

First insights on the diversity of the genus *Psychoda* Latreille (Diptera: Psychodidae) in Corsica using DNA barcoding[†]

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Summary. Among the 44 European *Psychoda* species identified to date, we expected to observe in Corsica species with large distribution ranges and/or frequent in adjacent territories (i.e. South mainland France, mainland Italy, and Sardinia). Each studied specimen was morphologically identified and then barcoded using cytochrome c oxidase subunit I (COI) and subunit II (COII) to confirm its identification, and to detect potential cryptic species. From 211 mounted specimens, 16 *Psychoda sensu lato* morphotypes were recorded in Corsica and mainland France. While 14 out of the 15 species recorded in Corsica are new records for the island, two species are also new records for France and Europe, *P. (Psychodocha) uncinula* Quate, 1954 and *P. (Chodopsycha) divaricata* Duckhouse, 1968. Moreover, our barcoding approach allowed us to detect 18 haplotypes in total, including three undescribed Corsican haplotype complexes, namely *P. (Tinearia) alternata*, *P. (Logima) satchelli* and *P. (Psychodocha) cinerea*. With only mitochondrial COI barcodes, it cannot be asserted whether these complexes represent the within-species coexistence of various haplotypes or the existence of distinct cryptic species. In BOLD (Barcode of Life Datasystems),

only 30 out of the 58 BINs corresponding to the genus *Psychoda* have a species name. All of the COI barcodes generated in this study already exist in BOLD, suggesting that the barcodes of most of the temperate *Psychoda* diversity are already known, but not properly named.

Résumé. Premières données sur la diversité du genre *Psychoda* Latreille (Diptera : Psychodidae) en Corse incluant les codes-barres ADN. Parmi les 44 espèces européennes actuelles de *Psychoda*, nous nous attendions à récolter en Corse des espèces à large aire de distribution et/ou fréquentes dans les territoires adjacents (i.e. sud de la France, Italie continentale et Sardaigne). Chaque spécimen étudié a été identifié morphologiquement et génétiquement à partir des séquences du cytochrome C oxydase sous-unité I (COI) et sous-unité II (COII) afin de détecter des espèces cryptiques potentielles. Un total de 211 spécimens a été examiné, représentant 16 morphotypes de *Psychoda sensu lato* de Corse et de France continentale. Ainsi, 14 des 15 espèces trouvées en Corse sont nouvelles pour l'île et deux le sont pour la France et l'Europe, *P. (Psychodocha) uncinula* Quate, 1954 et *P. (Chodopsycha) divaricata* Duckhouse, 1968. De plus, le séquençage des codes-barres ADN a permis de détecter un total de 18 haplotypes dont trois complexes génétiques : *P. (Tinearia) alternata*, *P. (Logima) satchelli* et *P. (Psychodocha) cinerea*. À partir des seuls codes-barres mitochondriaux du COI, il est difficile de trancher si ces complexes représentent la coexistence de différents haplotypes ou l'existence d'espèces cryptiques distinctes. Sur BOLD (Barcode of Life Datasystems), seulement 30 des 58 BINs correspondant au genre *Psychoda* ont un nom d'espèce attribué. Toutes les séquences de COI obtenues dans cette étude sont déjà présentes dans la base de données BOLD suggérant que la majorité des *Psychoda* tempérés a été séquencée mais pas proprement identifiée.

Keywords: cryptic species, haplotypes, *Psychoda sensu lato*, species complex

[†]This manuscript is dedicated to the memory of Phil(ip) Withers, a notable contributor to Psychodidae who died on July 5th 2020.

The genus *Psychoda* Latreille, 1796, the first described for Psychodidae, was properly delimited by Quate (1959), the first author to provide its diagnosis. The taxonomic definition of *Psychoda* has been extensively debated after Quate's work as discussed in Bravo et al. (2006), and is still controversial as it has been split between two (Vaillant 1972) and 13 intimately related genera (Ježek 1984, 2007; Ježek & van Harten 1996, 2005). In this work, we follow the definition of *Psychoda sensu lato* given by Bravo et al. (2006), considering the 13 described genera as subgenera of the genus *Psychoda*: subgenera *Psychoda* Latreille, 1796, *Tinearia* Schellenberg, 1803, *Logima* Eaton, 1904, *Copropsychoda* Vaillant, 1971, *Chodopsycha* Ježek, 1984, *Psycha* Ježek, 1984, *Psychodocha* Ježek, 1984, *Psychodula* Ježek, 1984, *Psychomora* Ježek, 1984, *Ypsydocha* Ježek, 1984, *Falsologima* Ježek & Harten, 1996, *Psychana* Ježek & Harten, 2005 and *Apsycha* Ježek, 2007.

Despite the taxonomic definition difficulty, new regional or national species lists are still regularly published, updating the distribution areas of known species and/or describing new species (Ježek 2004; Ježek & Yağci 2005; Salmela et al. 2007; Kvifte & Boumans 2014; Kroča & Ježek 2015; Kvifte et al. 2016; Ježek et al. 2018a, 2018b, 2018c; Kroča & Ježek 2019; Kvifte 2019). However, the *Psychoda* diversity remains unclear in many European regions, such as the island of Corsica. According to *Fauna Europaea* (Wagner 2013), the genus *Psychoda s. l.* is composed in Europe of 43 species (40 *Psychoda* and three *Tinearia*), plus *P. cultella* Salmela, Kvifte & More, 2012 not

yet listed (Salmela et al. 2012). When considering regions surrounding Corsica (Table 1), mainland of France and Italy have a *Psychoda s. l.* diversity of 23 to 24 species (Dahl et al. 1995; Wagner 2013; GBIF, <https://www.gbif.org/>; INPN, <https://inpn.mnhn.fr/accueil/index>; both databases consulted on November 8th 2021); and only eight species have been recorded in the neighbouring island of Sardinia (Salamanna, 1982, 1983). To the best of our knowledge, only one *Psychoda* species, *P. (Psychodocha) cinerea* Banks, 1894 is listed from Corsica out of the 377 aquatic insect species recorded from 41 Corsican littoral wetland areas (Moubayed-Breil et al. 2013). In Corsica, we expected to observe several other *Psychoda* species which have large distribution ranges (i.e. cosmopolitan or Palaearctic) such as *Psychoda (Tinearia) alternata* Say, 1924 and *P. (Logima) albipennis* Zetterstedt, 1850 (Ježek et al. 2018b), and/or frequent in adjacent territories (i.e. mainland France, mainland Italy, and Sardinia).

