  
177        2001; Quicke, 2015), the most recent catalogue of parasitoid species that attack  
178        *Drosophila* lists only 50 described species (Carton et al., 1986). This disparity suggests  
179        that the diversity of parasitic wasps attacking *Drosophila* is severely underestimated, an  
180        assertion supported by the results presented here. This is largely a consequence of the  
181        challenging nature of parasitoid taxonomy, in which morphological identification is  
182        intractable for many species, and the fact that taxonomic specialists are greatly  
183        outnumbered by the species they study.

184        Currently, only a few biological study systems have been characterized in  
185        sufficient breadth and depth to allow researchers to connect various levels of biological  
186        organization, from molecular mechanisms to food webs of interacting species.  
187        Parasitoids of *Drosophila* represent one such system (Prévost, 2009). Moreover, the  
188        practical feasibility of rearing parasitoids of *Drosophila* parasitoids under laboratory  
189        conditions has led to a number of fundamental discoveries in ecology (Carton et al.,  
190        1991; Terry et al. 2020; Thierry et al.,2021), evolution (Kraaijeveld & Godfray, 1997),  
191        immunology (Kim-Jo et al., 2019; Nappi & Carton, 2001; Schlenke et al., 2007),  
192        physiology (Melk & Govind, 1999), symbiosis (Xie et al., 2011, 2015), behavioral science  
193        (Lefèvre et al., 2012) and other fields. In contrast to this large body of laboratory  
194        studies, basic natural history of *Drosophila* parasitoids, especially their species richness  
195        (Kimura & Mitsui, 2020; Lue et al., 2018), is little known. Addressing this knowledge gap

196 is especially pressing given current efforts to use parasitoids in biological control efforts,  
197 such as those of the invasive pest spotted wing *Drosophila*, *Drosophila suzukii* (Abram et  
198 al., 2020; Daane et al., 2016; Giorgini et al., 2019; Wang et al., 2020 a&b).

199 Properly executed molecular identification has the potential to be much more  
200 efficient for the majority of researchers, and many laboratory strains are commonly  
201 identified using DNA sequences alone. While it is practical for researchers to assign  
202 species names based on a match to sequence records in genetic databases, this practice  
203 often causes a cascade of inaccuracies. To illustrate the extent of the problem, we note  
204 the example of *Ganaspis*, a genus of parasitoids commonly used in laboratories that  
205 includes both superficially indistinguishable species with highly divergent sequences  
206 that are often treated as conspecific, as well as specimens with identical sequences  
207 identified under different names (Figure 1).

208 To address these issues, we introduce a newly curated molecular reference database  
209 for *Drosophila* parasitoids —DROP— in which sequences are either linked to voucher  
210 specimens identified by taxonomists or have a traceable provenance (Figure 2). The first  
211 aim of DROP is to provide a reliable DNA sequence library for molecular identification of  
212 *Drosophila* parasitoids that enables cross-referencing of original taxonomic concepts  
213 with those of subsequent studies. We pay special attention to live parasitoid strains  
214 which are available for future experiments. The second aim is to standardize and  
215 expedite the linkage between specimens and available sequence data; we place a  
216 premium on museum vouchers as they allow for repeatable scientific research. In DROP,  
217 this goal is facilitated through a consolidated digital infrastructure of data associated

218 with laboratory strains, offering the opportunity for researchers to re-examine past  
219 experimental results in a permanent context. The third aim is to provide an up-to-date  
220 catalogue of the diversity of *Drosophila* parasitoids as a foundation for advancing the  
221 understanding of their taxonomy. Finally, the fourth aim of DROP is for our collaborative  
222 effort to serve as an inspiration to communities of researchers studying other groups of  
223 organisms who are experiencing difficulties with the reliability of molecular reference  
224 databases.

225

## 226 **Materials and Methods**

### 227 ***Drosophila* species and their parasitoids**

228 The phylogenetic and subgeneric structure within *Drosophila* and related genera is  
229 not yet fully resolved (O’Grady & DeSalle, 2018). Various subgenera, including  
230 *Scaptomyza*, *Zaprionus*, *Lordiphosa* and *Samoaia*, have been treated as both genera and  
231 subgenera, and researchers have yet to achieve consensus on these various hypotheses  
232 (O’Grady & DeSalle, 2018; Remsen & O’Grady, 2002; Yassin, 2013; Yassin & David,  
233 2010). Species in *Drosophila* subgenera and genera closely related to *Drosophila*  
234 commonly share niche space and natural histories and, as a result, are often attacked by  
235 overlapping or identical groups of parasitoids. For instance, the invasive African fig fly,  
236 *Zaprionus indianus* Gupta is attacked by *Pachycrepoideus vindemiae* (Rondani, 1875)  
237 and *Leptopilina boulardi* (Pfeiffer et al., 2019; Santos et al., 2016), all of which have been  
238 recorded from *Drosophila*. Therefore, we also include these groups within the contents  
239 of DROP.

240 Parasitoids of *Drosophila* belong to four superfamilies of Hymenoptera  
241 (Chalcidoidea, Cynipoidea, Ichneumonoidea, Diaprioidea) which evolved parasitism of  
242 *Drosophila* flies independently (Carton et al., 1986; Prévost, 2009). All the parasitoids  
243 known to attack *Drosophila* are solitary and attack either the larval or pupal stage; in  
244 both cases, they emerge from the fly's puparium. The known *Drosophila* larval  
245 parasitoids belong to two families, Braconidae (including the genera *Asobara*,  
246 *Aphaereta*, *Phaenocarpa*, *Tanycarpa*, *Aspilota*, *Opius*) and Figitidae (*Leptopilina*,  
247 *Ganaspis*, *Leptolamina*, *Kleidotoma*); all are koinobionts that allow the host to continue  
248 development while the parasitoid grows within it. The known *Drosophila* pupal  
249 parasitoids belong to three other families, Diapriidae (*Trichopria*, *Spilomicrus*),  
250 Pteromalidae (*Pachycrepoideus*, *Spalangia*, *Trichomalopsis*, *Toxomorpha*) and Encyrtidae  
251 (*Tachinaephagus*); they are all idiobionts that terminate host development immediately.  
252 Host-specificity across the *Drosophila* parasitoids is poorly characterized—while some  
253 can parasitize other families of Diptera (e.g., *Aphaereta aotea*) (Hughes & Woolcock,  
254 1976), most are thought to be limited to *Drosophila* hosts.

255

#### 256 **Data sources**

257 To assemble the DROP database, we targeted 20 genera that potentially parasitize  
258 frugivorous *Drosophila* species. We compiled DNA sequence and voucher data from four  
259 sources: 1) museum collections, 2) publications, 3) molecular biodiversity inventories  
260 publicly available in BOLD and GenBank, for which we managed to secure inspection of

261 the vouchers by taxonomists, and 4) conducted a sequencing and taxonomic inventory  
262 of laboratory strains.

263 We first gathered species information into a catalogue of *Drosophila* parasitoid  
264 species (Table 1) from 212 references (see DROP database reference table) and 36  
265 institutes (Table S2). To ensure reliable names for nominal species (sequences identified  
266 by a species name) in our database, we confirmed their taxonomic validity using the  
267 Ichneumonoidea 2015 digital catalogue (Yu et al., 2016;  
268 [https://web.archive.org/web/20161022093945/http://ichneumonoidea.name/global.ph](https://web.archive.org/web/20161022093945/http://ichneumonoidea.name/global.php)  
269 [p](http://ichneumonoidea.name/global.php)) and Hymenoptera Online (HOL; <http://hol.osu.edu/>), both of which are curated by  
270 taxonomic experts. To obtain reliable molecular identification data, we harvested 8,298  
271 DNA sequences from GenBank and BOLD (all compiled into BOLD system: DS-DROPAR  
272 dataset). These sequences represent 443 Barcode Index Numbers (BINs – a form of  
273 provisional taxa in BOLD) and 520 taxa, for a total of 963 operational taxonomic units  
274 (OTUs). We use the term “OTU” as a general and neutral designation encompassing  
275 described species, provisional species, undescribed species, cryptic species, and mis-  
276 identified species.

277 The majority of the harvested sequences were Braconidae (6690), Diapriidae  
278 (967), Figitidae (622), and Pteromalidae (19). Because of the concerns with generic  
279 databases (noted above and in Figure 1 and Table S1), we assembled a list of sequences  
280 with valid species names that could either be traceably linked to vouchers examined by  
281 taxonomists or referred to directly in publications authored by a recognized expert in  
282 the relevant taxon group. We then cross-checked species names with their

283 corresponding BINs in BOLD and flagged potential conflicts between species names and  
284 BINs (Table S1).

285 A core goal of DROP besides that of a tool for biodiversity research is to function as a  
286 platform that accommodates *Drosophila* parasitoids kept in culture (for experimental  
287 work) or in quarantine (for biological control applications). So far, there has been a lack  
288 of a coherent and reliable means of verifying species kept in laboratory settings, which  
289 can be a serious problem. Since lab cultures are routinely contaminated by neighboring  
290 cultures (e.g., through escapees), one species may be displaced by another even under a  
291 vigilant eye.

292 For lab and quarantine lines in DROP, we deposited DNA extractions and vouchers in  
293 the National Insect Collection, National Museum of Natural History, Smithsonian  
294 Institution (USNM; Washington, DC, USA). During their initial assembly for DROP,  
295 laboratory OTUs were designated by their strain name; most laboratory strains can be  
296 associated with provisional species but some cannot yet be assigned. Three females and  
297 three males of each strain were dry-mounted and individually assigned a USNMENT 'QR  
298 code' specimen label as representative vouchers. For each molecular voucher, three legs  
299 from a female wasp were removed for DNA extraction and sequencing (Supplementary  
300 Methods for details), and the rest of the body was assigned a USNMENT specimen label  
301 and preserved for morphological identification. Both DNA extraction and vouchers were  
302 entered into the database and uploaded to BOLD (DROP project: DS-LABS) with an  
303 associated GenBank ID [NOTE: the BOLD records will be pushed to GenBank at revision  
304 stage; these data are not embargoed].

305       Where possible, we identified OTU strains using a combination of morphological and  
306       sequence data, and characterized provisional species or species clusters using neighbor-  
307       joining trees (Figure S1) based on the COI gene sequences (Supplemental material). For  
308       establishing BIN limits in the context of DROP, we have adopted an initial percent cutoff  
309       at 2%. As Ratnasingham & Hebert (2013) pointed out, this is a good starting point for  
310       many taxa, but it also may be adjusted as more samples are acquired and compared.

311

### 312       ***Drosophila parasitoid database—DROP***

313       To compile the above information, we built a simple Structured Query Language  
314       (SQL) database in sqlite3 format using SQLiteStudio. There are eight linked tables in the  
315       database—species, strain, voucher, sequence, genome, transcriptome, proteome and  
316       reference—along with additional tables for linking these to reference table (Figure S2).  
317       The database incorporates all sample fields used by BOLD for compatibility and includes  
318       a number of new fields to accommodate a catalogue of *Drosophila* parasitoid species,  
319       laboratory strain information, and links from the DROP database to BOLD and GenBank  
320       records.

