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

Resource article

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Abstract

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

specimens are identified by taxonomists and vetted through direct comparison with primary type material. To initiate DROP, we curated 154 laboratory strains, 856 vouchers, 554 DNA sequences, 16 genomes, 14 transcriptomes, and 6 proteomes drawn from a total of 183 operational taxonomic units (OTUs): 114 described *Drosophila* parasitoid species and 69 provisional species. We found species richness of *Drosophila* parasitoids to be heavily underestimated and provide an updated taxonomic catalogue for the community. DROP offers accurate molecular identification and improves cross-referencing between individual studies that we hope will catalyze research on this diverse and fascinating model system. Our effort should also serve as an example for researchers facing similar molecular identification problems in other groups of organisms.

Key Words

Biodiversity, DNA sequences, Genomes, Integrative taxonomy, Molecular diagnostics, Biological control

Introduction

Building a knowledge base that encompasses ecology, evolution, genetics, and biological control is contingent on reliable taxonomic identifications. Molecular identification is commonly used in groups of organisms with cryptic species that are difficult to identify morphologically (Fagan-Jeffries et al., 2018; Miller et al., 2016; Novotny & Miller, 2014), for the molecular detection of species interactions (Baker et al., 2016; Condon et al., 2014; Gariepy et al., 2019; Hrček & Godfray, 2015; Hrcek et al., 2011), and for identification of species from environmental DNA samples (Shokralla et al., 2012). The accuracy of molecular identification, however, depends on the accuracy of identifications associated with sequences databased in existing online depositories (Fontes et al., 2021). The foundations of that accuracy are the voucher specimens which were sequenced and the collaboration of a taxonomic authority in the deposition of the sequence data.

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

154 Although the BOLD database function “BOLD-IDS” allows considerable database
155 curation (e.g. flagging of misidentified/contaminated records), it also automatically
156 includes sequences from GenBank, and may perpetuate the shortcomings previously
157 mentioned since these cannot be curated from within BOLD. As such, the quality of
158 sequences and the reliability of identifications obtained from BOLD-IDS can vary, and
159 depends on the curation by taxonomists focusing on individual taxa (Meiklejohn et al.,
160 2019). BOLD-IDS works well for taxa where qualified taxonomists have been involved
161 with assuring data quality; some insect examples include beetles (Hendrich et al., 2015),
162 butterflies (Escalante et al., 2010), geometrid moths (Hausmann et al., 2011, 2016;
163 Miller et al., 2016), true bugs (Raupach et al., 2014), and microgastrine wasps (Smith et
164 al., 2013).
165 Unfortunately, this is not the case of parasitoids (Insecta: Hymenoptera) of
166 *Drosophila* flies (Insecta: Drosophilidae). There are vast numbers of *Drosophila*
167 parasitoid sequences readily available in GenBank and BOLD, as these parasitoids and
168 their hosts are important model organisms in biology. As of this writing, there are
169 88,666 nucleotide sequences deposited in GenBank for *Leptopilina heterotoma*
170 (Thomson) and *L. boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae)
171 alone. However, less than 1 % of the identifications associated with these sequences
172 have been confirmed by taxonomists or are associated with voucher specimens
173 deposited in museum collections. With sequencing shifting from individual genes to
174 genomes we risk that the identification problems will soon apply to whole genomes.
175 ***Drosophila and their parasitoids***

176 The phylogenetic and subgeneric structure within *Drosophila* and related genera is
177 not yet fully resolved (O'Grady & DeSalle, 2018). Various subgenera, including
178 *Scaptomyza*, *Zaprionus*, *Lordiphosa* and *Samoaia*, have been treated as both genera and
179 subgenera, and researchers have yet to achieve consensus on these various hypotheses
180 (O'Grady & DeSalle, 2018; Remsen & O'Grady, 2002; Yassin, 2013; Yassin & David,
181 2010). Species in *Drosophila* subgenera and genera closely related to *Drosophila*
182 commonly share niche space and natural histories and, as a result, are often attacked by
183 overlapping or identical groups of parasitoids. For instance, the invasive African fig fly,
184 *Zaprionus indianus* Gupta is attacked by *Pachycrepoideus vindemiae* (Rondani, 1875)
185 and *Leptopilina boulardi* (Pfeiffer et al., 2019; Santos et al., 2016), all of which have been
186 recorded from *Drosophila*. Therefore, we also include these groups within the contents
187 of DROP.
188 Parasitoids of *Drosophila* belong to four superfamilies of Hymenoptera
189 (Chalcidoidea, Cynipoidea, Ichneumonoidea, Diaprioidea) which evolved parasitism of
190 *Drosophila* flies independently. All the parasitoids known to attack *Drosophila* are
191 solitary and attack either the larval or pupal stage; in both cases, they emerge from the
192 fly's puparium (Carton et al., 1986; Prévost, 2009). The known *Drosophila* larval
193 parasitoids belong to two families (Table 1), Braconidae (including the genera *Asobara,*
194 *Aphaereta, Phaenocarpa, Tanycarpa, Aspilota, Opius*) and Figitidae (*Leptopilina,*
195 *Ganaspis, Leptolamina, Kleidotoma*); all are koinobionts that allow the host to continue
196 development while the parasitoid grows within it. The known *Drosophila* pupal
197 parasitoids belong to three other families (Table 1), Diapriidae (*Trichopria, Spilomicrus*),

198 Pteromalidae (*Pachycrepoideus, Spalangia, Trichomalopsis, Toxomorpha*) and Encytidae
199 (*Tachinaephagus*); they are all idiobionts that terminate host development immediately.
200 Host-specificity across the *Drosophila* parasitoids is poorly characterized—while some
201 can parasitize other families of Diptera (e.g., *Aphaereta aotea*) (Hughes & Woolcock,
202 1976), most are thought to be limited to *Drosophila* hosts.
203 There are around 4000 described species of Drosophilidae, and *Drosophila* contains
204 more than a third of the family's described species (O'Grady & DeSalle, 2018). By
205 contrast, although parasitic wasps are generally a species-rich group (Dolphin & Quicke,
206 2001; Quicke, 2015), the most recent catalogue of parasitoid species that attack
207 *Drosophila* lists only 50 described species (Carton et al., 1986). This disparity suggests
208 that the diversity of parasitic wasps attacking *Drosophila* is severely underestimated, an
209 assertion supported by the results presented here. This is largely a consequence of the
210 challenging nature of parasitoid taxonomy, in which morphological identification is
211 intractable for many species, and the fact that taxonomic specialists are greatly
212 outnumbered by the species they study.
213 Currently, only a few biological study systems have been characterized in
214 sufficient breadth and depth to allow researchers to connect various levels of biological
215 organization, from molecular mechanisms to food webs of interacting species.
216 Parasitoids of *Drosophila* represent one such system (Prévost, 2009). Moreover, the
217 practical feasibility of rearing parasitoids of *Drosophila* under laboratory conditions has
218 led to a number of fundamental discoveries in ecology (Carton et al., 1991; Terry et al.,
219 2021), evolution (Kraaijeveld & Godfray, 1997), immunology (Kim-Jo et al., 2019; Nappi

220 & Carton, 2001; Schlenke et al., 2007), physiology (Melk & Govind, 1999), symbiosis (Xie
221 et al., 2011, 2015), behavioral science (Lefèvre et al., 2012) and other fields. In contrast
222 to this large body of laboratory studies, basic natural history of *Drosophila* parasitoids,
223 especially their species richness is little known (Kimura & Mitsui, 2020; Lue et al., 2018).
224 Addressing this knowledge gap is especially pressing given current efforts to use
225 parasitoids in biological control efforts, such as those of the invasive pest spotted wing
226 *Drosophila*, *Drosophila suzukii* (Abram et al., 2020; Daane et al., 2016; Giorgini et al.,
227 2019; Wang et al., 2020 a&b).