For the last decade, molecular data have been used to explore taxonomic questions at different hierarchical levels such as Psychodidae subfamilies (Curler & Moulton 2012) or Psychodinae tribes (Espindola et al. 2012; Kvifte 2018). Moreover, the monophyly of *Psychoda s. l.* was phylogenetically highly supported (Espindola et al. 2012; Grace-Lema et al. 2015; Kvifte 2018). DNA barcoding, based on a fragment of the mitochondrial gene Cytochrome c Oxidase subunit I (COI), has been shown to be an efficient complementary molecular information for species identification (Hebert et al. 2003). In a study on Finnmark Psychodidae, each species corresponded to a cluster of COI barcode sequences; moreover all sequences from *Psychoda s. l.* specimens grouped in a cluster different from the Pericomaini sequences (Kvifte & Andersen 2012). Interestingly, the two *Psychoda* species with the lowest support (~86% versus 100%) were *P. (Logima) albipennis* and *P. (Tinearia) lativentris* Berdén, 1952, two problematic species from a

morphological point of view (Kvifte & Andersen 2012). Also, DNA barcoding studies recently performed on Phlebotominae (Psychodidae) revealed the presence of several unnoticed complexes of morphospecies (Tharmatha et al. 2017), but conflating mitochondrial DNA barcodes and species without any other supporting evidence can be misleading or even erroneous (Haarto & Ståhls 2014; Teske et al. 2018).

Here we report new records of Corsican *Psychoda* collected in the Ajaccio region. Each studied specimen was morphologically identified and barcoded using COI and COII sequences to confirm its identification, and to detect haplotype species variability. Our specific aims were: i) to establish a first list of *Psychoda s. l.* species for Corsica; ii) to compare this *Psychoda* diversity to those of some neighbouring European regions; iii) to associate the DNA barcode of each specimen with a photographed slide preparation, and iv) to highlight and discuss taxonomical confusions resulting from previous studies based on morphological data alone.

Materials and methods

Sample collection

Between January and March 2017, November and December 2017, and January and March 2020, adult specimens of Psychodini (mainly *Psychoda* spp) were intensively collected from a compost bin in the Ajaccio region of Corsica (GPS coordinates: 41°54'45.9"N 8°39'10.6"E), using a mouth aspirator, and stored in 75% ethanol. Some additional specimens from two compost bins in mainland France (Montans, GPS: 43°51'06.6"N 1°49'25.9"E; Virazeil, GPS: 44°31'13.3"N 0°13'28.6"E) and from *Arum* sp. inflorescences (Rillieux-la-Pape, GPS: 45°50'13.98"N 4°54'18.75"E) were also collected for comparative purposes.

117

118 ***Morphological identifications***

119 All collected specimens were first sorted to morphotypes using a stereomicroscope. Thereafter,
120 some specimens of each morphotype and, when possible, for both sexes, were dissected and
121 mounted on glass slides for identification. For these specimens, the genitalia (cleaned with
122 potassium hydroxide), the head with the antennae, and one wing were mounted. The identification
123 to species level was thus achieved by the examination of the sexual appendages, antennal segments,
124 and the venation of the wing (following Ježek 1983, 1984; Vaillant 1988, 1989; Withers 1989;
125 Ježek 1990; Ježek & Van Harten 2005). Some morphological identifications were also confirmed
126 by two international specialists of the tribe, Jan Ježek (National Museum, Department of
127 Entomology, Czech Republic) and Gunnar M. Kvifte (Nord University, Norway). The unused parts
128 of these specimens, i.e. the thorax and the anterior part of the abdomen, were individually stored
129 in 75% ethanol for complementary molecular studies. Moreover, for each of the specimens selected
130 for DNA barcoding (see below), photographs of the corresponding slide preparations were also
131 deposited in BOLD (Table 2, Ratnasingham & Hebert 2007). All the mounted specimens are
132 preserved and available at the laboratory “Chimie et Biomasse” of the University of Corsica in
133 Ajaccio.

134

135 ***DNA Barcoding***

136 Some of the dissected specimens were also barcoded by sequencing a fragment of the Cytochrome
137 c Oxidase subunit I (COI) (Hebert et al. 2003). Genomic DNA was extracted from the thorax and
138 anterior part of the abdomen using the E.Z.N.A. Insect DNA Kit (Omega Biotek). A fragment of
139 the COI gene (658 bp) was amplified by PCR using the LCO1490

140 (GGTCAACAAATCATAAAGATATTGG) and HCO2198
 141 (TAAACTTCAGGGTGACCAAAAATCA) primers (Folmer et al. 1994). A smaller fragment
 142 (280 bp) was amplified for specimens of *Psychoda (Psycha) griseus* Tonnoir, 1922, for which
 143 the 658 bp fragment failed to be amplified. Specifically, we used the inner primers GriseusFor
 144 (CGAGCTGAATTAGGTCATCC) and GriseusRev (ACAGAAGGTCCTCCATG), as suggested
 145 by Anja Hoerger (University of Salzburg, Austria, pers. comm.). Thermocycling conditions for the
 146 658 bp fragment of the COI gene were: an initial denaturation at 94°C for 3 min, followed by 5
 147 cycles of 30 s denaturation at 94°C, 30 s annealing at 45°C, 1 min elongation at 72°C and 35 cycles
 148 of 1 min denaturation at 94°C, 1 min annealing at 51°C, 1 min elongation at 72°C, and a terminal
 149 elongation at 72°C for 10 min. The 280bp fragment of the COI gene was amplified using the
 150 following thermocycling conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles
 151 of 30 s denaturation at 94°C, 45 s annealing at 50°C, 1 min elongation at 72°C, and a terminal
 152 elongation at 72°C for 5 min. DNA sequences were purchased from Biofidal
 153 (<https://www.biofidal.com/>) and obtained by Sanger capillary DNA sequencing. COI sequences
 154 were deposited in the online databases GenBank and BOLD.
 155 We searched for each COI barcode in the GenBank using BLAST and in BOLD database to
 156 associate the sequences from our specimens with existing BINs, in order to find similar sequences
 157 that could potentially corroborate our identifications. BINs (Barcode Index Numbers) are aimed at
 158 being an automated objective registry of genetic diversity in DNA barcode databases
 159 (Ratnasingham & Hebert 2013). BINs represent clustered barcode sequences that create MOTUs
 160 (Molecular Operational Taxonomic Units) closely reflective of species groupings and are
 161 considered more robust than relying on a simple genetic divergence threshold (Ratnasingham &
 162 Hebert 2013).