321       DROP is available on Zenodo (<http://doi.org/10.5281/zenodo.4519656>) for  
322       permanent deposition and version control. In addition to the main database, the  
323       Zenodo repository includes additional files to facilitate easy use of the database. These  
324       files include: 1) the reference database in comma-separated text (.csv) and FASTA  
325       format ready to be used for molecular identification; 2) a species catalogue with  
326       taxonomic information; and 3) a list of laboratory strains with confirmed molecular



327 vouchers. DROP will be continued to be maintained by C-HL until further notice at the  
328 Zenodo repository and sequences generated in the future will also be deposited in BOLD  
329 (DROP project).

330

### 331 ***Species, provisional species, and OTU designations***

332 In addition to the inherent value of a formal taxonomic name, a reliable provisional  
333 taxon label can also be used for exchanging scientific information and conveying  
334 experimental results among researchers (Schindel & Miller, 2010). Based on the amount  
335 of sequence divergence between described species, we observed what appears to be a  
336 significant number of provisional OTUs in the initial dataset we compiled. Furthermore,  
337 among the data linked to a valid species name, some of these provisional OTUs are  
338 actively being used in research and have sequences available to the public. We  
339 therefore provide a list of potential new species with their molecular vouchers.

340 We use the following designation format for OTUs that refer to a provisional species:

341 “Drop\_strainX\_sp.1” or, when no other information is known, “DROP\_sp.1”. Where  
342 possible, these OTUs are linked to BINs within BOLD and to a voucher USNM specimen  
343 label number. If the genus of the OTU is known, the “Drop\_Leptopilina\_sp.1” format is  
344 followed. These designations can facilitate species identification as well as discovery and  
345 description of new species without compromising the existing taxonomy of the  
346 described OTUs in question. As more complete species descriptions become available,  
347 this provisional species framework can be updated while keeping the link to previous  
348 provisional species name.

## 349 **Results**

### 350 ***Overview of DROP***

351 We catalogued 182 OTUs in the DROP database with 113 described species of  
352 *Drosophila* parasitoids and 69 provisional species (Table 1). In total, we documented 154  
353 laboratory strains (Table S3), 853 vouchers from 36 institutions (Table S2). Among the  
354 described species, 98 have voucher information, of which 61 are traceable to type  
355 specimens, including 45 to holotypes (i.e., specimen used to root a name to the  
356 taxonomic author's concept of the species). *Leptopilina* is represented by the highest  
357 number of species with 45 OTUs, followed by *Asobara* with 26 OTUs. Within the 154  
358 catalogued lab strains, 86 were actively being used in ongoing research (i.e., a live strain  
359 being cultivated). These strains represent 39 OTUs: 11 described species and 28  
360 provisional species (Table S3, Figure S1).

361

### 362 ***Molecular Vouchers***

363 So far, DROP includes 545 DNA sequences and links to 16 genomes (Table 2.1), 11  
364 transcriptomes (Table 2.2), and 6 proteomes (Table 2.3). From the total of 8298 DNA  
365 sequences (dataset: DS-DROPAR) collected from public databases, only 322 sequences  
366 (less than 4% of available sequences) satisfied the criteria for validity we imposed for  
367 molecular vouchers (see material and methods) included in DROP. The DS-DROPAR  
368 dataset initially referred to 520 taxa names, but only 52 names were valid, linked to  
369 vouchers, or linked to a publication with evidence that the specimens had been  
370 identified by taxonomists. The remaining 223 of 545 DROP DNA sequences were

371 generated by this project (dataset: DS-LABS, DS-AUSPTOID) and came from 121 OTUs  
372 (12 provisional species and 101 lab strains).

373 The DROP database is largely made up of standard barcode COI sequences (340  
374 sequences), which includes 77 OTUs: 43 described species and 33 provisional species.  
375 We aimed to supplement COI with secondary markers (28SD2, 18S, ITS2) when possible,  
376 resulting in an additional 120 sequences from 26 OTUs: 15 described species and 11  
377 provisional species. There are currently 19 OTUs that have sequences from more than  
378 one genetic marker.

379

### 380 ***Species Delimitation in Laboratory Strains***

381 We used 298 COI sequences to resolve the identification of each laboratory  
382 strain, and where possible, indicated potential species clusters for *Drosophila*  
383 parasitoids (Fig. S1 and Table S3). Using a fixed 2% divergence cutoff, a total of 31 lab  
384 strain OTUs were assignable to a valid species name, and the remaining 70 strain OTUs  
385 were assigned to a provisional species. The taxonomic status of several of these  
386 provisional species is also being investigated using an integrative taxonomic approach  
387 involving morphological identification, genomic data, or other genetic data.

388

### 389 **Discussion**

390 In this paper, we introduce and describe a free and open-access database for the  
391 reliable molecular identification of *Drosophila* parasitoids. The guiding principle of DROP

392 is data credibility, based on the prerequisite that genetic data be associated with explicit  
393 criteria linking voucher specimens with taxonomic concepts of the original authors  
394 (Troudet et al., 2018). When incorporating information from public genetic databases,  
395 we include only sequences that have passed our filtering protocol. This protocol ensures  
396 each entry is associated with a valid scientific name, provisional name, or consistently  
397 applied OTU designation that can be used to integrate genetic and organismal data from  
398 independent studies.

399 The following discussion expands on the utility of DROP and how we hope it will  
400 benefit molecular species identification, connect research from various disciplines,  
401 support biological control applications, and serve as a long-term molecular voucher  
402 repository and clearinghouse for vetted data.

403

#### 404 ***Molecular (mis-)identification***

405 We observe that 17% of the described *Drosophila* parasitoid OTUs in BOLD and  
406 GenBank (dataset: DS-DROPAR) are associated with more than one BIN; these are  
407 examples of BIN-ID conflict. Roughly half of these OTUs are used as lab strains. This  
408 latter observation is disturbing, because it demonstrates that the criteria used to  
409 differentiate and reference species in active research programs are clouded. For  
410 example, BIN-ID conflicts were observed in the *Drosophila* parasitoids *Ganaspis*  
411 *brasiliensis* (Ihering) and *Asobara japonica* Belokobylskij (Table S1), both of which are in  
412 active use in numerous research programs (e.g. Moreau et al., 2009; Nomano et al.,  
413 2017; Reumer et al., 2012; Wang et al., 2020a & 2021) as well as in biological control

414 efforts against the invasive *D. suzukii* (e.g. Abram et al., 2020; Daane et al., 2016;  
415 Giorgini et al., 2019). All the BINs from *G. brasiliensis* carry the name *G. xanthopoda*  
416 (Figure 1). In such instances, assigning an identification by matching specimens to  
417 barcode records in the genetic database is problematic, as two names are applied to the  
418 same BIN. If sequences comprising the BIN are not linked to a voucher that can be  
419 examined, teasing apart the two names and how they are applied is impossible.  
420 Applying explicit, consistent criteria for species determination ensures that  
421 experimental results can be reliably repeated, and that any potentially novel  
422 observations will not be explained away as artifacts of identification. DROP addresses  
423 these concerns by linking reliable reference sequences and vouchers for *G. brasiliensis*  
424 (Figure 1) and from different studies: one with reference to the morphological  
425 description (Buffington & Forshage, 2016) and the other with reference to the genome  
426 (using voucher specimens from the morphological study; Blaimer et al., 2020).

427       We were not able to resolve all conflicts between BIN and species identity, for one  
428 or more of the following three reasons: First, many records lack reliably identified  
429 vouchers and have often been themselves used for molecular identification,  
430 proliferating errors. Second, in some cases, it is not possible to verify whether the  
431 genetic differences among BINs represent different species or simply intraspecific  
432 genetic variation (Bergsten et al., 2012), because BINs themselves are not a species  
433 concept. The only solution to this problem is to derive original sequence data from type  
434 specimens (which is often either impractical or impossible for a number of technical  
435 reasons), or from specimens whose conspecificity with the types has been corroborated.

436 Since species boundaries are always subject to testing, additional specimens from  
437 multiple collecting events (e.g., representing different seasons and geographic regions)  
438 may help provide the additional data to circumscribe a given species' limits. The third  
439 difficulty in resolving BIN-ID conflict derives from the data themselves: Although the  
440 mitochondrial COI gene is the locus most frequently chosen for identification of insects  
441 and other animals, its effectiveness varies among insect groups (Brower & DeSalle,  
442 2002; Gompert et al., 2008; Lin & Danforth, 2004). In part, this derives from gene-  
443 tree/species-tree conflict as a function of mitochondrial DNA introgression (Gompert et  
444 al., 2008; Klopstein et al., 2016), parthenogenesis (Reumer et al., 2012), and/or  
445 *Wolbachia* infection (Wachi et al., 2015; Xiao et al., 2012), any of which may lead to  
446 complications in species delimitation using mitochondrial loci. Ideally, studies should  
447 apply multiple loci, genomes, and comparative taxonomic data to clarify species  
448 boundaries. As *Drosophila* parasitoids are often maintained in laboratory cultures, it is  
449 also possible to use mating experiments to explore species boundaries under the  
450 paradigm of the biological species concept (Seehausen et al., 2020).

451

#### 452 ***DROP as a taxonomic tool***

453 DROP offers an empirical platform for species discovery and a useful tool for  
454 taxonomic research. The fact that the number of BINs reported here exceeds the  
455 number of described species (Table S1, Figure S3) highlights the need for taxonomic  
456 work. But such work cannot proceed on the basis of BINs or barcodes, but requires  
457 integrative taxonomic approach employing a combination of molecular and

458 morphological data. Describing new species on the sole basis of a barcode or BIN,  
459 without the benefit of independent character data, should, in general, be avoided. It  
460 risks creating nomenclatural synonymy if it is later determined that a sequence can be  
461 attributed to a specimen that bears a valid, available name. Moreover, BINs are based  
462 on distance analyses which, by definition, are incompatible with diagnoses per se  
463 (Ferguson, 2002; Prendini et al., 2002; Goldstein & DeSalle, 2011). Therefore, in  
464 taxonomic treatments, it is critical to clarify the range of applicability of a given BIN and  
465 it overlap with a taxonomic name (see example in Figure 1).

466         Public genetic databases have adopted a longstanding convention in treating  
467 undetermined OTUs and sequences, referring to provisional species with numbers, as  
468 for example “sp. 1”, and these are rarely linked to vouchers. For OTUs designated as  
469 provisional species, DROP enables cross-indexing of specimens, sequences and  
470 references with studies and provides researchers with valuable tools for taxonomic  
471 revisions, including the means of discovery, corroboration, and description of new  
472 species. For example, “drop\_Gan1\_sp.1” refers to voucher USNMENT01557320  
473 deposited in the USNM, Washington DC, COI sequence (DROP sequence\_id 2), BOLD  
474 process ID: DROP143-21, BIN number: XXXXXX (will update in the revision), 28D1  
475 sequences (DROP sequence\_id 289), and 28D2 sequences (DROP sequence\_id 303). In  
476 the future, when “drop\_Gan1\_sp.1” is described as a new species with a formal specific  
477 epithet, DROP will update the species status and holotype information while keeping  
478 this provisional species name as an informal “synonym.” We recognize tracking these

479 informal ‘tags’ through time can be problematic; however, linking these tags in DROP to  
480 a vouchered specimen and unique identifier will minimize confusion.