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

237 ***Aims***

238 To address these issues, we introduce a newly curated molecular reference database
239 for *Drosophila* parasitoids —DROP— in which sequences are either linked to voucher
240 specimens identified by taxonomists or have a traceable provenance (Figure 2). The first
241 aim of DROP is to provide a reliable DNA sequence library for molecular identification of

242 *Drosophila* parasitoids that enables cross-referencing of original taxonomic concepts
243 with those of subsequent studies. We pay special attention to live parasitoid strains
244 which are available for future experiments. The second aim is to standardize and
245 expedite the linkage between specimens and available sequence data; we place a
246 premium on museum vouchers as they allow for repeatable scientific research. In DROP,
247 this goal is facilitated through a consolidated digital infrastructure of data associated
248 with laboratory strains, offering the opportunity for researchers to re-examine past
249 experimental results in a permanent context. The third aim is to provide an up-to-date
250 catalogue of the diversity of *Drosophila* parasitoids as a foundation for advancing the
251 understanding of their taxonomy. Finally, the fourth aim of DROP is for our collaborative
252 effort to serve as an inspiration to communities of researchers studying other groups of
253 organisms who are experiencing difficulties with the reliability of molecular reference
254 databases.
255

256 **Materials and Methods**

257 ***Data sources***

258 To assemble the DROP database, we targeted 20 wasp genera that potentially
259 parasitize frugivorous *Drosophila* species. We compiled DNA sequence and voucher data
260 from four sources: 1) museum collections, 2) publications, for which we selected the
261 reference with taxonomist or parasitoid biologists as coauthors to ensure reliable
262 species identity, 3) molecular biodiversity inventories publicly available in BOLD and

263 GenBank, for which we managed to secure inspection of the vouchers by taxonomists,
264 and 4) a sequencing and taxonomic inventory of laboratory strains we conducted.
265 We first gathered species information into a catalogue of *Drosophila* parasitoid
266 species (Table 1) from 216 references (see DROP database reference table) and 36
267 institutes (Table S2). To ensure reliable names for nominal species (sequences identified
268 by a species name) in our database, we confirmed their taxonomic validity using the
269 Ichneumonoidea 2015 digital catalogue (Yu et al., 2016) and Hymenoptera Online (HOL;
270 http://hol.osu.edu/), both of which are curated by taxonomic experts. To obtain reliable
271 molecular identification data, we harvested 8,298 DNA sequences from GenBank and
272 BOLD (all compiled in BOLD as DS-DROPAR dataset dx.doi.org/10.5883/DS-DROPAR). As
273 of writing, these sequences represented 445 Barcode Index Numbers (BINs – a form of
274 dynamic provisionary taxa in BOLD, more detail in Ratnasingham & Hebert 2013), and
275 211 named taxa.
276 The majority of the harvested sequences were Braconidae (6690), Diapriidae
277 (967), Figitidae (622), and Pteromalidae (19). Because of the concerns with generic
278 databases (noted above and in Figure 1 and Table S1), we assembled a list of sequences
279 with valid species names that could either be traced back to vouchers examined by
280 taxonomists or were referred to directly in publications authored by a recognized expert
281 in the relevant taxon group. We then cross-checked species names with their
282 corresponding BINs in BOLD and flagged potential conflicts between species names and
283 BINs (Table S1).

284 A core goal of DROP besides that of a tool for biodiversity research is to function as a
285 platform that accommodates *Drosophila* parasitoids kept in laboratory strains (for
286 experimental work) or cultures in quarantine facilities (for biological control
287 applications). So far, there has been a lack of a coherent and reliable means of verifying
288 identification of species kept in laboratory settings, which can be a serious problem.
289 Since lab cultures are routinely contaminated by neighboring cultures (e.g., through
290 escapees), one species may be displaced by another even under a vigilant eye.
291 For lab and quarantine lines in DROP, we deposited DNA extractions and vouchers in
292 the National Insect Collection, National Museum of Natural History, Smithsonian
293 Institution (USNM; Washington, DC, USA). During their initial assembly of DROP,
294 laboratory OTUs (operational taxonomic unit) were designated by their strain name;
295 most laboratory strains can be associated with provisional species, but some cannot yet
296 be assigned. Three females and three males of each strain were dry-mounted and
297 individually assigned a USNMENT 'QR code' specimen label as representative vouchers.
298 For each molecular voucher, three legs from a female wasp were removed for DNA
299 extraction and sequencing (Supplementary Methods for details), and the rest of the
300 body was assigned a USNMENT specimen label and preserved for morphological
301 identification. Both DNA extraction and vouchers were entered into the database and
302 uploaded to BOLD (DROP project: DS-LABS dx.doi.org/10.5883/DS-LABS) with an
303 associated GenBank ID.
304 Where possible, we identified OTU strains using a combination of morphological and
305 sequence data, and characterized provisional species or species clusters using neighbor-

306 joining trees (Figure S1) based on the COI gene sequences (Supplemental material). For
307 establishing BIN limits in the context of DROP, we have adopted an initial percent cutoff
308 at 2%. We acknowledge that 2% genetic diverge cutoffs (or BINs) are unlikely to work
309 well across range of widely distributed species (Lin et al., 2015). But as Ratnasingham &
310 Hebert (2013) pointed out, 2% is a good starting point for many taxa, also it may need to
311 be adjusted as more samples are acquired and compared. Note that we use the term
312 "OTU" as a general and neutral designation encompassing described species, provisional
313 species, undescribed species, and cryptic species.
314
315 ***Drosophila parasitoid database—DROP***
316 To compile the above information, we built a simple Structured Query Language
317 (SQL) database in sqlite3 format using SQLiteStudio (step by step user instruction in
318 supplemental material). Sqlite3 is a cross-platform format which can be also be opened
319 using a number of other programs. There are eight linked tables in the database —
320 species, strain, voucher, sequence, genome, transcriptome, proteome and reference —
321 along with additional tables for linking these to reference table (Figure S2). The
322 database incorporates all sample fields used by BOLD for compatibility and includes a
323 number of new fields to accommodate a catalogue of *Drosophila* parasitoid species,
324 laboratory strain information, and links from the DROP database to BOLD and GenBank
325 records.
326 DROP is available on Zenodo (http://doi.org/10.5281/zenodo.4519656) for
327 permanent deposition and version control. In addition to the main database, the