When we had only one sequence from a given BIN (namely *P. uniformata*, *P. satchelli* and *P. alternata*), we selected randomly in BOLD database one 658bp sequence from the same BIN for the clustering analysis (Table 3). For two species (namely *P. gemina* and *P. cinerea*) the sequences from our specimens were attributed a BIN different from the BIN with the same identification in BOLD database. Consequently, two 658bp sequences corresponding to each of these two BINs were randomly chosen for the clustering analysis (Table 3). Based on the published latest phylogenetic study (Kvifte 2018), five additional Psychodini species, were also used as outgroups to root the tree (Table 3).

COI sequences were aligned using the ClustalW tool available into MEGA X (Kumar et al. 2018). Then, a Neighbour-Joining (NJ) tree was produced in MEGA X using the Kimura's 2 Parameter (K2P) distance model over 10,000 replicates to visualise COI sequences clustering (Kumar et al. 2018). We also amplified a partial fragment of the Cytochrome c Oxidase subunit II (COII, see lab protocol in supplementary material). However, as the specimens clustering and the Neighbour-Joining trees obtained using COI or COII were similar, the COII results are presented in the supplementary material.

Results

Morphological assessment

About 2,000 specimens were collected in total, most of them from the compost bin, and 211 of them were dissected and mounted. A total of 16 distinct morphotypes were identified to species level, 14 of which were found in Corsica, as well as two strictly continental species (Table 2). As reviewed in the introduction, only *Psychoda* (*Psychodocha*) *cinerea* had been previously listed from Corsica. Hence, 13 of the 14 species identified from Corsican specimens in Table 2 should

be considered as new records for Corsica. Finally, two species we identified, *P. (Chodopsycha) divaricata* Duckhouse, 1968 and *P. (Psychodocha) uncinula*, Quate, 1954 appear to be new records for Europe. Both species were found in Corsica, and *P. (Psychodocha) uncinula* was also found in mainland France (Table 2).

DNA Barcoding

GenBank accession numbers, Barcode Index Numbers (BINs) and BOLD ProcessID codes are listed in Table 2 and publicly available. The COI (658 bp) and COII (582 bp considering only the coding region) sequences were respectively obtained for 54 and 59 *Psychoda* specimens, covering all the 16 identified morphotypes (Table 2, Supplementary Table 1). All the COI sequences produced in the present study belonged to BINs already present in the online BOLD database. In contrast, only one COII sequence is available on GenBank for a non-identified *Psychoda* (Beckenbach & Borkent 2003); all of the COII sequences generated in this study were therefore novel (Supplementary Table 1).

Interestingly, the BIN algorithm generated 18 distinct BINs for the 16 identified morphotypes, among which 12 were already associated to a species name and six were not (BOLD accessed on June 12 2021) (Table 2). Most of the initial morphotype designations were confirmed except the specimens identified as *P. (Logima) satchelli* Quate, 1955 and *P. (Tinearia) alternata*, which were actually forming two distinct BINs within each species. No morphological differences were observed among the specimens identified as *P. (Logima) satchelli* on the antennae, wings or genitalia (male and female); the same was true for the specimens of *P. (Tinearia) alternata*. Interestingly, our unique sequence corresponding to a specimen morphologically identified as

Psychoda (Psychodocha) gemina (named afterwards *P. sp. aff. gemina*) from mainland France was attributed with a different BIN (BOLD:ACR3941) from the specimens identified as *P. (Psychodocha) gemina* Eaton, 1904 in BOLD database (BOLD:AAU4648). In the same manner, our sequences of *Psychoda (Psychodocha) cinerea* from both mainland France and Corsica belong to a BIN (BOLD:ACG4180) different from the BIN identified as *P. (Psychodocha) cinerea* (BOLD:ACF6513) in BOLD.

Neighbour-Joining tree

Bootstrap support values were high (e.g. 99%) for all the species clusters and low for internal nodes except for two cluster pairs presenting high sequence similarities (Figure 1) for the *P. (Tinearia) alternata* species (BOLD:AAF9306 and BOLD:ACM5462; 99%) and the *P. (Psychodocha) cinerea* species (BOLD:ACG4180 and BOLD:ACF6513; 99%). Interestingly, the Neighbour-Joining relationship between the two entities of the *P. (Logima) satchelli* species complex (BOLD:ACG1924 and BOLD:AAF9311) is not highly supported (67%), suggesting a lesser sequence similarity.

The NJ reconstruction based on the coding region of the COII sequences (Supplementary Figure) also confirmed the presence of 18 distinct genetic entities with strong support values ($\geq 99\%$) out of the 16 morphotypes. The high sequence similarity between the two entities of the *P. (Tinearia) alternata* species is also confirmed (100%). Interestingly, the unique *Psychoda* COII sequence available on GenBank clustered within the *P. (Logima) satchelli* species but does not match any of the two Corsican clusters corresponding to the COI BINs (BOLD:AAF9311 and BOLD:ACG1924, Supplementary Figure).