481

### 482 ***From molecular mechanisms to ecosystem structure***

483 The use of molecular tools in insect biodiversity studies has gradually expanded from  
484 barcoding single individuals to metabarcoding large environmental samples  
485 representing entire food webs (Jefferies et al., 2020; Littlefair et al., 2016). *Drosophila* and  
486 their parasitoids are among the few systems that currently allow us to explore  
487 thoroughly the mechanisms of species interactions at scales ranging from the molecular  
488 to the ecological. Here, we highlight two examples where information compiled in DROP  
489 enables the study of the *Drosophila*-parasitoid system across multiple levels of biological  
490 organization:

491 DROP includes a DNA reference library of Australian *Drosophila* parasitoids (DS-  
492 AUSPTOID in BOLD) that connects laboratory experiments and field research. Molecular  
493 vouchers of both hosts and parasitoids were collected along altitudinal gradients in the  
494 rainforest of northern Queensland, Australia (Jefferies et al., 2020). With this DNA reference  
495 library, researchers can detect interactions between *Drosophila* and their parasitoids  
496 using PCR-based approaches and parasitized pupae (Hrcek & Godfray, 2015; Jefferies et al.,  
497 2020). Surveying host-parasitoid interactions in this way will improve our understanding  
498 of how environmental change alters the structure of host-parasitoid networks (Morris et  
499 al., 2014; Staniczenko et al., 2017; Tylianakis et al., 2007) by accelerating data collection  
500 in the field. In addition, JH established lab cultures of both hosts and their parasitoids



501 from the same Australian sampling sites with the aim of conducting laboratory  
502 experiments (e.g. Thierry et al., 2021). Molecular vouchers of the lab strains were then  
503 submitted to DROP as a reference database (DS-LABS in BOLD) to ensure that criteria for  
504 species determination were applied consistently—and will continue to be applied  
505 consistently—between the natural community studies and the laboratory experiments.

506         The presence of a foundational DNA reference library and species catalogue in  
507 DROP will enable the process of exploring parasitoid biodiversity to become more  
508 efficient. For example, DROP includes molecular vouchers from *Drosophila* parasitoids  
509 that were collected across seasons and along latitudinal gradients in the eastern United  
510 States (Lue et al., 2016, 2018). These data proved to be extremely useful for identifying  
511 species in a more recent exploration of native parasitoid biodiversity across North  
512 America (e.g., Abram et al., 2020). There are additional uses for DROP: curated  
513 specimen collections may be used to document species distributions, phenology,  
514 understand micro-evolutionary patterns, observe the effects of climate change, and  
515 detect and track biological invasions (Funk, 2018; Schilthuizen et al., 2015; Tarli et al.,  
516 2018).

517

### 518 ***Taxonomic accuracy for biocontrol studies***

519         Unfortunately, the history of biological control includes many examples of  
520 misidentifications that have resulted in failures to employ or establish the expected  
521 control agent, thus hindering eventual success (Buffington et al., 2018; Rosen, 1986;  
522 Huffaker et al. 1962). In the context of biological control research on *Drosophila* pest

523 species, a simple, reliable, and rapid identification tool for their natural enemies is  
524 essential (Wang et al. 2020b). By anchoring the criteria for determining identities of  
525 organisms being considered for biological control programs, DROP annotation enables  
526 the direct examination of centers of origin for parasitoid species, their co-occurrence  
527 with natural enemies, and the optimal timing for potential introductions of such  
528 enemies (Abram et al., 2020; Daane et al., 2016; Girod et al., 2018a and b; Kimura, 2015;  
529 Mitsui et al., 2007). Because most sequences from DROP are already vetted for  
530 reliability, they can be used to identify biological control agents rapidly, before or after  
531 being brought into quarantine facilities for safety and efficacy testing. This will decrease  
532 the risk of non-target ecological impacts arising from misidentifications and facilitate  
533 regulatory review for releases of effective and specific natural enemies.

534 In addition to species identification, reference sequences from DROP may be used to  
535 create species-specific primers for the accurate identification of parasitoids, design  
536 multiplex PCR assays that rapidly distinguish species in natural or agricultural  
537 ecosystems (Ye et al., 2017), and apply high-throughput molecular identification  
538 diagnostics (Fagan-Jeffries et al., 2018). Applications of such specific primers have been  
539 used in bacteria, fungi, oomycetes and insect pests (Liu et al., 2017; Tedersoo et al.,  
540 2019; Tsai et al., 2020).

541

542 ***Long-term molecular voucher preservation***

543        During the curation of DROP, we found that holotype specimens were missing from  
544 museums for several iconic *Drosophila* parasitoid species: *Asobara tabida* (Nees von  
545 Esenbeck), *Leptopilina clavipes* (Hartig), and *Leptopilina longipes* (Hartig). This is not  
546 uncommon and impedes future taxonomic revisions regardless of whether molecular  
547 data are used. To avoid contributing to this problem, DROP uses museums as  
548 depositories for ensuring that sequenced vouchers of both described species and  
549 provisional species are permanently stored. In order to stabilize nomenclature, we  
550 further advocate the designation of neotypes (a replacement specimen for a missing  
551 holotype) that have museum-vouchered DNA barcodes and additional genomic  
552 extractions in storage.

553        Natural history museums are designed to maintain vouchers (including types) for  
554 long-term preservation, and increasingly they implement institutionalized workflows  
555 that link DNA sequences to specimens and specimen metadata (Prendini et al., 2002).  
556 We strongly encourage the deposition of voucher specimens from field surveys and  
557 experimental studies in museum collections, as has been urged by the Entomology  
558 Collections Network (ECN) and required in many PhD programs. No matter how quickly  
559 new molecular techniques are developed or refined, there is no substitute for a reliable  
560 database of voucher specimens when it comes to ensuring the repeatability of biological  
561 research (Funk et al., 2005; Lendemer et al., 2020).

562        Our results show that species richness of the parasitic wasps that attack *Drosophila*  
563 is severely underestimated, and only a fraction of them have been described. In DROP,  
564 38% of the OTUs are provisional species, and more than 46% of the named OTUs have

565 synonyms. Remarkably, *Leptopilina heterotoma*, one of the world's most studied  
566 parasitoids, has more than 20 synonyms! As is generally the case, the rate of species  
567 description and revision of *Drosophila* parasitoids lags far behind that with which  
568 molecular sequence data are generated. Ensuring a consistent application of OTU  
569 recognition is therefore essential. With DROP, researchers may ensure consistency is  
570 their application of scientific names, and that those names are valid, making the  
571 daunting process of describing *Drosophila* parasitoids more accurate and efficient.

572 In addition to the collection of physical museum resources, a central role  
573 taxonomists play in DROP and its curation is that of fostering better integration of  
574 taxonomy with experimental and biodiversity research. Our intention is to perpetuate  
575 DROP beyond this introductory publication. We hope that experts in all areas of  
576 *Drosophila*-parasitoid biology and related fields will join us in this effort.

577

## 578 **Conclusion**

579 Taxonomic confusion presents many obstacles in experimental and biodiversity  
580 studies. One way of addressing this impediment is to provide a reliable DNA library with  
581 traceable vouchers (Astrin et al., 2013). We developed DROP as a resource and platform  
582 for gathering and sharing reliable genomic sequence data for *Drosophila* parasitoids. We  
583 hope it will serve as a model for researchers working with organisms which present  
584 similar difficulties. While compiling DROP, we found that the high number of provisional  
585 *versus* named OTUs suggests that the diversity of parasitic wasps attacking *Drosophila* is  
586 greatly underestimated. With this in mind, DROP represents the start of an important

587 knowledge base that will strengthen future studies of natural host-parasitoid  
588 interactions, population dynamics, biocontrol, and the impact of climate change on  
589 biodiversity and ecosystem services.

590

## 591 **Acknowledgements**

592 The DROP project was developed during the 2018 Entomology Society of  
593 America conference, during the symposium “Drosophila parasitoids: from molecular to  
594 ecosystem level”. We thank Dr. Elijah Talamas for valuable comments on earlier drafts.  
595 We also thank Chris Jeffs for providing Australian field samples. We are also thankful for  
596 funding support from the Czech Science Foundation (17-27184Y). Additional fund for  
597 sequencing was provided by MLB, OTL, and PPAS. Mention of trade names or  
598 commercial products in this publication is solely for the purpose of providing specific  
599 information and does not imply recommendation or endorsement by the USDA. USDA is  
600 an equal opportunity provider and employer.

601

## 602 **References**

- 603 Abram, P. K., Mcpherson, A. E., Kula, R., Hueppelsheuser, T., Perlman, S. J., Curtis, C. I.,  
604 Fraser, J. L., ... Buffington, M. (2020). New records of *Leptopilina*, *Ganaspis*, and  
605 *Asobara* species associated with *Drosophila suzukii* in North America, including  
606 detections of *L. japonica* and *G. brasiliensis*. *Journal of Hymenoptera Research*, 78,  
607 1-17, <https://doi.org/10.3897/jhr.78.55026>  
608 Astrin, J. J., Zhou, X., & Misof, B. (2013). The importance of biobanking in molecular  
609 taxonomy, with proposed definitions for vouchers in a molecular context. *ZooKeys*,  
610 365(SPEC.ISSUE), 67–70. <https://doi.org/10.3897/zookeys.365.5875>  
611 Baker, C. C. M., Bittleston, L. S., Sanders, J. G., & Pierce, N. E. (2016). Dissecting host-  
612 associated communities with DNA barcodes. *Philosophical Transactions of the*  
613 *Royal Society B: Biological Sciences*, 371(1702).  
614 <https://doi.org/10.1098/rstb.2015.0328>