328 Zenodo repository includes additional files to facilitate easy use of the database. These
329 files include: 1) the reference database in comma-separated text (.csv) and FASTA
330 format ready to be used for molecular identification; 2) a species catalogue with
331 taxonomic information; and 3) a list of laboratory strains with confirmed molecular
332 vouchers. DROP will be continued to be curated and maintained by C-HL at the Zenodo
333 repository and sequences generated in the future will also be deposited in BOLD (DROP
334 project). If the curator changes, this will be announced in the README.md file in Zenodo
335 repository. As the database relies on vouchers, we will aim for it to be continued to be
336 maintained by taxonomists with direct access to museums.
337
338 ***Species, provisional species, and OTU designations***
339 In addition to the inherent value of a formal taxonomic name, a reliable provisional
340 taxon label can also be used for exchanging scientific information and conveying
341 experimental results among researchers (Schindel & Miller, 2010). Based on the amount
342 of sequence divergence between described species, we observed what appears to be a
343 significant number of provisional OTUs in the initial dataset we compiled. Furthermore,
344 among the data linked to a valid species name, some of these provisional OTUs are
345 actively being used in research and have sequences available to the public. We
346 therefore provide a list of provisional species (potential new species) with their
347 molecular vouchers.
348 We use the following designation format for OTUs that refer to a provisional species:
349 “Drop_strainX_sp.1” or, when no other information is known, “DROP_sp.1”. Where

350 possible, these OTUs are linked to a voucher USNM specimen label number. If the genus
351 of the OTU is known, the "Drop_Leptopilina_sp.1" format is followed. These
352 designations can facilitate species identification as well as discovery and description of
353 new species without compromising the existing taxonomy of the described OTUs in
354 question. As more complete species descriptions become available, this provisional
355 species framework can be updated while keeping the link to previous provisional species
356 name through deposited vouchers.
357
358 **Results**
359 ***Overview of DROP***
360 We catalogued 183 OTUs in the DROP database with 114 described species of
361 *Drosophila* parasitoids and 69 provisional species (Table 1). In total, we documented 154
362 laboratory strains (Table S3), and 853 vouchers from 36 institutions (Table S2). Among
363 the described species, 98 have voucher information, of which 61 are traceable to type
364 specimens, including 45 to holotypes (i.e., specimen used to root a name to the
365 taxonomic author's concept of the species). *Leptopilina* is represented by the highest
366 number of species with 45 OTUs, followed by *Asobara* with 26 OTUs. Within the 154
367 catalogued lab strains, 86 were actively being used in ongoing research (i.e*.,* a live strain
368 being cultivated). These strains represent 39 OTUs: 11 described species and 28
369 provisional species (Table S3, Figure S1).
370
371 ***Molecular Vouchers***

372 So far, DROP includes 545 DNA sequences and links to 16 genomes (Table S4), 14
373 transcriptomes (Table S5), and 6 proteomes (Table S6). From the total of 8298 DNA
374 sequences (BOLD dataset: DS-DROPAR) collected from public databases, only 322
375 sequences (less than 4% of available sequences) satisfied the reliability criteria we
376 imposed for molecular vouchers to be included in DROP (see Materials and Methods).
377 The DS-DROPAR dataset dx.doi.org/10.5883/DS-DROPAR initially referred to 211 taxon
378 names, but only 52 names were valid, linked to vouchers, or linked to a publication with
379 evidence that the specimens had been identified by taxonomists. The remaining 223 of
380 545 DROP DNA sequences were generated by DROP project (datasets: DS-LABS
381 dx.doi.org/10.5883/DS-LABS and DS-AUSPTOID dx.doi.org/10.5883/DS-AUSPTOID) and
382 came from 121 OTUs (101 lab strains and 12 provisional species).
383 The DROP database is largely made up of standard barcode COI sequences (349
384 sequences), which includes 77 OTUs: 43 described species and 33 provisional species.
385 We aimed to supplement COI with secondary markers (28SD2, 18S, ITS2) when possible,
386 resulting in an additional 120 sequences from 26 OTUs: 15 described species and 11
387 provisional species. There are currently 19 OTUs that have sequences from more than
388 one genetic marker.
389
390 ***Species Delimitation in Laboratory Strains***
391 We used 298 COI sequences to resolve the identification of each laboratory
392 strain, and where possible, indicated potential species clusters (Fig. S1 and Table S3).
393 Using a fixed 2% divergence cutoff, a total of 31 lab strain OTUs were assignable to a

394 valid species name, and the remaining 70 strain OTUs were assigned to a provisional
395 species. The taxonomic status of several of these provisional species is also being
396 investigated using an integrative taxonomic approach involving morphological
397 identification, genomic data, or other genetic data.

398

399 **Discussion**

400 In this paper, we introduce and describe a free and open-access database for the
401 reliable molecular identification of *Drosophila* parasitoids. The guiding principle of DROP
402 is data credibility, based on the prerequisite that genetic data are explicitly associated
403 with voucher specimens and taxonomic concepts of the original authors (Troudet et al.,
404 2018). When incorporating information from public genetic databases, we included only
405 sequences that have passed our filtering protocol. This protocol ensures each entry is
406 associated with a valid scientific name, provisional name, or consistently applied OTU
407 designation that can be used to integrate genetic and organismal data from
408 independent studies.

409 The following discussion expands on the utility of DROP and how we hope it will
410 benefit molecular species identification, connect research from various disciplines,
411 support biological control applications, and serve as a long-term molecular voucher
412 repository and clearinghouse for vetted data. We also provide specific guidance for
413 users how best to refer to DROP in their publications to allow cross-linking between
414 studies.

415

416 ***Molecular (mis-)identification***

417 We observe that 17% of the described *Drosophila* parasitoid OTUs in BOLD and
418 GenBank (dataset: DS-DROPAR) are associated with more than one BIN; these are
419 examples of BIN-ID conflict. Roughly half of these OTUs are used as lab strains. This
420 latter observation is disturbing, because it demonstrates that the criteria used to
421 differentiate and reference species in active research programs are clouded. For
422 example, BIN-ID conflicts were observed in the *Drosophila* parasitoids *Ganaspis*
423 *brasiliensis* (Ihering) and *Asobara japonica* Belokobylskij (Table S1), both of which are in
424 active use in numerous research programs (e.g. Moreau et al., 2009; Nomano et al.,
425 2017; Reumer et al., 2012; Wang et al., 2020a & 2021) as well as in biological control
426 efforts against the invasive *D. suzukii* (e.g. Abram et al., 2020; Daane et al., 2016;
427 Giorgini et al., 2019). All the BINs from *G. brasiliensis* carry the name *G. xanthopoda*
428 (Figure 1). In such instances, assigning an identification by matching specimens to
429 barcode records in the genetic database is problematic, as two names are applied to the
430 same BIN. If sequences comprising the BIN are not linked to a voucher that can be
431 examined, teasing apart the two names and how they are applied is impossible.
432 Applying explicit, consistent criteria for species determination ensures that
433 experimental results can be reliably repeated, and that any potentially novel
434 observations will not be explained away as artifacts of identification. DROP addresses
435 these concerns by linking reliable reference sequences and vouchers for *G. brasiliensis*
436 (Figure 1) between different studies: one with reference to the morphological

437 description (Buffington & Forshage, 2016) and the other with reference to the genome
438 (using voucher specimens from the morphological study; Blaimer et al., 2020).
439 We were not able to resolve all conflicts between BIN and species identity, for one
440 or more of the following three reasons: First, many records lack reliably identified
441 vouchers and have often been themselves used for molecular identification,
442 proliferating errors. Second, in some cases, it is not possible to verify whether the
443 genetic differences among BINs represent different species or simply intraspecific
444 genetic variation (Bergsten et al., 2012), because BINs themselves are not a species
445 concept. The only solution to this problem is to derive original sequence data from type
446 specimens (which is often either impractical or impossible for a number of technical
447 reasons), or from specimens whose conspecificity with the types has been corroborated.
448 Since species boundaries are always subject to testing, additional specimens from
449 multiple collecting events (ideally representing different seasons and geographic
450 regions) may help provide the additional data to circumscribe a given species' limits. The
451 third difficulty in resolving BIN-ID conflict derives from the data themselves: Although
452 the mitochondrial COI gene is the locus most frequently chosen for identification of
453 insects and other animals, its effectiveness varies among insect groups (Brower &
454 DeSalle, 2002; Gompert et al., 2008; Lin & Danforth, 2004). In part, this derives from
455 gene-tree/species-tree conflict as a function of mitochondrial DNA introgression
456 (Gompert et al., 2008; Klopfstein et al., 2016), parthenogenesis (Reumer et al., 2012),
457 and/or *Wolbachia* infection (Ferrer-Suay et al., 2018; Wachi et al., 2015; Xiao et al.,
458 2012), any of which may lead to complications in species delimitation using