Discussion

Psychoda diversity

Our sampling over nine months at a single site near Ajaccio produced a checklist of 16 *Psychoda sensu lato* species for Corsica, which represent 66% of the known diversity for mainland France (Wagner 2013; Table 1) and twice the diversity of the neighbouring island of Sardinia (Salamanna 1983; Table 1). The regional widespread species (France, Italy and Sardinia) such as *P. (Logima) albipennis*, *P. (Tinearia) alternata*, *P. (Psychodocha) cinerea*, *P. (Tinearia) lativentris*, *P. (Psychodula) minuta* Banks, 1984 and *P. (Psychomora) trinodulosa* Tonnoir, 1922 were also recovered from Corsica (Table 1). Several species such as *P. (Psycha) grisescens*, *P. (Psychoda) phalaenoides* and *P. (Logima) satchelli* recorded from mainland France, Corsica and mainland Italy are not reported from Sardinia suggesting probably a lesser sampling effort (Table 1). *Fauna Europaea* (as reported in Table 1) lists *Psychoda (Logima) surcoufi* Tonnoir, 1922 as present in Europe; however, this species is synonymous with the American *Psychoda (Logima) sigma* Kincaid 1899 according to Del Rosario (1936) and several authors (Ježek 2003; Prpić 2015; Kvifte 2019). Note that Withers (1988) also recognized these taxa as synonyms, but omitted to consider the principle of priority, thus considering wrongly *P. (Logima) surcoufi* Tonnoir, 1922 as the valid name instead of *P. (Logima) sigma* Kincaid, 1899. The corresponding BIN (BOLD:AAU4664) is represented by 591 COI sequences in BOLD from specimens collected in Australia, New Zealand, Norway, Germany, Canada, Kenya, Colombia and now France. Interestingly, no specimen of *P. (Logima) sigma* from the type locality in the American Pacific coast (Kincaid 1899) has yet been sequenced.

255 ***New European records***

256 Our survey adds two new *Psychoda* species to the fauna of Europe, namely *Psychoda*
257 (*Chodopsycha*) *divaricata* and *P. (Psychodocha)* *uncinula*. The first species, *P. (Chodopsycha)*
258 *divaricata*, is represented by 12 sequenced specimens in BOLD and corresponds to a BIN
259 (BOLD:ACN1858) including specimens distributed in Argentina, Brazil and now France.
260 Similarly, *P. (Psychodocha)* *uncinula* with 128 sequenced specimens in BOLD corresponds to a
261 BIN (BOLD:ACD9559) comprising specimens from the USA, China, Russia and now France.
262 Interestingly, *P. (Psychodocha)* *uncinula*, described from Hawaii, USA (Quate 1954), has never
263 been recorded in Europe, while it was by far the most abundant species in the compost bin in our
264 two sampling sites of mainland France. Moreover, the genitalia of both males and females of this
265 species are very distinctive, when compared to the European species listed in *Fauna Europaea*,
266 and thus this species should not be easily missed by specialists. However, *P. (Psychodocha)* *itoco*
267 Tokunaga & Komyo, 1954 – a species described from Japan where it is commonly found
268 (Tokunaga & Komyo 1954; Tokunaga 1957) – has been recorded in Czech Republic and Norway,
269 with a first occurrence reported in 1994 (Ježek 2003; Salmela et al. 2007; Kvifte 2019).
270 Interestingly, on the basis of the drawings of Quate (1954), Tokunaga & Komyo (1954) and
271 Tokunaga (1957), it appears that the genitalia of both sexes are almost indistinguishable from those
272 of *P. (Psychodocha)* *uncinula*. Thus, possible synonymy between *P. (Psychodocha)* *itoco* and *P.*
273 (*Psychodocha*) *uncinula* should be considered. If this is the case, the valid name of this taxon ought
274 to be *P. (Psychodocha)* *uncinula* Quate, 1954, with *P. (Psychodocha)* *itoco* Tokunaga & Komyo,
275 1954 as a junior synonym, since the description of *P. (Psychodocha)* *uncinula* was published a few
276 months earlier (March 1954) than *P. (Psychodocha)* *itoco* (July/September 1954). Further surveys
277 will be interesting to better understand the distribution of these new non-European psychodid

species and to study their colonization routes. Finally, the *Psychoda sensu lato* diversity presented here will certainly increase in the next few years, as insect diversity is studied over Corsica by the program “*la Planète revisitée*” coordinated by the Muséum national d’Histoire naturelle (<http://laplaneterevisitee-corse.mnhn.fr/>).

Our molecular study was not designed to resolve the relationships among taxa (contrary to Espíndola et al. 2012; Kvifte 2018) and we thus cannot argue about the phylogenetic value of the splitting of the genus *Psychoda* proposed by Ježek (1984) and supported by Espíndola et al. (2012). One interesting result is that *Psychoda* (*Psychodocha*) *gemina* appears to be sister (e.g. the genetically most dissimilar) to remaining *Psychoda sensu lato* in both published molecular phylogenies and the present study (Espíndola et al. 2012; Kvifte 2018; Figure 1). As for Phlebotominae (Tharmatha et al. 2017), the COI barcoding approach also revealed the presence of three unnoticed haplotype complexes. However, while the *P. (Tinearia) alternata* and *P. (Psychodocha) cinerea* species were highly supported in both COI and COII NJ trees ($\geq 99\%$), the relationship between the two haplotypes identified as *P. (Logima) satchelli* was less supported (67% and 85% for the COI and COII, respectively). Based on only our COI barcodes, it cannot be stated whether this variability is due to the coexistence of two mitochondrial lineages for a given species or the existence of two species morphologically very similar (e.g. cryptic). Consequently, in the present paper, we refrain from proposing any synonymies, species resurrection or description of new species as highlighted only by the COI barcoding. Such statements would need the revision of the corresponding type specimens and related species but also complementary genetic data such as nuclear genes since mitochondrial DNA barcodes without any other supporting evidence can be misleading for species delimitation (Haarto & Ståhls 2014; Teske et al. 2018). However, such

revision would certainly clarify the taxonomic boundaries of the species composing the three revealed haplotype complexes which are discussed separately in the following paragraphs.