- 615 Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M.,  
616 Hendrich, ... Vogler, A. P. (2012). The effect of geographical scale of sampling on  
617 DNA barcoding. *Systematic Biology*, 61 (5), 851-869.  
618 <https://doi.org/10.1093/sysbio/sys037>
- 619 Blaimer, B. B., Gotzek, D., Brady, S. G., & Buffington, M. (2020). Comprehensive  
620 phylogenomic analyses re-write the evolution of parasitism within cynipoid wasps.  
621 *BMC Ecology and Evolution*, 20 (155). <https://doi.org/10.1186/s12862-020-01716-2>
- 622 Brower, A. V. Z., & DeSalle, R. (2002). Patterns of mitochondrial versus nuclear DNA  
623 sequence divergence among nymphalid butterflies: The utility of wingless as a  
624 source of characters for phylogenetic inference. *Insect Molecular Biology*, 7 (1), 73-  
625 82. <https://doi.org/10.1046/j.1365-2583.1998.71052.x>
- 626 Buffington, M., & Forshage, M. (2016). Redescription of *Ganaspis brasiliensis* (Ihering,  
627 1905), new combination, (Hymenoptera: Figitidae) a natural enemy of the Invasive  
628 *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). *Proceedings of the*  
629 *Entomological Society of Washington*, 118(1), 1–13. <https://doi.org/10.4289/0013-8797.118.1.1>
- 631 Buffington, M., Talamas, E. J., & Hoelmer, K. A. (2018). Team Trissolcus: Integrating  
632 taxonomy and biological control to combat the brown marmorated stink bug.  
633 *American Entomologist*, 64 (4), 224–232
- 634 Carton, Y., Boulétreau, M., van Alphen, J. J. M., & van Lenteren, J. C. (1986). The  
635 *Drosophila* parasitic wasps. In Ashburner M, Carson HL, Thompson JN (Eds), *The*  
636 *genetics and biology of Drosophila*, (3), 348–394.
- 637 Carton, Y., Haouas, S., Marrakchi, M., & Hochberg, M. (1991). Two competing parasitoid  
638 species coexist in sympatry. *Oikos*, 60, 222-230. <https://doi.org/10.2307/3544869>
- 639 Condon, M. A., Scheffer, S. J., Lewis, M. L., Wharton, R., Adams, D.C., & Forbes, A. A.  
640 (2014). Lethal interactions between parasites and prey increase niche diversity in a  
641 tropical community. *Science*, 343(6176), pp.1240-1244.
- 642 Daane, K. M., Wang, X.-G., Biondi, A., Miller, B. E., Miller, J. C., Riedl, H., Shearer, P. W.,  
643 ... Walton, V. M. (2016). First exploration of parasitoids of *Drosophila suzukii* in  
644 South Korea as potential classical biological agents. *Journal of Pest Science* 89, 823–  
645 835, doi:10.1007/s10340-016-0740-0.
- 646 Dolphin, K., & Quicke, D. L. J. (2001). Estimating the global incompletely described  
647 parasitoid wasps. *Biological Journal Of The Linnean Society*, 73 (3), 279-286,  
648 <https://doi.org/10.1006>
- 649 Escalante, P., Ibarra-Vazquez, A., & Rosas-Escobar, P. (2010). Tropical montane  
650 nymphalids in Mexico: DNA barcodes reveal greater diversity. *Mitochondrial DNA*,  
651 21, 30-37, <https://doi.org/10.3109/19401736.2010.535527>
- 652 Fagan-Jeffries, E. P., Cooper, S. J. B., Bertozzi, T., Bradford, T. M., & Austin, A. D. (2018).  
653 DNA barcoding of microgastrine parasitoid wasps (Hymenoptera: Braconidae) using  
654 high-throughput methods more than doubles the number of species known for  
655 Australia. *Molecular Ecology Resources*, 18(5), 1132–1143.  
656 <https://doi.org/10.1111/1755-0998.12904>
- 657 Ferguson, J. W. H. (2002). On the use of genetic divergence for identifying species.  
658 *Biological Journal of the Linnean Society*, 75, 509–16.

- 659 Forbes, A. A., Bagley, R. K., Beer, M. A., Hippee, A. C., & Widmayer, H. A. (2018).  
660 Quantifying the unquantifiable: Why Hymenoptera, not Coleoptera, is the most  
661 speciose animal order. *BMC Ecology*, 18(1), 1–11. [https://doi.org/10.1186/s12898-](https://doi.org/10.1186/s12898-018-0176-x)  
662 [018-0176-x](https://doi.org/10.1186/s12898-018-0176-x)
- 663 Funk, V. A. (2018). Collections-based science in the 21st Century. *Journal of Systematics*  
664 *and Evolution*, 56(3), 175–193. <https://doi.org/10.1111/jse.12315>
- 665 Funk, V. A., Hoch, P. C., Prather, L. A., & Wagner, W. L. (2005). The importance of  
666 vouchers. *Taxon*, 54(1), 127–129. <https://doi.org/10.2307/25065309>
- 667 Garipey, T. D., Bruin, A., Konopka, J., Scott-Dupree, C., Fraser, H., Bon, M. C., & Talamas,  
668 E. (2019). A modified DNA barcode approach to define trophic interactions  
669 between native and exotic pentatomids and their parasitoids. *Molecular Ecology*,  
670 28(2), 456–470. <https://doi.org/10.1111/mec.14868>
- 671 Giorgini, M., Wang, X.-G., Wang, Y., Chen, F. S., Hougardy, E., Zhang, H. M., Chen, Z.  
672 Q., ... Guerrieri, E. (2019). Exploration for native parasitoids of *Drosophila suzukii* in  
673 China reveals a diversity of parasitoid species and narrow host range of the  
674 dominant parasitoid. *Journal of Pest Science*, 92(2), 509–522.  
675 <https://doi.org/10.1007/s10340-018-01068-3>
- 676 Girod, P., Borowiec, N., Buffington, M., Chen, G., Fang, Y., Kimura, M. T., Peris-Felipo, F.  
677 J., ... Kenis, M. (2018). The parasitoid complex of *D. suzukii* and other fruit feeding  
678 *Drosophila* species in Asia. *Scientific Reports*, 8(1), e11839.  
679 <https://doi.org/10.1038/s41598-018-29555-8>
- 680 Girod, P., Lierhmann, O., Urvois, T., Turlings, T. C. J., Kenis, M., & Haye, T. (2018). Host  
681 specificity of Asian parasitoids for potential classical biological control of *Drosophila*  
682 *suzukii*. *Journal of Pest Science* 91,1241–1250, [https://doi.org/10.1007/s10340-](https://doi.org/10.1007/s10340-018-1003-z)  
683 [018-1003-z](https://doi.org/10.1007/s10340-018-1003-z)
- 684 Goldstein, P. Z., & DeSalle, R. (2011). Integrating DNA barcode data and taxonomic  
685 practice: Determination, discovery, and description. *BioEssays*, 33(2),135-147,  
686 <https://doi.org/10.1002/bies.201000036>
- 687 Gompert, Z., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2008). Widespread mito-nuclear  
688 discordance with evidence for introgressive hybridization and selective sweeps in  
689 *Lycaeides*. *Molecular Ecology*, 17(24), 5231-5244, [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-294X.2008.03988.x)  
690 [294X.2008.03988.x](https://doi.org/10.1111/j.1365-294X.2008.03988.x)
- 691 Grissell, E. (1999). Hymenopteran biodiversity: some alien notions. *American*  
692 *Entomologist*, 45,236-244.
- 693 Hardy, I. C., van Alphen, J. J. M., & Godfray, H. C. J. (1994). Parasitoids: Behavioral and  
694 evolutionary ecology. *The Journal of Animal Ecology*, 63(4), 1009-1010,  
695 <https://doi.org/10.2307/5282>
- 696 Hausmann, A., Haszprunar, G., & Hebert, P. D. N. (2011). DNA barcoding the geometrid  
697 fauna of bavaria (Lepidoptera): Successes, surprises, and questions. *PLoS ONE*, 6(2),  
698 1–9. <https://doi.org/10.1371/journal.pone.0017134>
- 699 Hausmann, A., Miller, S. E., Holloway, J. D., Dewaard, J. R., Pollock, D., Prosser, S. W. J.,  
700 & Hebert, P. D. N. (2016). Calibrating the taxonomy of a megadiverse insect family:  
701 3000 DNA barcodes from geometrid type specimens (Lepidoptera, Geometridae).  
702 *Genome*, 59(9), 671–684. <https://doi.org/10.1139/gen-2015-0197>

- 703 Hebert, P. D. N., Ratnasingham, S., & DeWaard, J. R. (2003). Barcoding animal life:  
704 Cytochrome c oxidase subunit 1 divergences among closely related species.  
705 *Proceedings of the Royal Society B: Biological Sciences*, 270 (Suppl.), 96-99,  
706 <https://doi.org/10.1098/rsbl.2003.0025>
- 707 Hendrich, L., Morinière, J., Haszprunar, G., Hebert, P. D. N., Hausmann, A., Köhler, F., &  
708 Balke, M. (2015). A comprehensive DNA barcode database for Central European  
709 beetles with a focus on Germany: Adding more than 3500 identified species to  
710 BOLD. *Molecular Ecology Resources*, 15(4), 795-818, <https://doi.org/10.1111/1755-0998.12354>
- 711
- 712 Hrček, J., & Godfray, H. C. J. (2015). What do molecular methods bring to host-parasitoid  
713 food webs? *Trends in Parasitology*, 31(1), 30–35.  
714 <https://doi.org/10.1016/j.pt.2014.10.008>
- 715 Hrccek, J., Miller, S. E., Quicke, D. L. J., & Smith, M. A. (2011). Molecular detection of  
716 trophic links in a complex insect host-parasitoid food web. *Molecular Ecology*  
717 *Resources*, 11(5), 786–794. <https://doi.org/10.1111/j.1755-0998.2011.03016.x>
- 718 Huffaker, C. B., Kennett, C. E., Finney, G. L. (1962). Biological control of olive scale,  
719 *Pwrlatoria oleae* (Cohree), in California by imported *Aphytis maculicornis* (Masi)  
720 (Hymenoptera: Aphelinidae). *Hilgardia*, 32 (13): 541-636. DOI:  
721 10.3733/hilg.v32n13p541
- 722 Hughes, R. D., Woolcock, L. T. (1976). *Aphaereta aotea* sp. N. (Hymenoptera:  
723 Braconidae), an Alysine parasite of dung breeding flies. *Journal of Australian*  
724 *Entomological Society*, 15, 191-196.
- 725 Jeffs, C. T., Terry, J. C. D., Higgie, M., Jandová, A., Konvičková, H., Brown, J. J., Lue, C.-H.,  
726 ... Lewis, O. T. (2020). Molecular analyses reveal consistent food web structure with  
727 elevation in rainforest *Drosophila* - parasitoid communities. *Ecography*, 43, 1-11,  
728 <https://doi.org/10.1111/ecog.05390>
- 729 Kim-Jo, C., Gatti, J. L., & Poirié, M. (2019). *Drosophila* cellular immunity against  
730 parasitoid wasps: A complex and time-dependent process. *In Frontiers in*  
731 *Physiology*, <https://doi.org/10.3389/fphys.2019.00603>
- 732 Kimura, M. T. (2015). Prevalence of exotic frugivorous *Drosophila* species, *D. simulans*  
733 and *D. immigrans* (Diptera: Drosophilidae), and its effects on local parasitoids in  
734 Sapporo, northern Japan. *Applied Entomology and Zoology*, 50(4), 509–515.  
735 <https://doi.org/10.1007/s13355-015-0361-8>
- 736 Kimura, M. T., & Mitsui, H. (2020). *Drosophila* parasitoids (Hymenoptera) of Japan. *In*  
737 *Entomological Science*, 23(4), 359-368, <https://doi.org/10.1111/ens.12432>
- 738 Klopstein, S., Kropf, C., & Baur, H. (2016). Wolbachia endosymbionts distort DNA  
739 barcoding in the parasitoid wasp genus *Diplazon* (Hymenoptera: Ichneumonidae).  
740 *Zoological Journal of the Linnean Society*, 177(3), 541–557.  
741 <https://doi.org/10.1111/zoj.12380>
- 742 Kraaijeveld, A. R., & Godfray, H. C. J. (1997). Trade-off between parasitoid resistance and  
743 larval competitive. *Nature*, 389, 278-280, <https://doi.org/10.1038/38483>
- 744 Lefèvre, T., De Roode, J. C., Kacsoh, B. Z., & Schlenke, T. A. (2012). Defence strategies  
745 against a parasitoid wasp in *Drosophila*: Fight or flight? *Biology Letters*, 8(2), 230-  
746 233, <https://doi.org/10.1098/rsbl.2011.0725>