459 mitochondrial loci. Ideally, studies should apply multiple loci, genomes, and comparative
460 taxonomic data to clarify species boundaries. As *Drosophila* parasitoids are often
461 maintained in laboratory cultures, it is also possible to use mating experiments to
462 explore species boundaries under the paradigm of the biological species concept
463 (Seehausen et al., 2020).
464
465 ***DROP as a taxonomic tool***
466 DROP offers an empirical platform for species discovery and a useful tool for
467 taxonomic research. The fact that the number of BINs reported here exceeds the
468 number of described species (Table S1, Figure S3) highlights the need for taxonomic
469 work. But such work cannot proceed on the basis of BINs or barcodes, but requires
470 integrative taxonomic approach employing a combination of molecular and
471 morphological data. Describing new species on the sole basis of a barcode or BIN,
472 without the benefit of independent character data, should, in general, be avoided
473 (Meier et al., 2021). It risks creating nomenclatural synonymy if it is later determined
474 that a sequence can be attributed to a specimen that bears a valid, available name.
475 Moreover, BINs are based on distance analyses which, by definition, are incompatible
476 with diagnoses per se (Ferguson, 2002; Prendini et al., 2002; Goldstein & DeSalle, 2011).
477 Therefore, in taxonomic treatments, it is critical to clarify the range of applicability of a
478 given BIN and its overlap with a taxonomic name (see example in Figure 1). DROP allows
479 cross-linking between studies and therefore provides researchers with valuable tools for

480 taxonomic revisions, including the means of discovery, corroboration, and description of
481 new species.
482
483 ***How to use DROP to ensure cross-linking between studies and reliable molecular***
484 ***identification?***
485 Public genetic databases have adopted a longstanding convention in treating
486 undetermined OTUs and sequences, referring to provisional species with numbers, as
487 for example "sp. 1", and these are rarely linked to vouchers. For OTUs designated as
488 provisional species, DROP enables cross-indexing of specimens, sequences and
489 references between any studies (ecological, taxonomic, evolutionary, genetic, etc). The
490 best way to ensure cross-linking is depositing a voucher in DROP, together with a
491 sequence or genome from the same individual (or individual from the same strain or
492 series). For example, one can write:
493
494 *Provisional species "drop_Gan1_sp.1" refers to voucher USNMENT01557320*
495 *deposited in the USNM, Washington DC, COI sequence (DROP sequence_id: 2, BOLD*
496 *Process ID: DROP143-21), 28SD1 sequence (DROP sequence_id: 289), and 28SD2*
497 *sequence (DROP sequence_id: 303).*
498
499 Similarly, laboratory strains can be reported in the same way, just adding the
500 DROP lab strain_id. It is important to periodically recheck identification of laboratory
501 strains as cultures are easily cross-contaminated, and deposit vouchers of laboratory

502 strains associated with experiments to DROP. In the future, when e.g.
503 “drop_Gan1_sp.1” is described as a new species with a formal specific epithet, DROP
504 curator will update the species status and holotype information while keeping this
505 provisional species name as an informal “synonym.”
506 A weaker and thus much less preferred way of cross-linking is to state in the
507 study that the identification of organisms was performed based on molecular
508 identification match of a sequence to DROP sequences. This is the only available option
509 for environmental DNA studies. For example, one can write:
510
511 *Provisional species “drop_Gan1_sp.1” was identified based on 99.9% blast match*
512 *of COI to DROP sequence_id: 2 (BOLD Process ID: DROP143-21).*
513
514 DROP deposition in Zenodo allows referencing of DROP either through general
515 doi (the doi we use throughout this paper), which takes the user always to the latest
516 database version, or through a doi specific to DROP version. When referencing DROP
517 please primarily cite this paper, but for reproducibility it is also good practice to include
518 doi of the specific DROP version used.
519 There are two basic ways of molecular identification which should ideally be
520 used in combination: sequence matching (blast), and tree-building methods which
521 investigate membership to a cluster. Further, there are a number of decisions to be
522 made with each method, concerning locus (or loci) and thresholds. DROP leaves these
523 decisions up to the users, only provides raw sequences or links to them. Practically, the

524 choice of loci is currently mostly limited to COI, but in the future it is likely that
525 molecular identifications will be based on multiple loci or whole genomes. Over time we
526 will also get a better idea about what thresholds are more appropriate than a fixed 2%
527 cut off. For rarer parasitoid genera which attack also other hosts besides *Drosophila*
528 (e.g. *Opius*, or *Spalangia* wasps) we suggest caution in the identification using only
529 DROP sequences as DROP does not include all sequences from these genera, but just
530 from species which are already known to attack *Drosophila*.
531
532 ***From molecular mechanisms to ecosystem structure***
533 The use of molecular tools in insect biodiversity studies has gradually expanded from
534 barcoding single individuals to metabarcoding large environmental samples
535 representing entire food webs (Jeffs et al., 2020; Littlefair et al., 2016). *Drosophila* and
536 their parasitoids are among the few systems that currently allow us to explore
537 thoroughly the mechanisms of species interactions at scales ranging from the molecular
538 to the ecological. Here, we highlight two examples where information compiled in DROP
539 enables the study of the *Drosophila*-parasitoid system across multiple levels of biological
540 organization:

541 DROP includes a DNA reference library of Australian *Drosophila* parasitoids (dataset:
542 DS-AUSPTOID dx.doi.org/10.5883/DS-AUSPTOID) that connects laboratory experiments
543 and field research. Molecular vouchers of both hosts and parasitoids were collected
544 along altitudinal gradients in the rainforest of northern Queensland, Australia (Jeffs et
545 al., 2021). With this DNA reference library, researchers can detect interactions between

546 *Drosophila* and their parasitoids using PCR-based approaches and parasitized pupae
547 (Hrcek & Godfray, 2015; Jeffs et al., 2020). Surveying host-parasitoid interactions in this
548 way will improve our understanding of how environmental change alters the structure
549 of host-parasitoid networks (Morris et al., 2014; Staniczenko et al., 2017; Tylianakis et
550 al., 2007) by accelerating data collection in the field. In addition, JH established lab
551 cultures of both hosts and their parasitoids from the same Australian sampling sites with
552 the aim of conducting laboratory experiments (e.g. Thierry et al., 2021). Molecular
553 vouchers of the lab strains were then submitted to DROP as a reference database
554 (datasets: DS-LABS dx.doi.org/10.5883/DS-LABS) to ensure that criteria for species
555 determination were applied consistently—and will continue to be applied consistently—
556 between the natural community studies and the laboratory experiments.
557 The presence of a foundational DNA reference library and species catalogue in
558 DROP will enable the process of exploring parasitoid biodiversity to become more
559 efficient. For example, DROP includes molecular vouchers from *Drosophila* parasitoids
560 that were collected across seasons and along latitudinal gradients in the eastern United
561 States (Lue et al., 2016, 2018). These data proved to be extremely useful for identifying
562 species in a more recent exploration of native parasitoid biodiversity across North
563 America (e.g., Abram et al., 2020). There are additional uses for DROP: curated
564 specimen collections may be used to document species distributions, phenology,
565 understand micro-evolutionary patterns, observe the effects of climate change, and
566 detect and track biological invasions (Funk, 2018; Schilthuizen et al., 2015; Tarli et al.,
567 2018).