Psychoda alternata species

Our sequence of *Psychoda (Logima) alternata* from mainland France belongs to a BIN (BOLD:AAF9306) known from 1,129 COI published sequences, 1,075 of them been identified as *P. (Logima) alternata*. The sequenced specimens were sampled from all over the world, suggesting a cosmopolitan distribution of this haplotype (Americas, Europe, Africa, and Asia). Its nearest BIN is another taxon (BOLD:ACM5462) which has been less sampled (39 published sequences) and only scarcely identified as *P. (Logima) alternata* (4 sequences). The specimens from this BIN present a narrower distribution: Egypt, Bulgaria, Turkey, Israel, Germany and now Corsica (France), and none are listed from the Americas. As the type locality of *P. (Logima) alternata* is in Pennsylvania, USA (Say 1824), we propose that specimens from mainland France (BOLD:AAF9306) likely represent *P. (Logima) alternata* as traditionally accepted, while specimens from Corsica (BOLD:ACM5462) represent a different haplotype centred around the Mediterranean (plus Germany). Complementary morphological and genetic studies are needed to establish whether the haplotype of the Corsican specimens corresponds or not to a new (cryptic) species.

Psychoda cinerea species

Our sequences of *Psychoda (Psychodocha) cinerea* from both mainland France and Corsica belong to a BIN (BOLD:ACG4180) known from 67 European COI sequences (Germany, Denmark, Belarus, Bulgaria, France, Montenegro) and associated with different tentative identifications: *P.*

cinerea (two sequences), *P. cf. cinerea* (four sequences) or *P. phalaenoides* (19 sequences). The nearest BIN is another taxon identified as *P. (Psychodocha) cinerea* (BOLD:ACF6513) and represented by 155 COI sequences (153 of them been identified as *P. cinerea*) from Canada, United States, Egypt, New Zealand, Germany, Norway, Belarus, South Africa, Bulgaria, France, Israel and Lebanon. The nearest BIN of the latter is a non-public sequence (BOLD:ADD3093) from the Balearic Islands (Spain). It thus appears that the *P. (Psychodocha) cinerea* is in fact a complex of three distinct haplotypes. As the type locality of *P. (Psychodocha) cinerea* is New York, USA (Banks 1894), it can be supposed that specimens from the Balearic Islands (BOLD:ADD3093) and from mainland France and Corsica (BOLD:ACG4180), both BINs absent from the Americas, represent two different old-world haplotype mitochondrial lineages. Further complementary morphological and genetic studies are needed to establish whether these haplotypes are new (cryptic) species, particularly the one only reported from the Balearic Islands.

Psychoda satchelli species

In Corsica, we collected two taxa that appear to be affiliated with *P. (Logima) satchelli* Quate, 1955 corresponding to two BINs (BOLD:ACG1924 and BOLD:AAF9311). We obtained six sequences of the BIN BOLD:ACG1924, already known from 123 published sequences, all from Europe: Slovakia, Germany, Norway, Austria, Denmark, Bulgaria, and now Corsica (France). The specimens in BOLD:ACG1924 have been identified as *P. albipennis* (five sequences), *P. satchelli* (four sequences) or *P. cf. satchelli* (31 sequences). The second taxon (BOLD:AAF9311) is represented by 273 published sequences, identified as *P. satchelli* (19 sequences) or *P. cf. satchelli* (one sequence). Note that we only obtained one COI sequence from Corsica. However, this appears to be due to amplification issues, since we obtained six sequences of *P. (Logima) satchelli*

corresponding to this BIN number when using the COII gene. Its distribution range is more cosmopolitan with samples from the United States, Canada, Slovakia, Norway, Germany, Bulgaria, and now Corsica (France). Interestingly, the nearest BINs of both genetic clusters containing specimens identified as *P. (Logima) satchelli* are two BINs (BOLD:ACD1254 and BOLD:ACS7943) with no public records available, each represented by only one sequence from Canada. Moreover, a published COII sequence belongs to this cluster (Beckenbach & Borkent 2003) but does not match to any of the two Corsican taxa. Consequently, it seems that the *P. (Logima) satchelli* is a complex of four distinct haplotypes. It is likely that the BIN BOLD:AAF9311 is the best candidate for representing *P. (Logima) satchelli*, since it is the only taxa known from the USA, the country from which the type specimen has been collected (Quate 1955). The other haplotype represents a different European mitochondrial lineage. Complementary morphological and genetic studies are needed to establish whether the two haplotypes of the Corsican specimens correspond or not to distinct (cryptic) species.

Psychoda gemina confusion

Our sample of *Psychoda (Psychodocha)* sp. aff. *gemina* from mainland France belongs to a BIN (BOLD:AAU4648) corresponding to 1,349 sequences reported in BOLD from non-identified specimens and recorded from the USA, New Zealand, Australia, Kenya, South Africa and now France. The nearest BIN (BOLD:AAP4717) is an Australian unidentified Psychodidae (42 sequences). However, the probable and best candidate for *Psychoda (Psychodocha) gemina*, described from England (Eaton 1904), is BOLD:ACR3941 known from 192 sequences, 165 of them identified as *P. gemina*, and collected from several European countries, such as Norway, Sweden, Finland, Germany, Bulgaria, Denmark, Belarus and France. The nearest BINs to *P.*

(*Psychodocha*) *gemina* are two unidentified *Psychoda* species from Costa Rica (BOLD:ADB1380 and BOLD:ADF 3686). It therefore appears that the relationship between *P. (Psychodocha) gemina* and *Psychoda (Psychodocha)* sp. aff. *gemina* (F168 SPE, Virazeil, France) which appear morphologically similar to us, if any, remains unelucidated.