- 747 Lendemer, J., Thiers, B., Monfils, A. K., Zaspel, J., Ellwood, E. R., Bentley, A., LeVan, K., ...  
748 Aime, M. C. (2020). The extended specimen network: A strategy to enhance US  
749 biodiversity collections, promote research and education. *BioScience*, 70(1), 23-30,  
750 <https://doi.org/10.1093/biosci/biz140>
- 751 Lin, C. P., & Danforth, B. N. (2004). How do insect nuclear and mitochondrial gene  
752 substitution patterns differ? Insights from Bayesian analyses of combined datasets.  
753 *Molecular Phylogenetics and Evolution*, 30(3), 686-702,  
754 [https://doi.org/10.1016/S1055-7903\(03\)00241-0](https://doi.org/10.1016/S1055-7903(03)00241-0)
- 755 Littlefair, J. E., Clare, E. L., & Naaum, A. (2016). Barcoding the food chain: From Sanger to  
756 high-throughput sequencing1. *Genome*, 59(11), 946–958.  
757 <https://doi.org/10.1139/gen-2016-0028>
- 758 Liu, L. J., Pang, A. H., Feng, S. Q., Cui, B. Y., Zhao, Z. H., Kučerová, Z., Stejskal, V., ... Li, Z.  
759 H. (2017). Molecular Identification of ten species of stored-product psocids through  
760 microarray method based on ITS2 rDNA. *Scientific Reports*, 7(1): 16694,  
761 <https://doi.org/10.1038/s41598-017-16888-z>
- 762 Lue, C.-H., Borowy, D., Buffington, M. L., & Leips, J. (2018). Geographic and seasonal  
763 variation in species diversity and community composition of frugivorous *Drosophila*  
764 (Diptera: Drosophilidae) and their *Leptopilina* (Hymenoptera: Figitidae) parasitoids.  
765 *Environmental Entomology*, 47(5): 1096-1106. <https://doi.org/10.1093/ee/nvy114>
- 766 Lue, C.-H., Driskell, A. C., Leips, J., & Buffington, M. L. (2016). Review of the genus  
767 *Leptopilina* (Hymenoptera, Cynipoidea, Figitidae, Eucoilinae) from the Eastern  
768 United States, including three newly described species. *Journal of Hymenoptera*  
769 *Research*, 53: 35-76. <https://doi.org/10.3897/jhr.53.10369>
- 770 Meiklejohn, K. A., Damaso, N., & Robertson, J. M. (2019). Assessment of BOLD and  
771 GenBank – Their accuracy and reliability for the identification of biological  
772 materials. *PLoS ONE*, 14(6): e0217084.  
773 <https://doi.org/10.1371/journal.pone.0217084>
- 774 Melk, J. P., & Govind, S. (1999). Developmental analysis of *Ganaspis xanthopoda*, a larval  
775 parasitoid of *Drosophila melanogaster*. *Journal of Experimental Biology*, 202, 1885-  
776 1896
- 777 Miller, S. E., Hausmann, A., Hallwachs, W., & Janzen, D. H. (2016). Advancing taxonomy  
778 and bioinventories with DNA barcodes. *Philosophical Transactions of the Royal*  
779 *Society Biological Sciences*, 371(1702): 20150339. doi: 10.1098/rstb.2015.0339
- 780 Mitsui, H., van Achterberg, K., Nordlander, G., & Kimura, M. T. (2007). Geographical  
781 distributions and host associations of larval parasitoids of frugivorous *Drosophilidae*  
782 in Japan. *Journal of Natural History*, 41(25–28), 1731–1738.  
783 <https://doi.org/10.1080/00222930701504797>
- 784 Moreau, S. J. M., Vinchon, S., Cherqui, A., & Prévost, G. (2009). Components of *Asobara*  
785 venoms and their effects on hosts. In *Advances in Parasitology*, Prévost G (Ed). 70,  
786 217-232, [https://doi.org/10.1016/S0065-308X\(09\)70008-9](https://doi.org/10.1016/S0065-308X(09)70008-9)
- 787 Morris, R. J., Gripenberg, S., Lewis, O. T., & Roslin, T. (2014). Antagonistic interaction  
788 networks are structured independently of latitude and host guild. *Ecology Letters*,  
789 17(3), 340-349, <https://doi.org/10.1111/ele.12235>
- 790 Nappi, A. J., & Carton, Y. (2001). Immunogenetic aspects of the cellular immune

- 791 response of *Drosophila* against parasitoids. *Immunogenetics*, 52(3–4), 157–164.  
792 <https://doi.org/10.1007/s002510000272>
- 793 Nomano, F. Y., Kasuya, N., Matsuura, A., Suwito, A., Mitsui, H., Buffington, M.L., &  
794 Kimura, M. T. (2017). Genetic differentiation of *Ganaspis brasiliensis*  
795 (Hymenoptera: Figitidae) from East and Southeast Asia. *Applied Entomology and*  
796 *Zoology*, 52(3), 429–437. <https://doi.org/10.1007/s13355-017-0493-0>
- 797 Novotny, V., & Miller, S. E. (2014). Mapping and understanding the diversity of insects in  
798 the tropics: Past achievements and future directions. *Austral Entomology*, 53(3),  
799 259–267. <https://doi.org/10.1111/aen.12111>
- 800 O’Grady, P. M., & DeSalle, R. (2018). Phylogeny of the genus *Drosophila*. *Genetics*,  
801 209(1), 1–25. <https://doi.org/10.1534/genetics.117.300583>
- 802 Pentinsaari, M., Ratnasingham, S., Miller, S. E., & Hebert, P. D. N. (2020). BOLD and  
803 GenBank revisited – Do identification errors arise in the lab or in the sequence  
804 libraries? *PLoS One*, 15(4): e0231814. <https://doi.org/10.1371/journal.pone.0231814>
- 805
- 806 Pfeiffer, D. G., Shrader, M. E., Wahls, J. C. E., Willbrand, B. N., Sandum, I., van der Linde,  
807 K., Laub, C. A., ... Day, E. R. (2019). African Fig Fly (Diptera: Drosophilidae): Biology,  
808 expansion of geographic range, and its potential status as a soft fruit pest. *Journal*  
809 *of Integrated Pest Management*, 10(1), 1–8. <https://doi.org/10.1093/jipm/pmz018>
- 810 Prendini, L., Hanner, R., & DeSalle, R. (2002). Obtaining, storing and archiving specimens  
811 and tissue samples for use in molecular studies. *In Techniques in Molecular*  
812 *Systematics and Evolution*. [https://doi.org/10.1007/978-3-0348-8125-8\\_11](https://doi.org/10.1007/978-3-0348-8125-8_11)
- 813 Prévost, G. (2009). Parasitoids of *Drosophila*. *In Advances in parasitology*.  
814 [https://doi.org/10.1016/S0065-308X\(09\)70018-1](https://doi.org/10.1016/S0065-308X(09)70018-1)
- 815 Quicke, D. L. J. (2015). The Braconid and Ichneumonid parasitoid wasps: Biology,  
816 systematics, evolution and ecology. Wiley-Blackwell,  
817 <https://doi.org/10.1002/9781118907085>
- 818 Ratnasingham, S., & Hebert, P. D. N. (2007). BARCODING: bold: The Barcode of Life Data  
819 System (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, 7(3), 355–364,  
820 <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- 821 Ratnasingham, S., & Hebert, P. D. N. (2013). A DNA-based registry for all animal species:  
822 the barcode index number (BIN) system. *PLoS ONE*, 8(7): e66213.  
823 <https://doi.org/10.1371/journal.pone.0066213>
- 824 Raupach, M. J., Hendrich, L., Kuchler, S. M., Deister, F., Moriniere, J., & Gossner, M. M.  
825 (2014). Building-Up of a DNA Barcode Library for true bugs (Insecta: Hemiptera:  
826 Heteroptera) of Germany reveals taxonomic uncertainties and surprises. *PLoS ONE*,  
827 9(9), 1–13. <https://doi.org/10.1371/journal.pone.0106940>
- 828 Remsen, J., & O’Grady, P. (2002). Phylogeny of Drosophilinae (Diptera: Drosophilidae),  
829 with comments on combined analysis and character support. *Molecular*  
830 *Phylogenetics and Evolution*, 24(2), 249–264, [https://doi.org/10.1016/S1055-](https://doi.org/10.1016/S1055-7903(02)00226-9)  
831 [7903\(02\)00226-9](https://doi.org/10.1016/S1055-7903(02)00226-9)
- 832 Reumer, B. M., van Alphen, J. J. M., & Kraaijeveld, K. (2012). Occasional males in  
833 parthenogenetic populations of *Asobara japonica* (Hymenoptera: Braconidae): Low  
834 *Wolbachia* titer or incomplete coadaptation. *Heredity*, 108(3), 341–346,