568

569 ***Taxonomic accuracy for biocontrol studies***

570 Unfortunately, the history of biological control includes many examples of
571 misidentifications that have resulted in failures to employ or establish the expected
572 control agent, thus hindering eventual success (Buffington et al., 2018; Rosen, 1986;
573 Huffaker et al. 1962). In the context of biological control research on *Drosophila* pest
574 species, a simple, reliable, and rapid identification tool for their natural enemies is
575 essential (Wang et al. 2020b). By anchoring the criteria for determining identities of
576 organisms being considered for biological control programs, DROP annotation enables
577 the direct examination of centers of origin for parasitoid species, their co-occurrence
578 with natural enemies, and the optimal timing for potential introductions of such
579 enemies (Abram et al., 2020; Daane et al., 2016; Girod et al., 2018a and b; Kimura, 2015;
580 Mitsui et al., 2007). Because most sequences from DROP are already vetted for
581 reliability, they can be used to identify biological control agents rapidly, before or after
582 being brought into quarantine facilities for safety and efficacy testing. This will decrease
583 the risk of non-target ecological impacts arising from misidentifications and facilitate
584 regulatory review for releases of effective and specific natural enemies.

585 In addition to species identification, reference sequences from DROP may be used as
586 a starting point to create species-specific primers for the accurate identification of
587 parasitoids, design multiplex PCR assays that rapidly distinguish species in natural or
588 agricultural ecosystems (Ye et al., 2017), and apply high-throughput molecular
589 identification diagnostics (Fagan-Jeffries et al., 2018).

590

591 ***Long-term molecular voucher preservation***

592 During the curation of DROP, we found that holotype specimens were missing from
593 museums for several iconic *Drosophila* parasitoid species: *Asobara tabida* (Nees von
594 Esenbeck), *Leptopilina clavipes* (Hartig), and *Leptopilina longipes* (Hartig). This is not
595 uncommon and impedes future taxonomic revisions regardless of whether or not
596 molecular data are used. To avoid contributing to this problem, DROP uses museums as
597 depositories for ensuring that sequenced vouchers of both described species and
598 provisional species are permanently stored. In order to stabilize nomenclature, we
599 further advocate the designation of neotypes (a replacement specimen for a missing
600 holotype or type series) that have museum-vouchered DNA barcodes and additional
601 genomic extractions in storage.

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

611 Our results show that species richness of the parasitic wasps that attack *Drosophila*
612 is severely underestimated, and only a fraction of them have been described. In DROP,
613 38% of the OTUs are provisional species, and more than 46% of the named OTUs have
614 synonyms. Remarkably, *Leptopilina heterotoma*, one of the world's most studied
615 parasitoids, has more than 20 synonyms! As is generally the case, the rate of species
616 description and revision of *Drosophila* parasitoids lags far behind that with which
617 molecular sequence data are generated. Ensuring a consistent application of OTU
618 recognition is therefore essential. With DROP, researchers may ensure consistency in
619 their application of scientific names, and that those names are valid, making the
620 daunting process of describing *Drosophila* parasitoids more accurate and efficient. In
621 addition to the collection of physical museum resources, a central role taxonomists play
622 in DROP and its curation is that of fostering better integration of taxonomy with
623 experimental and biodiversity research. Our intention is to perpetuate DROP beyond
624 this introductory publication. We hope that experts in all areas of *Drosophila*-parasitoid
625 biology and related fields will join us in this effort.
626
627 ***Conclusion***

628 Taxonomic confusion presents many obstacles in experimental and biodiversity
629 studies. One way of addressing this impediment is to provide a reliable DNA library with
630 traceable vouchers (Astrin et al., 2013). Compared to BOLD and GenBank, DROP is a
631 small database that provides some advantages over an immense genetic database. For
632 example, it is easier for the research community to have direct communication amongst

633 themselves, when there is a strong focus on a few specific taxa (Weigand et al., 2019). A
634 good database has to maintain good quality of molecular data, but even more
635 challenging is to maintain quality of identification from different sources (Fontes et al.,
636 2021). In a big database, setting up a universal standard that satisfied all the taxa and
637 researchers desires is particularly challenging. The curated nature of DROP will allow us
638 to make strong rules to govern this data and assure users of its fidelity. While GenBank
639 and BOLD each perform some amount of curation, it could be difficult to agree on
640 curators for the whole range of animal and plant species catalogued there. We
641 developed DROP as a resource and platform for gathering and sharing reliable genomic
642 sequence data for *Drosophila* parasitoids. We hope it will serve as a model for
643 researchers working with organisms which present similar difficulties. While compiling
644 DROP, we found that the high number of provisional *versus* named OTUs suggests that
645 the diversity of parasitic wasps attacking *Drosophila* is greatly underestimated. With this
646 in mind, DROP represents the start of an important knowledge base that will strengthen
647 future studies of natural host-parasitoid interactions, population dynamics, biocontrol,
648 and the impact of climate change on biodiversity and ecosystem services.

649

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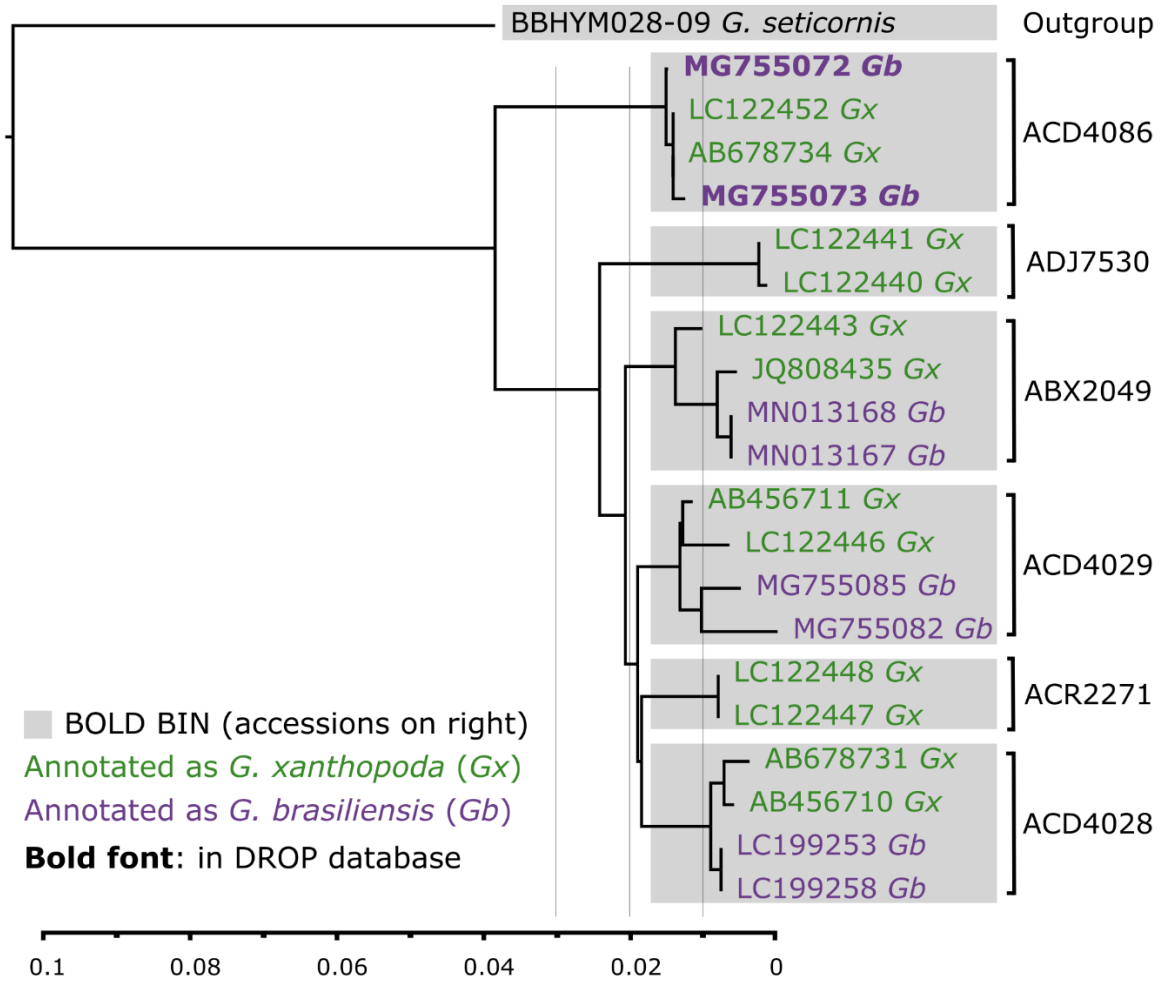
Data Accessibility