Conclusion

It is interesting to note that, among the 16 *Psychoda sensu lato* morphotypes recorded from Corsica and mainland France, the COI barcoding highlighted the presence of possible as many as 18 distinct taxa. While 14 out of 15 species recorded in Corsica are new for the island, two species are new for France and Europe (*P. (Psychodocha) uncinula* and *P. (Chodopsycha) divaricata*). These new records show that distribution ranges can be highly dynamic through expansion with climate change or introductions through increases in global trade. Moreover, our DNA barcoding approach allowed for the detection of at least three distinct haplotype complexes, possibly representing some taxa that are most likely undescribed. A study looking at morphological differences between the specimens corresponding to these BINs is further required. But even in such case, it can be difficult to determine which taxa from the complex corresponds to the reference species, since no barcode sequence has been yet generated from specimens from the type localities. It is also interesting to note that all the COI barcodes generated in this study are already present on BOLD database, suggesting that most of the temperate Psychodid diversity has likely already been sequenced but not properly named. In BOLD (as of January 12th 2022), there are 13,240 published sequences identified as belonging to the genus *Psychoda* corresponding to 58 BINs; however, only 30 of these BINs have a species name, including four BINs associated with several species names. Consequently, a great effort should be made in re-analyse the morphological characters of

specimens corresponding to these BINs in order to better understand the morphological variability in *Psychoda sensu lato*. A complementary molecular study using nuclear genes of specimens corresponding to different BINS would be informative in resolving the question about haplotype or species diversity. It is fascinating that even though Psychodinae have been studied in temperate regions for over 125 years (Eaton 1893 and afterwards), many questions remain, concerning not only their taxonomy, but also their biogeography, their ecology and their evolutionary history.

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557 Table 1. European *Psychoda sensu lato* species list from *Fauna Europaea* (<https://fauna-eu.org/>),
558 accessed on November 8th 2021. Species presence (X) or absence (-) are given for mainland
559 France, Corsica, mainland Italy and Sardinia. Subgenera names have been added following Bravo
560 et al. (2006). Note that *Tinearia* is listed in both *Fauna Europaea*, Global Biodiversity Information
561 Facility (GBIF: <https://www.gbif.org/>) and l’Inventaire National du Patrimoine Naturel (INPN:
562 <https://inpn.mnhn.fr/accueil/index>) as a different genus.

<i>Psychoda</i> species	France	Corsica	Italy	Sardinia
<i>Psychoda</i> (<i>Logima</i>) <i>albipennis</i>	X	–	X	X ¹
<i>P.</i> (<i>Tinearia</i>) <i>alternata</i>	X	–	X	X
<i>P.</i> (<i>Psychoda</i>) <i>alticola</i>	X	–	–	–
<i>P.</i> (<i>Psychomora</i>) <i>armillariphila</i>	X	–	–	–
<i>P.</i> (<i>Copropsychoda</i>) <i>brevicornis</i>	X	–	–	–
<i>P.</i> (<i>Psychodocha</i>) <i>cinerea</i>	X ²	X ³	X	X
<i>P.</i> (<i>Apsyscha</i>) <i>coprophila</i>	X	–	–	–
<i>P.</i> (<i>Psychoda</i>) <i>crassipenis</i>	X ⁴	–	–	–
<i>P.</i> (<i>Psychoda</i>) <i>dolomitica</i>	–	–	X	–
<i>P.</i> (<i>Logima</i>) <i>erminea</i>	X ²	–	X ⁵	–
<i>P.</i> (<i>Logima</i>) <i>fumetaria</i>	X	–	–	–
<i>P.</i> (<i>Psychodocha</i>) <i>gemina</i>	X	–	X ⁵	–
<i>P.</i> (<i>Psycha</i>) <i>griseus</i>	X	–	X	–
<i>P.</i> (<i>Tinearia</i>) <i>lativentris</i>	X	–	X	X ¹
<i>P.</i> (<i>Chodopsycha</i>) <i>lobata</i>	X	–	X	–
<i>P.</i> (<i>Psychodula</i>) <i>minuta</i>	X	–	X	X
<i>P.</i> (<i>Psychomora</i>) <i>mycophila</i>	X	–	–	–
<i>P.</i> (<i>Logima</i>) <i>parthenogenetica</i>	X	–	X	X
<i>P.</i> (<i>Psychoda</i>) <i>phalaenoides</i>	X	–	X	–
<i>P.</i> (?) <i>pontina</i>	–	–	X	–
<i>P.</i> (<i>Tinearia</i>) <i>pseudoalternicula</i>	–	–	X	X
<i>P.</i> (<i>Apsyscha</i>) <i>pusilla</i>	–	–	X	–
<i>P.</i> (<i>Chodopsycha</i>) <i>quatei</i>	–	–	X	–
<i>P.</i> (?) <i>sarcophila</i>	X	–	–	–
<i>P.</i> (<i>Logima</i>) <i>satchelli</i>	–	–	X	–
<i>P.</i> (<i>Ypsychoda</i>) <i>setigera</i>	X	–	X ⁵	–
<i>P.</i> (<i>Logima</i>) <i>surcoufi</i>	X	–	–	–
<i>P.</i> (?) <i>tridentata</i>	–	–	X	–
<i>P.</i> (<i>Psychomora</i>) <i>trinodulosa</i>	X	–	X ⁰	X
<i>P.</i> (<i>Psychoda</i>) <i>uniformata</i>	–	–	X	–
<i>P.</i> (<i>Psycha</i>) <i>vaillanti</i>	–	–	X	–
<i>P.</i> (<i>Psychoda</i>) <i>villosa</i>	–	–	X	–

<i>P. (Psychoda) virgo</i>	X	–	–	–
<i>P. (Logima) zetterstedti</i>	X ⁵	–	X ⁶	–
Total	24	1	23-24	8

¹species not listed from Sardinia in *Fauna Europaea* but listed in Salamanna1982 (cited as *P. severini*).

²species not listed from France in *Fauna Europaea* but listed in l'INPN Naturel and the GBIF.

³species not listed from Corsica in *Fauna Europaea* but listed in Moubayed-Breil et al. 2013.

⁴species not listed from France in *Fauna Europaea* but listed in Albre et al. 2003 and Chartier et al. 2013.

⁰species listed from Italy in *Fauna Europaea* but not listed in Dahl et al. 1995.

⁵species not listed from Italy in *Fauna Europaea* but listed in Dahl et al. 1995.