- 835 <https://doi.org/10.1038/hdy.2011.82>
- 836 Rosen, D. (1986). The role of taxonomy in effective biological control programs.
- 837 *Agriculture, Ecosystems & Environment*, 15(2-3), 121-129.
- 838 [https://doi.org/10.1016/0167-8809\(86\)90085-X](https://doi.org/10.1016/0167-8809(86)90085-X)
- 839 Santos, W. G. N., Fernandes, E. C., Souza, M. M., Guimarães, J. A., & Araujo, E. L. (2016).
- 840 First record of Eucoilinae (Hymenoptera: Figitidae), parasitoids of African fig fly
- 841 *Zaprionus indianus* Gupta (Diptera: Drosophilidae), in the Caatinga biome.
- 842 *Semina:Ciencias Agrarias*, 37(5), 3055–3058. [https://doi.org/10.5433/1679-](https://doi.org/10.5433/1679-0359.2016v37n5p3055)
- 843 [0359.2016v37n5p3055](https://doi.org/10.5433/1679-0359.2016v37n5p3055)
- 844 Schilthuizen, M., Vairappan, C. S., Slade, E. M., Mann, D. J., & Miller, J. A. (2015).
- 845 Specimens as primary data: Museums and “open science.” *Trends in Ecology and*
- 846 *Evolution*, 30(5), 237–238. <https://doi.org/10.1016/j.tree.2015.03.002>
- 847 Schlenke, T. A., Morales, J., Govind, S., & Clark, A. G. (2007). Contrasting infection
- 848 strategies in generalist and specialist wasp parasitoids of *Drosophila melanogaster*.
- 849 *PLoS Pathogens*, 3(10):e158, <https://doi.org/10.1371/journal.ppat.0030158>
- 850 Schindel, D., & Miller, S. E. (2010). Provisional Nomenclature the on-ramp to taxonomic
- 851 names. In: Polaszek, A. (Ed), *Systema Nature*, 250: The Linnaean Ark. CRC, Boca
- 852 Raton, 109-115.
- 853 Seehausen, M. L., Ris, N., Driss, L., Racca, A., Girod, P., Warot, S., Borowiec, N., Tosevski,
- 854 I., & Kenis, M. (2020). Evidence for a cryptic parasitoid species reveals its suitability
- 855 as a biological control agent. *Scientific reports*, 10: 19096.
- 856 <https://doi.org/10.1038/s41598-020-76180-5>
- 857 Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation
- 858 sequencing technologies for environmental DNA research. *In Molecular Ecology*,
- 859 21(8), 1794-1805, <https://doi.org/10.1111/j.1365-294X.2012.05538.x>
- 860 Smith, M. A., Fisher, B. L., & Hebert, P. D. N. (2005). DNA barcoding for effective
- 861 biodiversity assessment of a hyperdiverse arthropod group: The ants of
- 862 Madagascar. *Philosophical Transactions of the Royal Society Biological Sciences*,
- 863 360(1462), 1825-1834, <https://doi.org/10.1098/rstb.2005.1714>
- 864 Smith, M. A., Fernandez-Triana, J. L., Eveleigh, E., Gomez, J., Guclu, C., Hallwachs, W.,
- 865 Hebert, P. D. N., ... Zaldivar-Riveron, A. (2013). DNA barcoding and the taxonomy of
- 866 Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly
- 867 20000 sequences. *Molecular Ecology Resources*, 13, 168-276,
- 868 <https://doi.org/10.1111/1755-0988.12038>
- 869 Staniczenko, P. P. A., Reed-Tsochas, F., Lewis, O. T., Tylanakis, J. M., Albrecht, M.,
- 870 Coudrain, V., & Klein, A. M. (2017). Predicting the effect of habitat modification on
- 871 networks of interacting species. *Nature Communications*, 8, 792,
- 872 <https://doi.org/10.1038/s41467-017-00913-w>
- 873 Tarli, V. D., Grandcolas, P., & Pellens, R. (2018). The informative value of museum
- 874 collections for ecology and conservation: A comparison with target sampling in the
- 875 Brazilian Atlantic forest. *PLoS ONE*, 13(11).
- 876 <https://doi.org/10.1371/journal.pone.0205710>
- 877 Tedersoo, L., Drenkhan, R., Anslan, S., Morales-Rodriguez, C., & Cleary, M. (2019). High-
- 878 throughput identification and diagnostics of pathogens and pests: Overview and

- 879 practical recommendations. *Molecular Ecology Resources*, 19(1), 47–76.  
880 <https://doi.org/10.1111/1755-0998.12959>
- 881 Terry, J. C. D., Chen, J., & Lewis, O. T. (2020). The effect of natural enemies on the  
882 coexistence of competing species - an empirical test using Bayesian modern  
883 coexistence theory. *bioRxiv*: <https://doi.org/10.1101/2020.08.27.270389>
- 884 Thierry, M., Pardikes, N. A., Lue, C.-H., Lewis, O. L., & Hrcek, J. (2021). Experimental  
885 warming influences species abundances in a *Drosophila* host community through  
886 direct effects on species performance rather than altered competition and  
887 parasitism. *PLOS ONE* (In Press).
- 888 Troudet, J., Vignes-Lebbe, R., Grandcolas, P., & Legendre, F. (2018). The increasing  
889 disconnection of primary biodiversity data from specimens: How does it happen  
890 and how to handle it? *Systematic Biology*, 67(6), 1110–1119.  
891 <https://doi.org/10.1093/sysbio/syy044>
- 892 Tsai, C.-L., Chu, I.-H., Chou, M.-H., Chareonviriyaphap, T., Chiang, M.-Y., Lin, P.-A., Lu, K.-  
893 H., & Yeh, W.-B. (2020). Rapid identification of the invasive fall armyworm  
894 *Spodoptera frugiperda* (Lepidoptera, Noctuidae) using species-specific primers in  
895 multiplex PCR. *Scientific Reports*, 10(1), 1–9. [https://doi.org/10.1038/s41598-020-](https://doi.org/10.1038/s41598-020-73786-7)  
896 [73786-7](https://doi.org/10.1038/s41598-020-73786-7)
- 897 Tylanakis, J. M., Tscharntke, T., & Lewis, O. T. (2007). Habitat modification alters the  
898 structure of tropical host-parasitoid food webs. *Nature*, 445(7124), 202–205.  
899 <https://doi.org/10.1038/nature05429>
- 900 Wachi, N., Nomano, F. Y., Mitsui, H., Kasuya, N., & Kimura, M. T. (2015). Taxonomy and  
901 evolution of putative thelytokous species of Leptopilina (Hymenoptera: Figitidae)  
902 from Japan, with description of two new species. *Entomological Science*, 18(1), 41–  
903 54. <https://doi.org/10.1111/ens.12089>
- 904 Wang, X.-G., Biondi, A., & Daane, K. M. (2020). Functional responses of three candidate  
905 Asian larval parasitoids evaluated for classical biological control of *Drosophila*  
906 *suzukii*. *Journal of Economic Entomology*, 113(1): 73–80. doi: 10.1093/jee/toz265
- 907 Wang, X.-G., Biondi, A., Nance, A. N., Zappalà, L., Hoelmer, K. A., & Daane, K. M. (2021).  
908 Assessment of *Asobara japonica* as a potential biological control agent for the  
909 spotted wing drosophila, *Drosophila suzukii*. *Entomologia Generalis (In Press)* doi:  
910 10.1127/entomologia/2020/1100
- 911 Wang, X.-G., Lee, J., Daane, K.M., Buffington, M., & Hoelmer, K. A. (2020). Biological  
912 control of *Drosophila suzukii*. *CAB Reviews* 54, 10.1079/PAVSNNR202015054
- 913 Xiao, J. H., Wang, N. X., Murphy, R. W., Cook, J., Jia, L. Y., & Huang, D. W. (2012).  
914 *Wolbachia* infection and dramatic intraspecific mitochondrial DNA divergence in a  
915 fig wasp. *Evolution*, 66, 1907-1916, [https://doi.org/10.1111/j.1558-](https://doi.org/10.1111/j.1558-5646.2011.01561.x)  
916 [5646.2011.01561.x](https://doi.org/10.1111/j.1558-5646.2011.01561.x)
- 917 Xie, J., Tiner, B., Vilchez, I., & Mateos, M. (2011). Effect of the *Drosophila* endosymbiont  
918 *Spiroplasma* on parasitoid wasp development and on the reproductive fitness of  
919 wasp-attacked fly survivors. *Evolutionary Ecology*, 25, 1065-1079,  
920 <https://doi.org/10.1007/s10682-010-9453-7>
- 921 Xie, J., Winter, C., Winter, L., & Mateos, M. (2015). Rapid spread of the defensive  
922 endosymbiont *Spiroplasma* in *Drosophila hydei* under high parasitoid wasp

923 pressure. *FEMS Microbiology Ecology*, 91(2), 1-11,  
924 <https://doi.org/10.1093/femsec/iu017>  
925 Yassin, A. (2013). Phylogenetic classification of the Drosophilidae Rondani (Diptera): The  
926 role of morphology in the postgenomic era. *Systematic Entomology*,  
927 <https://doi.org/10.1111/j.1365-3113.2012.00665.x>  
928 Yassin, A., & David, J. R. (2010). Revision of the Afrotropical species of *Zaprionus*  
929 (Diptera, Drosophilidae), with descriptions of two new species and notes on  
930 internal reproductive structures and immature stages. *ZooKeys*, 51, 33-72,  
931 <https://doi.org/10.3897/zookeys.51.380>  
932 Ye, Z., Vollhardt, I. M. G., Girtler, S., Wallinger, C., Tomanovic, Z., & Traugott, M. (2017).  
933 An effective molecular approach for assessing cereal aphid-parasitoid-  
934 endosymbiont networks. *Scientific Reports*, 7(1), 1–12.  
935 <https://doi.org/10.1038/s41598-017-02226-w>  
936  
937

#### 938 **Data Accessibility**

939 The DROP database is freely accessible at Zenodo depository  
940 (<http://doi.org/10.5281/zenodo.4519656>). New sequences have been deposited in  
941 BOLD in datasets DS-LABS, and DS-AUSPTOID. [NOTE: the doi records will be update at  
942 revision stage]