The DROP database is freely accessible at Zenodo depository (http://doi.org/10.5281/zenodo.4519656). Sequences from GenBank and BOLD, all compiled in BOLD, DROP project, DS-DROPAR dataset dx.doi.org/10.5883/DS-DROPAR. New sequences have been deposited in BOLD, DROP project (datasets: DS-LABS dx.doi.org/10.5883/DS-LABS and DS-AUSPTOID dx.doi.org/10.5883/DS-AUSPTOID).

Author Contributions

The initial project idea was originated by C-HL, MLB, JH, MM, TS, JV, SG, and PPAS. Molecular work was conducted by C-HL, SS, ML, AJ, and AD. BOLD and GenBank data was harvested by TAE and C-HL. Figures were made by AL and C-HL. Laboratory and field sample preparations were conducted by MTK, YC, TS, MM, SG, JV, EG, MG, XW, KM, KMD, PA, NAP, MT, JJB, MP, FMJ, WDT, JSD, BW, OTL, PPAS, JL and AL. Taxonomic concepts and interpretations were conducted by RRK, MLB, CH-L, PG, and SEM. DROP database was built by JH and C-HL. All authors contributed to review and final revisions of the manuscript, which was written primarily by C-HL, MLB and JH.

1026 **Figures:**



1027

1028 Figure 1: An example of difficulties of molecular identification demonstrated on
1029 *Ganaspis xanthopoda* and *G. brasiliensis*. Only two sequences (in bold text) can be
1030 reliably used for identification and are included in DROP database. To select the
1031 sequences, we searched the BINs associated with the organism's name "*Ganaspis*
1032 *xanthopoda"* (green) or *"Ganaspis brasiliensis"* (purple) in BOLD. From each BIN, two
1033 sequences from each species were selected to build a neighbor-joining tree (bottom axis
1034 indicated % genetic divergence). There was a total of 6 BINs (gray boxes) in this
1035 sequence complex. Of these, 4 BINs contained both species names and without
1036 examination of vouchers, identification would be impossible. In DROP, vouchers from
1037 two sequences, **MG755073** and **MG755072**, were deposited in CNR-IPSP (Table S2),
1038 examined by taxonomists and identified as *G. brasiliensis*. These two COI sequences can
1039 now be used to reliably identify *G. brasiliensis*. For *G. xanthopoda,* there were no
1040 available vouchers or reliable sequences that passed DROP standards to use for
1041 identification. Species delimitation between *G. brasiliensis* and *G. xanthopoda* is
1042 convoluted, varies according to arbitrary % genetic divergence (gray vertical lines), and
1043 needs an integrative taxonomic revision.

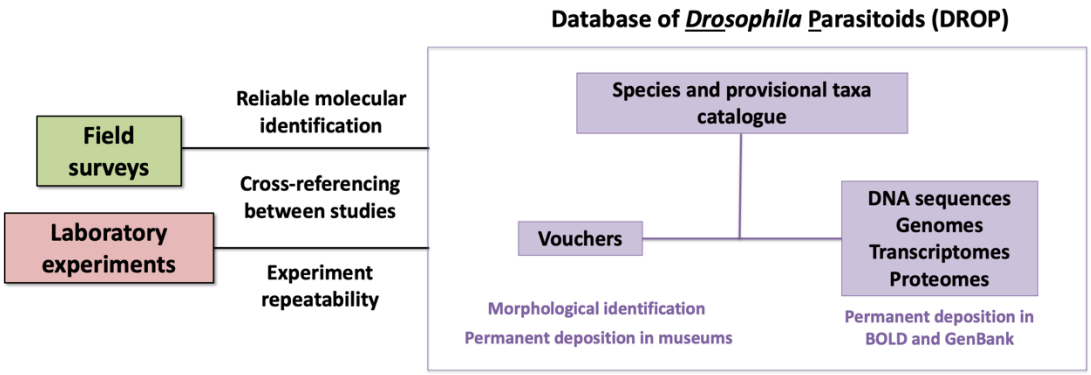


Figure 2: Concept of a centralized, vetted, curated database for *Drosophila* Parasitoids (DROP) we developed. First, we provide a species and provisional species catalog with correct taxonomy. Second, to provide a reliable genetic reference library, we link genetic data (DNA sequences, genomes, transcriptomes,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

Tables:

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

Superfamily	Family	Genus	Species_Name	Author
Chalcidoidea	Encyrtidae		*drop_Cha2_sp12*	
Chalcidoidea	Encyrtidae	*Tachinaephagus*	*drop_ IR1_sp41*	Kimura
Chalcidoidea	Encyrtidae	*Tachinaephagus*	*drop_BG1_sp42*	Kimura
Chalcidoidea	Encyrtidae	*Tachinaephagus*	*zealandicus*	Ashmead 1904
Chalcidoidea	Pteromalidae		*drop_Pte69_sp11*	
Chalcidoidea	Pteromalidae	*Pachycrepoideus*	*vindemmiae*	(Rondani, 1875)
Chalcidoidea	Pteromalidae	*Spalangia*	*drop_IR1_sp38*	Kimura
Chalcidoidea	Pteromalidae	*Spalangia*	*drop_NG1_sp39*	Kimura
Chalcidoidea	Pteromalidae	*Spalangia*	*drop_SK1_sp40*	Kimura
Chalcidoidea	Pteromalidae	*Spalangia*	*drosophilae*	Ashmead 1887
Chalcidoidea	Pteromalidae	*Spalangia*	*erythromera*	Foerster 1850
Chalcidoidea	Pteromalidae	*Trichomalopsis*	*dubia*	(Ashmead, 1896)
Chalcidoidea	Pteromalidae	*Trichomalopsis*	*microptera*	(Lindeman, 1887)
Chalcidoidea	Pteromalidae	*Trichomalopsis*	*nigricola*	Boucek