⁶species not listed from France and Italy in *Fauna Europaea* but listed in Ježek & Yağci 2005.

Table 2. List of specimens mounted and barcoded (COI) presented in this study. For each specimen, the specimen code corresponds to its morphological identification (P: *Psychoda*, *: mainland France specimen, M: Male, F: Female), the corresponding BINs from BOLD database, the GenBank accession number, the specimen ProcessID code (BOLD) and the locality: Ajaccio (Corsica), Montans (mainland France), Rillieux-la-Pape (mainland France) and Virazeil (mainland France).

Specimen code	BIN	GenBank	ProcessID	Locality
<i>Clogmia albipunctata</i> SPE	AAF9305	MT745768	PSYKO026-20	Ajaccio
<i>P alternata</i> * F170 SPE	AAF9306	MT745813	PSYKO029-20	Montans
<i>P satchelli</i> F86 SPE	AAF9311	MT745803	PSYKO021-19	Ajaccio
<i>P minuta</i> F75 SPE	AAF9314	MT745794	PSYKO019-19	Ajaccio
<i>P minuta</i> M115 SPE	AAF9314	MT745795	PSYKO044-20	Ajaccio
<i>P minuta</i> M85 SPE	AAF9314	MT745796	PSYKO045-20	Ajaccio
<i>P phalaenoides</i> F127 SPE	AAF9317	MT745800	PSYKO049-20	Ajaccio
<i>P phalaenoides</i> F146-SPE	AAF9317	MT745801	PSYKO050-20	Ajaccio
<i>P phalaenoides</i> * F186 SPE	AAF9317	MT745819	PSYKO57-20	Rillieux-la-Pape
<i>P phalaenoides</i> M137 SPE	AAF9317	MT745802	PSYKO051-20	Ajaccio
<i>P albipennis</i> * F171 SPE	AAL7819	MT745810	PSYKO027-20	Montans
<i>P albipennis</i> F25 SPE	AAL7819	MT745769	PSYKO011-19	Ajaccio
<i>P albipennis</i> F4 SPE	AAL7819	MT745770	PSYKO008-19	Ajaccio
<i>P albipennis</i> F48 SPE	AAL7819	MT745771	PSYKO015-19	Ajaccio
<i>P albipennis</i> F64 SPE	AAL7819	MT745772	PSYKO010-19	Ajaccio
<i>P albipennis</i> M42 SPE	AAL7819	MT745773	PSYKO001-19	Ajaccio
<i>P albipennis</i> M63 SPE	AAL7819	MT745774	PSYKO009-19	Ajaccio
<i>P grisescens</i> F138 SPE	AAM9202	MT745814	PSYKO041-20	Ajaccio
<i>P grisescens</i> F140 SPE	AAM9202	MT745815	PSYKO042-20	Ajaccio
<i>P grisescens</i> M141 SPE	AAM9202	MT745816	PSYKO043-20	Ajaccio
<i>P trinodulosa</i> F57 SPE	AAN8770	MT745806	PSYKO017-19	Ajaccio
<i>P trinodulosa</i> F90 SPE	AAN8770	MT745807	PSYKO022-19	Ajaccio
<i>P sp aff gemina</i> * F168 SPE	AAU4648	MT745817	PSYKO034-20	Virazeil
<i>P sigma</i> F15 SPE	AAU4664	MT745804	PSYKO052-20	Ajaccio
<i>P sigma</i> M18 SPE	AAU4664	MT745805	PSYKO053-20	Ajaccio
<i>P uniformata</i> F179 SPE	AAZ5592	MT745818	PSYKO040-20	Ajaccio
<i>P lativentris</i> F11 SPE	ABA0879	MT745792	PSYKO006-19	Ajaccio
<i>P lativentris</i> F46 SPE	ABA0879	MT745793	PSYKO013-19	Ajaccio
<i>P uncinula</i> * F178 SPE	ACD9559	MT745812	PSYKO037-20	Virazeil
<i>P uncinula</i> F35 SPE	ACD9559	MT745787	PSYKO004-19	Ajaccio

<i>P uncinula</i> * M177 SPE	ACD9559	MT745811	PSYKO038-20	Virazeil
<i>P uncinula</i> M56 SPE	ACD9559	MT745788	PSYKO039-20	Ajaccio
<i>P uncinula</i> M62 SPE	ACD9559	MT745789	PSYKO018-19	Ajaccio
<i>P satchelli</i> F79 SPE	ACG1924	MT745781	PSYKO035-20	Ajaccio
<i>P satchelli</i> F94 SPE	ACG1924	MT745782	PSYKO025-19	Ajaccio
<i>P satchelli</i> M105 SPE	ACG1924	MT745783	PSYKO020-19	Ajaccio
<i>P satchelli</i> M116 SPE	ACG1924	MT745784	PSYKO002-19	Ajaccio
<i>P satchelli</i> M117 SPE	ACG1924	MT745785	PSYKO036-20	Ajaccio
<i>P satchelli</i> M38 SPE	ACG1924	MT745786	PSYKO003-19	Ajaccio
<i>P cinerea</i> F136 SPE	ACG4180	MT745777	PSYKO032-20	Ajaccio
<i>P cinerea</i> F154 SPE	ACG4180	MT745778	PSYKO033-20	Ajaccio
<i>P cinerea</i> * F175 SPE	ACG4180	MT745808	PSYKO030-20	Virazeil
<i>P cinerea</i> F92 SPE	ACG4180	MT745779	PSYKO024-19	Ajaccio
<i>P cinerea</i> * M169 SPE	ACG4180	MT745809	PSYKO031-20	Virazeil
<i>P cinerea</i> M66 SPE	ACG4180	MT745780	PSYKO012-19	Ajaccio
<i>P mycophila</i> F131 SPE	ACG6331	MT745797	PSYKO046-20	Ajaccio
<i>P mycophila</i> F157 SPE	ACG6331	MT745798	PSYKO047-20	Ajaccio
<i>P mycophila</i> M159 SPE	ACG6331	MT745799	PSYKO048-20	Ajaccio
<i>P alternata</i> F40 SPE	ACM5462	MT745775	PSYKO007-19	Ajaccio
<i>P alternata</i> M91 SPE	ACM5462	MT745776	PSYKO023-19	Ajaccio
<i>P divaricata</i> F110 SPE	ACN1858	MT745790	PSYKO014-19	Ajaccio
<i>P divaricata</i> M52 SPE	ACN1858	MT745791	PSYKO016-19	Ajaccio
<i>P crassipenis</i> * F187 SPE	ACP0335	MT745820	PSYKO54-20	Rillieux-la-Pape
<i>P crassipenis</i> * F188 SPE	ACP0335	MT745821	PSYKO55-20	Rillieux-la-Pape
<i>P crassipenis</i> * F189 SPE	ACP0335	MT745822	PSYKO56-20	Rillieux-la-Pape