943

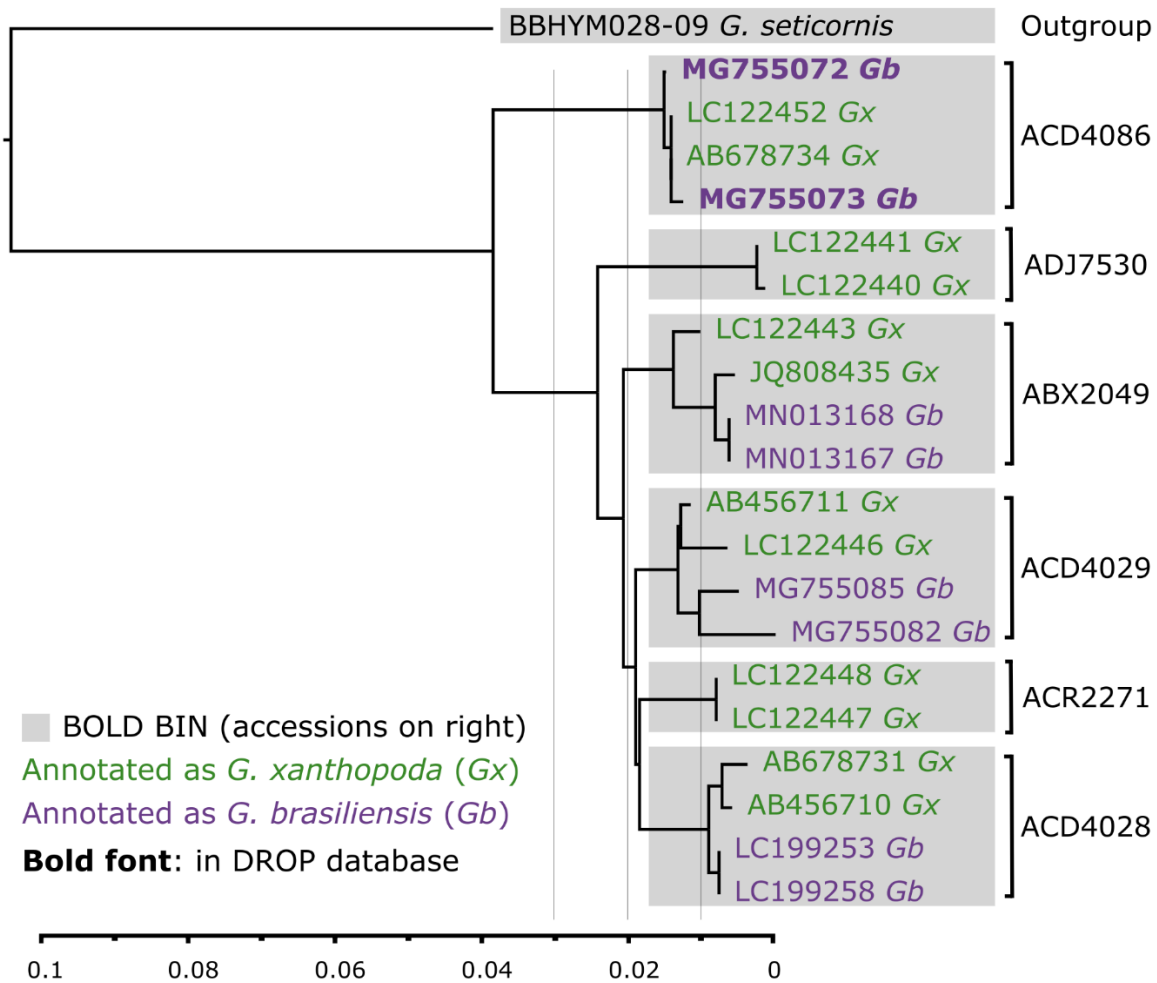
#### 944 **Author Contributions**

945 The initial project idea was originated by C-HL, MLB, JH, MM, TS, JV, SG, and  
946 PPAS. Molecular work was conducted by C-HL, SS, ML, AJ, and AD. BOLD and GenBank  
947 data was harvested by TAE and C-HL. Figures were made by AL and C-HL. Laboratory and  
948 field sample preparations were conducted by MTK, YC, TS, MM, SG, JV, EG, MG, XW,  
949 KM, KMD, PA, NAP, MT, JJB, MP, FMJ, WDT, JSD, BW, OTL, PPAS, JL and AL. Taxonomic  
950 concepts and interpretations were conducted by RRK, MLB, CH-L, PG, and SEM. DROP

951 database was built by JH and C-HL. All authors contributed to review and final revisions  
952 of the manuscript, which was written primarily by C-HL, MLB and JH.  
953

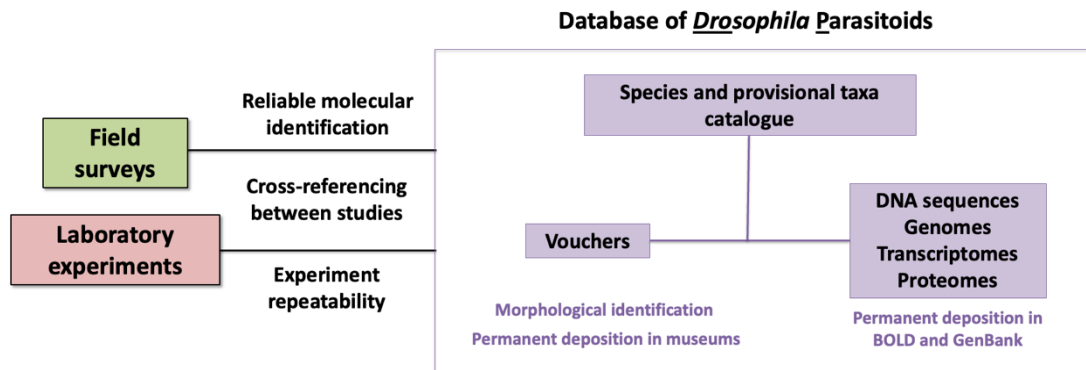
954

Figures:



955

956 Figure 1: An example of difficulties of molecular identification demonstrated on  
 957 *Ganaspis xanthopoda* and *G. brasiliensis*. Only two sequences (in bold text) can be  
 958 reliably used for identification and are included in DROP database. To select the  
 959 sequences, we searched the BINs associated with the organism's name "*Ganaspis*  
 960 *xanthopoda*" (green) or "*Ganaspis brasiliensis*" (purple) in BOLD. From each BIN, two  
 961 sequences from each species were selected to build a neighbor-joining tree (bottom axis  
 962 indicated % genetic divergence). There was a total of 6 BINs (gray boxes) in this  
 963 sequence complex. Of these, 4 BINs contained both species names and without  
 964 examination of vouchers identification would be impossible. In DROP, vouchers from  
 965 two sequences, **MG755073** and **MG755072**, were deposited in CNR-IPSP (Table S2),  
 966 examined by taxonomists and identify as *G. brasiliensis*. These two COI sequences can  
 967 now be used to reliably identify *G. brasiliensis*. For *G. xanthopoda*, there were no  
 968 available vouchers or reliable sequences that passed DROP standards to use for  
 969 identification. Species delimitation between *G. brasiliensis* and *G. xanthopoda* is  
 970 convoluted (see discussion), varies according to arbitrary % genetic divergence (gray  
 971 vertical lines), and needs future an integrative taxonomic revision.



972

973 **Figure 2:** Concept of a centralized, vetted, integrated database for *Drosophila*  
 974 *Parasitoids* (DROP) we developed. First, we provide a species and provisional species  
 975 catalog with correct taxonomy. Second, to provide a reliable genetic reference library,  
 976 genetic data (DNA sequences, genomes, transcriptomes, proteomes) link to a voucher  
 977 connected to the species catalog. Third, we link the two primary sources of data (field  
 978 surveys and laboratory experiments) by requiring a permanent deposition of vouchers  
 979 and sequences in order to be included in DROP.

980

981

982 **Tables:**

983 **Table 1:** List of species and provisional species included in DROP. For additional  
 984 taxonomic details, see DROP.

985

Superfamily	Family	Genus	Species_Name	Author
Chalcidoidea	Encyrtidae		<i>drop_Cha2_sp12</i>	
Chalcidoidea	Encyrtidae	<i>Tachinaephagus</i>	<i>drop_IR1_sp41</i>	Kimura
Chalcidoidea	Encyrtidae	<i>Tachinaephagus</i>	<i>drop_BG1_sp42</i>	Kimura
Chalcidoidea	Encyrtidae	<i>Tachinaephagus</i>	<i>zealandicus</i>	Ashmead 1904
Chalcidoidea	Pteromalidae		<i>drop_Pte69_sp11</i>	
Chalcidoidea	Pteromalidae	<i>Pachycrepoideus</i>	<i>vindemmiae</i>	(Rondani, 1875)
Chalcidoidea	Pteromalidae	<i>Spalangia</i>	<i>drop_IR1_sp38</i>	Kimura
Chalcidoidea	Pteromalidae	<i>Spalangia</i>	<i>drop_NG1_sp39</i>	Kimura
Chalcidoidea	Pteromalidae	<i>Spalangia</i>	<i>drop_SK1_sp40</i>	Kimura
Chalcidoidea	Pteromalidae	<i>Spalangia</i>	<i>drosophilae</i>	Ashmead 1887
Chalcidoidea	Pteromalidae	<i>Spalangia</i>	<i>erythromera</i>	Foerster 1850
Chalcidoidea	Pteromalidae	<i>Trichomalopsis</i>	<i>dubia</i>	(Ashmead, 1896)
Chalcidoidea	Pteromalidae	<i>Trichomalopsis</i>	<i>microptera</i>	(Lindeman, 1887)
Chalcidoidea	Pteromalidae	<i>Trichomalopsis</i>	<i>nigricola</i>	Boucek



Chalcidoidea	Pteromalidae	<i>Trichomalopsis</i>	<i>sarcophagae</i>	(Gahan, 1914)
Chalcidoidea	Pteromalidae	<i>Vrestovia</i>	<i>brevior</i>	Boucek 1993
Chalcidoidea	Pteromalidae	<i>Vrestovia</i>	<i>fidenas</i>	(Walker, 1848)
Chalcidoidea	Pteromalidae		<i>drop_PacAtl_sp46</i>	
Chalcidoidea	Pteromalidae		<i>drop_PachyPort_sp45</i>	
Chalcidoidea			<i>drop_CH_sp64</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>brasiliensis</i>	(Ihering, 1905)
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gan_sp51</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gan_sp52</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gan_sp53</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gsp1_sp67</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gsp2_sp68</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gsp50_sp66</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_IR1_sp25</i>	Kimura
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_IR2_sp26</i>	Kimura
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_Gan1_sp1</i>	
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>drop_TK1_sp27</i>	Kimura
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>hookeri</i>	Crawford 1913
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>mahensis</i>	Kieffer 1911
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>mellipes</i>	(Say, 1826)
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>mundata</i>	Forster 1869
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>seticornis</i>	(Hellen, 1960)
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>tenuicornis</i>	Kieffer 1904
Cynipoidea	Figitidae	<i>Ganaspis</i>	<i>xanthopoda</i>	(Ashmead, 1896)
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>bicolor</i>	(Giraud, 1860)
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>dolichocera</i>	Thomson 1877
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>drop_TK1_sp28</i>	Kimura
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>filicornis</i>	(Cameron, 1889)
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>icarus</i>	(Quinlan, 1964)
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>psiloides</i>	Westwood 1833
Cynipoidea	Figitidae	<i>Kleidotoma</i>	<i>tetratoma</i>	(Hartig, 1841)
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>drop_Fig64_sp5</i>	
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>drop_Lmn_sp6</i>	
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>drop_TK1_sp29</i>	Kimura
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>gressitti</i>	Yoshimoto & Yasumatsu 1965
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>papuensis</i>	Yoshimoto 1963
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>ponapensis</i>	Yoshimoto 1962
Cynipoidea	Figitidae	<i>Leptolamina</i>	<i>seychellensis</i>	(Kieffer, 1911)

Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>atraticeps</i>	(Kieffer, 1911)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>australis</i>	(Belizin, 1966)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>boulardi</i>	(Barbotin, Carton & Kelner-Pillault, 1979)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>clavipes</i>	(Hartig, 1841)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>cupulifera</i>	(Kieffer, 1916)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>decemflagella</i>	Lue & Buffington 2017
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp54</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp55</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp56</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp57</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp58</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp59</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp60</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp61</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Lep_sp62</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_BG1_sp34</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Fig059_sp4</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Fig124_sp2</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_Fig58_sp3</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_IR1_sp30</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_NG1_sp33</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_SK1_sp35</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_STL_sp7</i>	
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_TK2_sp31</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>drop_TK3_sp32</i>	Kimura
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>fimbriata</i>	(Kieffer, 1901)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>freyae</i>	Allemand & Nordlander 2002
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>guineaensis</i>	Allemand & Nordlander 2002
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>heterotoma</i>	(Thomson, 1862)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>japonica japonica</i>	Novkovic & Kimura 2011
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>lasallei</i>	Buffington & Guerrieri 2020
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>leipsi</i>	Lue & Buffington 2018
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>lonchaeae</i>	(Cameron, 1912)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>longipes</i>	(Hartig, 1841)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>mahensis</i>	(Kieffer, 1911)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>maia</i>	Lue & Buffington 2016
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>maria</i>	(Girault, 1930)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>orientalis</i>	Allemand & Nordlander 2002

Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>pacifica</i>	Novkovic & Kimura 2011
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>rufipes</i>	(Cameron, 1908)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>rugipunctata</i>	(Yoshimoto, 1962)
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>ryukyuensis</i>	Novkovic & Kimura 2011
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>tokioensis</i>	Wachi & Kimura 2015
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>tsushimaensis</i>	Wachi & Kimura 2015
Cynipoidea	Figitidae	<i>Leptopilina</i>	<i>victoriae</i>	Nordlander 1980
Cynipoidea	Figitidae	<i>Rhoptromeris</i>	<i>heptoma</i>	(Hartig, 1840)
Cynipoidea	Figitidae	<i>Rhoptromeris</i>	<i>nigriventris</i>	Nordlander 1978
Cynipoidea	Figitidae	<i>Rhoptromeris</i>	<i>rufiventris</i>	(Giraud, 1860)
Cynipoidea	Figitidae	<i>Rhoptromeris</i>	<i>villosa</i>	(Hartig, 1840)
Cynipoidea	Figitidae		<i>drop_Lg500_sp43</i>	
Ichneumonoidea	Braconidae	<i>Alysia</i>	<i>drop_SP1_sp24</i>	Kimura
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>aotea</i>	Hughes & Woolcock 1976
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>drop_SP1_sp15</i>	Kimura
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>drop_TK1_sp13</i>	Kimura
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>drop_TM1_sp14</i>	Kimura
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>minuta</i>	(Nees, 1811)
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>pallipes</i>	(Say, 1829)
Ichneumonoidea	Braconidae	<i>Aphaereta</i>	<i>scaptomyzae</i>	Fischer 1966
Ichneumonoidea	Braconidae	<i>Areotetes</i>	<i>striatiferus</i>	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	<i>Areotetes</i>	<i>carinuliferus</i>	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>ajbelli</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>albiclava</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>antipoda</i>	(Ashmead, 1900)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>bactrocerae</i>	(Gahan, 1952)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>brevicauda</i>	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>citri</i>	(Fischer, 1963)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>drop_KG1_sp16</i>	Kimura
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>drop_NG1_sp17</i>	Kimura
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>drop_SK2_sp20</i>	Kimura
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>drop_SP1_sp18</i>	Kimura
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>drop_Sp2_sp19</i>	Kimura
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>elongata</i>	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>gahani</i>	(Papp, 1969)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>japonica</i>	Belokobylskij 1998
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>kenyaensis</i>	Peris-Felipo 2014
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>leverii</i>	(Nixon, 1939)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>mesocauda</i>	van Achterberg & Guerrieri 2016

Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>orientalis</i>	Viereck 1913
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>persimilis</i>	(Prince, 1976)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>pleuralis</i>	(Ashmead, 1905)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>rossica</i>	Belokobylskij 1998
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>rufescens</i>	(F <sup>r</sup> ster, 1862)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>tabida</i>	(Nees, 1834)
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>triangulata</i>	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>turneri</i>	Peris-Felipo 2014
Ichneumonoidea	Braconidae	<i>Asobara</i>	<i>unicolorata</i>	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>albertica</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>andyaustini</i>	Wharton 2002
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>angusta</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>concolor</i>	Nees 1812
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>parecur</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Aspilota</i>	<i>villosa</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Dinotrema</i>	<i>barrattae</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Dinotrema</i>	<i>longworthi</i>	Berry 2007
Ichneumonoidea	Braconidae	<i>Dinotrema</i>	<i>philipi</i>	Berry 2007
Ichneumonoidea	Braconidae		<i>drop_Aso_sp8</i>	
Ichneumonoidea	Braconidae	<i>Opiognathus</i>	<i>pactus</i>	(Haliday, 1837)
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>bellus</i>	Gahan 1930
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>cinerariae</i>	Fischer
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>crenuliferus</i>	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>monilipalpis</i>	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>ocreatus</i>	(Papp)
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>pallipes</i>	Wesmael 1835
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>pteridiophilus</i>	Wharton & Austin 1990
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>pterus</i>	Wharton & Austin 1990
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>trimaculatus</i>	Spinola
Ichneumonoidea	Braconidae	<i>Opius</i>	<i>youi</i>	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	<i>Phaenocarpa</i>	<i>conspurcator</i>	(Haliday, 1838)
Ichneumonoidea	Braconidae	<i>Phaenocarpa</i>	<i>drop_IR1_sp22</i>	Kimura
Ichneumonoidea	Braconidae	<i>Phaenocarpa</i>	<i>drop_TK1_sp21</i>	Kimura
Ichneumonoidea	Braconidae	<i>Phaenocarpa</i>	<i>tacita</i>	Stelfox 1941
Ichneumonoidea	Braconidae	<i>Phaenocarpa</i>	<i>drosophilae</i>	(Fischer 1975)
Ichneumonoidea	Braconidae	<i>Tanycarpa</i>	<i>bicolor</i>	(Nees, 1814)
Ichneumonoidea	Braconidae	<i>Tanycarpa</i>	<i>chors</i>	Belokobylskij 1998
Ichneumonoidea	Braconidae	<i>Tanycarpa</i>	<i>drop_NG1_sp23</i>	Kimura
Ichneumonoidea	Braconidae	<i>Tanycarpa</i>	<i>punctata</i>	van Achterberg 1976

Ichneumonoidea	Braconidae		<i>drop_Aly_sp47</i>	
Ichneumonoidea	Braconidae		<i>drop_Aly_sp48</i>	
Ichneumonoidea	Braconidae		<i>drop_Aly_sp49</i>	
Ichneumonoidea	Braconidae		<i>drop_Aly_sp50</i>	
Ichneumonoidea	Braconidae		<i>drop_Aly_sp63</i>	
Ichneumonoidea	Braconidae		<i>drop_Aso_sp69</i>	
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>anastrephae</i>	Costa Lima 1940
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_BG1_sp37</i>	Kimura
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_Dia70_sp65</i>	
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_Tri_sp44</i>	
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_Bdia_sp10</i>	
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_Dia127_sp9</i>	
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drop_TK1_sp36</i>	Kimura
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>drosophilae</i>	(Kieffer, 1912)
Diaprioidea	Diapriidae	<i>Trichopria</i>	<i>modesta</i>	(Ratzeburg, 1848)

986

987 **Table 2.1:** *Drosophila* parasitoid whole-genome sequences included in DROP. For  
 988 additional details, see DROP.

989

Genus	Species_Name	Species_id	Genome_id	Voucher_id	GenBank_id
<i>Ganaspis</i>	<i>brasiliensis</i>	19	8	868	GCA_009823575.1
<i>Ganaspis</i>	<i>brasiliensis</i>	19	16	872	SRX8882993
<i>Ganaspis</i>	<i>brasiliensis</i>	19	17	871	SRX8882992
<i>Ganaspis</i>	<i>drop_Gsp1_sp67</i>	182	15	873	SRX8882994
<i>Ganaspis</i>	<i>drop_Gsp2_sp68</i>	183	14	874	SRX8882995
<i>Ganaspis</i>	<i>drop_Gsp50_sp66</i>	181	9	869	GCA_011057455.1
<i>Leptolamina</i>	<i>ponapensis</i>	48	13	875	SRX8883008
<i>Leptopilina</i>	<i>boulardi</i>	4	5	865	GCA_011634795.1
<i>Leptopilina</i>	<i>boulardi</i>	4	6	866	GCA_003121605.1
<i>Leptopilina</i>	<i>boulardi</i>	4	12	876	SRX8883009
<i>Leptopilina</i>	<i>clavipes</i>	5	7	867	GCA_001855655.1
<i>Leptopilina</i>	<i>heterotoma</i>	6	1	861	GCA_010016045.1
<i>Leptopilina</i>	<i>heterotoma</i>	6	2	862	GCA_009602685.1
<i>Leptopilina</i>	<i>heterotoma</i>	6	3	863	GCA_009026005.1
<i>Leptopilina</i>	<i>heterotoma</i>	6	4	864	GCA_009025955.1
<i>Leptopilina</i>	<i>japonica japonica</i>	13	11	877	SRX8883011

990

991

992

993 **Table 2.2:** *Drosophila* parasitoid transcriptome data included in DROP.  
994

Genus	Species_Name	Strain_id	Transcriptome_id	Voucher_id	Genbank_id
<i>Leptopilina</i>	<i>boulardi</i>	126	2	858	2183568
<i>Leptopilina</i>	<i>boulardi</i>	127	3	859	2183567
<i>Leptopilina</i>	<i>boulardi</i>	151	8	882	15642271
<i>Leptopilina</i>	<i>boulardi</i>	151	9	883	15642270
<i>Leptopilina</i>	<i>heterotoma</i>	147	1	857	2183569
<i>Leptopilina</i>	<i>heterotoma</i>	152	5	884	2046288
<i>Leptopilina</i>	<i>heterotoma</i>	61	6	880	11581553
<i>Leptopilina</i>	<i>heterotoma</i>	60	7	881	11662592
<i>Leptopilina</i>	<i>boulardi</i>	11	10	908	GAJA00000000.1
<i>Leptopilina</i>	<i>heterotoma</i>	14	11	909	GAJC00000000.1
<i>Ganaspis</i>	<i>hookeri</i>	7	12	910	GAIW00000000.1

995

996 **Table 2.3:** *Drosophila* parasitoid proteomes data included in DROP  
997

Genus	Species_Name	Strain_id	Proteomes_id	Voucher_id	Assession_id
<i>Leptopilina</i>	<i>heterotoma</i>	152	1	885	PRIDE: PXD005639
<i>Leptopilina</i>	<i>heterotoma</i>	61	2	886	PRIDE: PXD005632
<i>Leptopilina</i>	<i>boulardi</i>	27	3	911	Upon request to julien.varaldi@univ-lyon1.fr
<i>Leptopilina</i>	<i>boulardi</i>	11	4	912	PRIDE: PDX023836
<i>Leptopilina</i>	<i>heterotoma</i>	14	5	913	PRIDE: PDX023824
<i>Ganaspis</i>	<i>hookeri</i>	7	6	914	PRIDE: PDX023825

998