Chalcidoidea	Pteromalidae	*Trichomalopsis*	*sarcophagae*	(Gahan, 1914)
Chalcidoidea	Pteromalidae	*Vrestovia*	*brevior*	Boucek 1993
Chalcidoidea	Pteromalidae	*Vrestovia*	*fidenas*	(Walker, 1848)
Chalcidoidea	Pteromalidae		*drop_ PacAtl_sp46*	
Chalcidoidea	Pteromalidae		*drop_ PachyPort_sp45*	
Chalcidoidea			*drop_ CH_sp64*	
Cynipoidea	Figitidae	*Ganaspis*	*brasiliensis*	(Ihering, 1905)
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gan_sp51*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gan_sp52*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gan_sp53*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gsp1_sp67*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gsp2_sp68*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ Gsp50_sp66*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_ IR1_sp25*	Kimura
Cynipoidea	Figitidae	*Ganaspis*	*drop_ IR2_sp26*	Kimura
Cynipoidea	Figitidae	*Ganaspis*	*drop_Gan1_sp1*	
Cynipoidea	Figitidae	*Ganaspis*	*drop_TK1_sp27*	Kimura
Cynipoidea	Figitidae	*Ganaspis*	*hookeri*	Craword 1913
Cynipoidea	Figitidae	*Ganaspis*	*mahensis*	Kieffer 1911
Cynipoidea	Figitidae	*Ganaspis*	*mellipes*	(Say, 1826)
Cynipoidea	Figitidae	*Ganaspis*	*mundata*	Forster 1869
Cynipoidea	Figitidae	*Ganaspis*	*seticornis*	(Hellen, 1960)
Cynipoidea	Figitidae	*Ganaspis*	*tenuicornis*	Kieffer 1904
Cynipoidea	Figitidae	*Ganaspis*	*xanthopoda*	(Ashmead, 1896)
Cynipoidea	Figitidae	*Kleidotoma*	*bicolor*	(Giraud, 1860)
Cynipoidea	Figitidae	*Kleidotoma*	*dolichocera*	Thomson 1877
Cynipoidea	Figitidae	*Kleidotoma*	*drop_TK1_sp28*	Kimura
Cynipoidea	Figitidae	*Kleidotoma*	*filicornis*	(Cameron, 1889)
Cynipoidea	Figitidae	*Kleidotoma*	*icarus*	(Quinlan, 1964)
Cynipoidea	Figitidae	*Kleidotoma*	*psiloides*	Westwood 1833
Cynipoidea	Figitidae	*Kleidotoma*	*tetratoma*	(Hartig, 1841)
Cynipoidea	Figitidae	*Leptolamina*	*drop_Fig64_sp5*	
Cynipoidea	Figitidae	*Leptolamina*	*drop_Lmn_sp6*	
Cynipoidea	Figitidae	*Leptolamina*	*drop_TK1_sp29*	Kimura
Cynipoidea	Figitidae	*Leptolamina*	*gressitti*	Yoshimoto & Yasumatsu 1965
Cynipoidea	Figitidae	*Leptolamina*	*papuensis*	Yoshimoto 1963
Cynipoidea	Figitidae	*Leptolamina*	*ponapensis*	Yoshimoto 1962
Cynipoidea	Figitidae	*Leptolamina*	*seychellensis*	(Kieffer, 1911)

Cynipoidea	Figitidae	*Leptopilina*	*atraticeps*	(Kieffer, 1911)
Cynipoidea	Figitidae	*Leptopilina*	*australis*	(Belizin, 1966)
Cynipoidea	Figitidae	*Leptopilina*	*boulardi*	(Barbotin, Carton & Kelner-Pillault, 1979)
Cynipoidea	Figitidae	*Leptopilina*	*clavipes*	(Hartig, 1841)
Cynipoidea	Figitidae	*Leptopilina*	*cupulifera*	(Kieffer, 1916)
Cynipoidea	Figitidae	*Leptopilina*	*decemflagella*	Lue & Buffington 2017
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp54*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp55*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp56*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp57*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp58*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp59*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp60*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp61*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_ Lep_sp62*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_BG1_sp34*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*drop_Fig059_sp4*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_Fig124_sp2*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_Fig58_sp3*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_IR1_sp30*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*drop_NG1_sp33*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*drop_SK1_sp35*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*drop_STL_sp7*	
Cynipoidea	Figitidae	*Leptopilina*	*drop_TK2_sp31*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*drop_TK3_sp32*	Kimura
Cynipoidea	Figitidae	*Leptopilina*	*fimbriata*	(Kieffer, 1901)
Cynipoidea	Figitidae	*Leptopilina*	*freyae*	Allemand & Nordlander 2002
Cynipoidea	Figitidae	*Leptopilina*	*guineaensis*	Allemand & Nordlander 2002
Cynipoidea	Figitidae	*Leptopilina*	*heterotoma*	(Thomson, 1862)
Cynipoidea	Figitidae	*Leptopilina*	*japonica japonica*	Novkovic & Kimura 2011
Cynipoidea	Figitidae	*Leptopilina*	*lasallei*	Buffington & Guerrieri 2020
Cynipoidea	Figitidae	*Leptopilina*	*leipsi*	Lue & Buffington 2018
Cynipoidea	Figitidae	*Leptopilina*	*lonchaeae*	(Cameron, 1912)
Cynipoidea	Figitidae	*Leptopilina*	*longipes*	(Hartig, 1841)
Cynipoidea	Figitidae	*Leptopilina*	*mahensis*	(Kieffer, 1911)

Cynipoidea	Figitidae	*Leptopilina*	*maia*	Lue & Buffington 2016
Cynipoidea	Figitidae	*Leptopilina*	*maria*	(Girault, 1930)
Cynipoidea	Figitidae	*Leptopilina*	*orientalis*	Allemand & Nordlander 2002
Cynipoidea	Figitidae	*Leptopilina*	*pacifica*	Novkovic & Kimura 2011
Cynipoidea	Figitidae	*Leptopilina*	*rufipes*	(Cameron, 1908)
Cynipoidea	Figitidae	*Leptopilina*	*rugipunctata*	(Yoshimoto, 1962)
Cynipoidea	Figitidae	*Leptopilina*	*ryukyuensis*	Novkovic & Kimura 2011
Cynipoidea	Figitidae	*Leptopilina*	*tokioensis*	Wachi & Kimura 2015
Cynipoidea	Figitidae	*Leptopilina*	*tsushimaensis*	Wachi & Kimura 2015
Cynipoidea	Figitidae	*Leptopilina*	*victoriae*	Nordlander 1980
Cynipoidea	Figitidae	*Rhoptromeris*	*heptoma*	(Hartig, 1840)
Cynipoidea	Figitidae	*Rhoptromeris*	*nigriventris*	Nordlander 1978
Cynipoidea	Figitidae	*Rhoptromeris*	*rufiventris*	(Giraud, 1860)
Cynipoidea	Figitidae	*Rhoptromeris*	*villosa*	(Hartig, 1840)
Cynipoidea	Figitidae		*drop_Lg500_sp43*	
Ichneumonoidea	Braconidae	*Alysia*	*drop_SP1_sp24*	Kimura
Ichneumonoidea	Braconidae	*Aphaereta*	*aotea*	Hughes & Woolcock 1976
Ichneumonoidea	Braconidae	*Aphaereta*	*drop_SP1_sp15*	Kimura
Ichneumonoidea	Braconidae	*Aphaereta*	*drop_TK1_sp13*	Kimura
Ichneumonoidea	Braconidae	*Aphaereta*	*drop_TM1_sp14*	Kimura
Ichneumonoidea	Braconidae	*Aphaereta*	*minuta*	(Nees, 1811)
Ichneumonoidea	Braconidae	*Aphaereta*	*pallipes*	(Say, 1829)
Ichneumonoidea	Braconidae	*Aphaereta*	*scaptomyzae*	Fischer 1966
Ichneumonoidea	Braconidae	*Areotetes*	*striatiferus*	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	*Areotetes*	*carinuliferus*	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	*Asobara*	*ajbelli*	Berry 2007
Ichneumonoidea	Braconidae	*Asobara*	*albiclava*	Berry 2007
Ichneumonoidea	Braconidae	*Asobara*	*antipoda*	(Ashmead, 1900)
Ichneumonoidea	Braconidae	*Asobara*	*bactrocerae*	(Gahan, 1952)
Ichneumonoidea	Braconidae	*Asobara*	*brevicauda*	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	*Asobara*	*citri*	(Fischer, 1963)
Ichneumonoidea	Braconidae	*Asobara*	*drop_KG1_sp16*	Kimura
Ichneumonoidea	Braconidae	*Asobara*	*drop_NG1_sp17*	Kimura
Ichneumonoidea	Braconidae	*Asobara*	*drop_SK2_sp20*	Kimura