578
579

Table 3. List of published specimens used as outgroups, bold reference or closest clade affiliation. For each specimen, the taxonomic name indicated in the corresponding database (GenBank or BOLD) is given, with the corresponding BIN, GenBank sequence accession, and/or the specimen ProcessID code (BOLD).

Taxon name	BIN	GenBank	ProcessID
<i>Philosepedon</i> sp.	ABA0882	JQ349584	–
Psychodidae	ACY6029	MG298342	–
Diptera	ACX1419	–	AMTPE864-15
<i>Trichopsychoda hirtella</i>	ACM7618	MF966162	–
<i>Trichopsychoda hirtella</i>	ACM7618	–	GMGMA102-14
<i>Psychoda cinerea</i>	ACF6513	KR989081	–
<i>P. cinerea</i>	ACF6513	MG296511	–
<i>P. gemina</i>	ACR3941	–	GMGRF1794-13
<i>P. gemina</i>	ACR3941	MF966166	–
<i>P. satchelli</i>	AAF9311	–	GMGRF390-13
<i>P. alternata</i>	AAF9306	HQ583069	–
Psychodinae	AAZ5592	–	GMEGM093-14
<i>Psychoda</i> sp.	AAU4648	–	AACTA7603-20

Figure legends

Figure 1. Neighbour-Joining tree based on 68 COI sequences resulting from 10,000 replicates based on the K2P distances. The brackets indicate the different BINs. (P: *Psychoda*, *: mainland France, SPE: sequences generated in the present study). Specimen information is given in Tables 2 and 3.

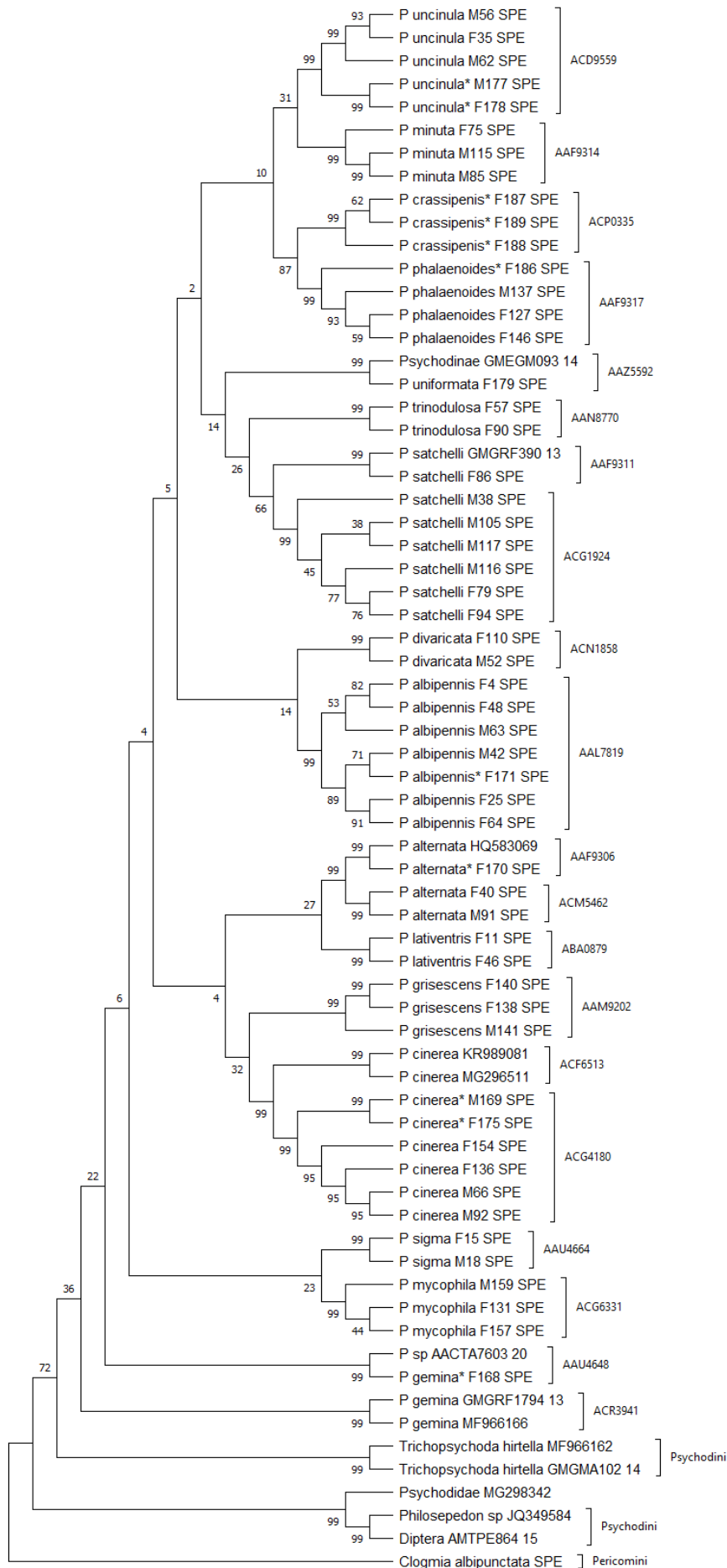


Fig. 1