Ichneumonoidea	Braconidae	*Asobara*	*drop_SP1_sp18*	Kimura
Ichneumonoidea	Braconidae	*Asobara*	*drop_Sp2_sp19*	Kimura
Ichneumonoidea	Braconidae	*Asobara*	*elongata*	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	*Asobara*	*gahani*	(Papp, 1969)
Ichneumonoidea	Braconidae	*Asobara*	*japonica*	Belokobylskij 1998
Ichneumonoidea	Braconidae	*Asobara*	*kenyaensis*	Peris-Felipo 2014
Ichneumonoidea	Braconidae	*Asobara*	*leveri*	(Nixon, 1939)
Ichneumonoidea	Braconidae	*Asobara*	*mesocauda*	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	*Asobara*	*orientalis*	Viereck 1913
Ichneumonoidea	Braconidae	*Asobara*	*persimilis*	(Prince, 1976)
Ichneumonoidea	Braconidae	*Asobara*	*pleuralis*	(Ashmead, 1905)
Ichneumonoidea	Braconidae	*Asobara*	*rossica*	Belokobylskij 1998
Ichneumonoidea	Braconidae	*Asobara*	*rufescens*	(Fˆrster, 1862)
Ichneumonoidea	Braconidae	*Asobara*	*tabida*	(Nees, 1834)
Ichneumonoidea	Braconidae	*Asobara*	*triangulata*	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	*Asobara*	*turneri*	Peris-Felipo 2014
Ichneumonoidea	Braconidae	*Asobara*	*unicolorata*	van Achterberg & Guerrieri 2016
Ichneumonoidea	Braconidae	*Aspilota*	*albertica*	Berry 2007
Ichneumonoidea	Braconidae	*Aspilota*	*andyaustini*	Wharton 2002
Ichneumonoidea	Braconidae	*Aspilota*	*angusta*	Berry 2007
Ichneumonoidea	Braconidae	*Aspilota*	*concolor*	Nees 1812
Ichneumonoidea	Braconidae	*Aspilota*	*parecur*	Berry 2007
Ichneumonoidea	Braconidae	*Aspilota*	*villosa*	Berry 2007
Ichneumonoidea	Braconidae	*Dinotrema*	*barrattae*	Berry 2007
Ichneumonoidea	Braconidae	*Dinotrema*	*longworthi*	Berry 2007
Ichneumonoidea	Braconidae	*Dinotrema*	*philipi*	Berry 2007
Ichneumonoidea	Braconidae		*drop_Aso_sp8*	
Ichneumonoidea	Braconidae	*Opiognathus*	*pactus*	(Haliday, 1837)
Ichneumonoidea	Braconidae	*Opius*	*bellus*	Gahan 1930
Ichneumonoidea	Braconidae	*Opius*	*cinerariae*	Fischer
Ichneumonoidea	Braconidae	*Opius*	*crenuliferus*	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	*Opius*	*monilipalpis*	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	*Opius*	*ocreatus*	(Papp)
Ichneumonoidea	Braconidae	*Opius*	*pallipes*	Wesmael 1835
Ichneumonoidea	Braconidae	*Opius*	*pteridiophilus*	Wharton & Austin 1990

Ichneumonoidea	Braconidae	*Opius*	*pterus*	Wharton & Austin 1990
Ichneumonoidea	Braconidae	*Opius*	*trimaculatus*	Spinola
Ichneumonoidea	Braconidae	*Opius*	*youi*	Li & van Achterberg 2013
Ichneumonoidea	Braconidae	*Phaenocarpa*	*conspurcator*	(Haliday, 1838)
Ichneumonoidea	Braconidae	*Phaenocarpa*	*drop_ IR1_sp22*	Kimura
Ichneumonoidea	Braconidae	*Phaenocarpa*	*drop_TK1_sp21*	Kimura
Ichneumonoidea	Braconidae	*Phaenocarpa*	*tacita*	Stelfox 1941
Ichneumonoidea	Braconidae	*Phaenocarpa*	*drosophilae*	(Fischer 1975)
Ichneumonoidea	Braconidae	*Tanycarpa*	*bicolor*	(Nees, 1814)
Ichneumonoidea	Braconidae	*Tanycarpa*	*chors*	Belokobylskij 1998
Ichneumonoidea	Braconidae	*Tanycarpa*	*drop_NG1_sp23*	Kimura
Ichneumonoidea	Braconidae	*Tanycarpa*	*punctata*	van Achterberg 1976
Ichneumonoidea	Braconidae		*drop_ Aly_sp47*	
Ichneumonoidea	Braconidae		*drop_ Aly_sp48*	
Ichneumonoidea	Braconidae		*drop_ Aly_sp49*	
Ichneumonoidea	Braconidae		*drop_ Aly_sp50*	
Ichneumonoidea	Braconidae		*drop_ Aly_sp63*	
Ichneumonoidea	Braconidae		*drop_ Aso_sp69*	
Diaprioidea	Diapriidae	*Trichopria*	*anastrephae*	Costa Lima 1940
Diaprioidea	Diapriidae	*Trichopria*	*drop_ BG1_sp37*	Kimura
Diaprioidea	Diapriidae	*Trichopria*	*drop_ Dia70_sp65*	
Diaprioidea	Diapriidae	*Trichopria*	*drop_ Tri_sp44*	
Diaprioidea	Diapriidae	*Trichopria*	*drop_Bdia_sp10*	
Diaprioidea	Diapriidae	*Trichopria*	*drop_Dia127_sp9*	
Diaprioidea	Diapriidae	*Trichopria*	*drop_TK1_sp36*	Kimura
Diaprioidea	Diapriidae	*Trichopria*	*drosophilae*	(Kieffer, 1912)
Diaprioidea	Diapriidae	*Trichopria*	*modesta*	(Ratzeburg, 1848)

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