The InBIO Barcoding Initiative Database: DNA barcodes of Portuguese Diptera 02 - Limoniidae, Pediciidae and Tipulidae

Sónia Ferreira‡, Pjotr Oosterbroek§, Jaroslav Starý‖, Pedro Sousa‡, Vanessa A. Mata‡, Luis P da Silva ‡, Joana Paupério‡, Pedro Beja‡,¶

‡ CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vila do Conde, Portugal
§ Naturalis Biodiversity Center, Leiden, Netherlands
‖ Olomouc-Nedvězí & Silesian Museum, Nádražní okruh 31, CZ-746 01 Opava, Olomouc, Czech Republic
¶ CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Vila do Conde, Portugal

Corresponding author: Sónia Ferreira (hiporame@gmail.com)

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Abstract

Background

The InBIO Barcoding Initiative (IBI) Diptera 02 dataset contains records of 412 crane fly specimens belonging to the Diptera families: Limoniidae, Pediciidae and Tipulidae. This dataset is the second release by IBI on Diptera and it greatly increases the knowledge on the DNA barcodes and distribution of crane flies from Portugal. All specimens were collected in Portugal, including six specimens from the Azores and Madeira archipelagos. Sampling took place from 2003 to 2019. Specimens have been morphologically identified to species level by taxonomists and belong to 83 species in total. The species, represented in this dataset, correspond to about 55% of all the crane fly species known from Portugal and 22% of crane fly species known from the Iberian Peninsula. All DNA extractions and
most specimens are deposited in the IBI collection at CIBIO, Research Center in Biodiversity and Genetic Resources.

**New information**

Fifty-three species were new additions to the Barcode of Life Data System (BOLD), with another 18 species' barcodes added from under-represented species in BOLD. Furthermore, the submitted sequences were found to cluster in 88 BINs, 54 of which were new to BOLD. All specimens have their DNA barcodes publicly accessible through BOLD online database and its collection data can be accessed through the Global Biodiversity Information Facility (GBIF). One species, *Gonomyia tenella* (Limonidae), is recorded for the first time from Portugal, raising the number of crane flies recorded in the country to 145 species.

**Keywords**

Diptera, Limoniidae, Pediciidae, Tipulidae, occurrence records, Portugal, DNA barcode, COI

**Introduction**

Portugal is part of the Mediterranean hotspot of biodiversity, yet Portuguese biodiversity remains poorly studied and genetic data are scarcer still. To tackle this problem, the Research Network in Biodiversity and Evolutionary Biology (InBIO) created the InBIO Barcoding Initiative (IBI), making use of in-house High Throughput Sequencing knowledge to construct a reference collection of morphologically identified Portuguese specimens and corresponding DNA barcodes ([Ferreira et al. 2018](#)). Invertebrates, especially insects, were given priority in the IBI due to their share of overall biodiversity and importance in ecosystems functioning (e.g. [Weisser and Siemann 2004](#), [Losey and Vaughan 2006](#), [Mata et al. 2016](#), [da Silva et al. 2019](#)) and due to the lack of available DNA barcodes in public databases representative of Portuguese Invertebrates (e.g. [Corley and Ferreira 2017](#), [Corley et al. 2017](#), [Ferreira et al. 2019](#), [Weigand et al. 2019](#)).

DNA barcoding is a molecular biology method for species identification that relies on the comparison of a short mitochondrial DNA sequence of interest to a library of sequences with known species identity ([Hebert et al. 2003](#)). The construction of comprehensive reference libraries is therefore essential and these require the morphological identification of vouchers by expert taxonomists ([Kress et al. 2015](#), [Ferreira et al. 2018](#)). DNA barcoding has expanded beyond single organism and species identification, to broader metabarcoding studies ([Porter and Hajibabaei 2020](#)). DNA barcodes are now a ubiquitous tool in ecological and biological conservation studies, as well as, for example, in forensic applications ([Pečnikar and Buzan 2013](#), [Kress et al. 2015](#), [DeSalle and Goldstein 2019](#)).
The order Diptera is one of the most diverse, widespread and common of the holometabolic insects, having more than 158,000 described species (Pape et al. 2009, Evenhuis and Pape 2021). Within Diptera, the crane flies (Tipuloidea) are further classified into four families, Cylindrotomidae, Limoniidae, Pediciidae and Tipulidae (Starý 1992; but see Petersen et al. 2010 and Starý 2021) and are one of the most diverse groups, with over 15,630 recognised species (Oosterbroek 2021). Adult Tipuloidea can superficially resemble mosquitoes, with their slender bodies and long antennae, wings, legs and abdomen, but can be identified by the presence of two complete anal veins in the wings, a V-shaped transverse suture on the mesothorax and the absence of ocelli (de Jong et al. 2007). Larvae of Tipuloidea are mainly identified by the presence of a hemicephalous, retractible head capsule ocelli (de Jong et al. 2007). Larvae of most species are found in aquatic habitats, from fast-flowing streams to brackish water or in semi-aquatic habitats, such as organic sludge along the edge of water bodies or saturated mosses and hepatics. Those that are terrestrial are mostly still found in humid habitats, like leaf-litter (Pritchard 1983, de Jong et al. 2007), although a few also live in dry soils. Contrary to immature forms, all adult Tipuloidea, mostly short-lived after emergence, are terrestrial (Pritchard 1983, de Jong et al. 2007), while in the larvae stage, most species feed on algae or decaying plant material and associated microflora and some groups also feed on mosses and hepatics, though several Limnophilinae and Pediciinae larvae are predatory (Pritchard 1983, de Jong et al. 2007). Most species do not feed after reaching adulthood, although adults generally drink water to offset body evaporation (Pritchard 1983, de Jong et al. 2007). A few species are known to be important crop pests, as their larvae, when in large numbers, can damage crops by feeding on their roots or seedlings (Alford 2012, Alford 2014, Blackshaw and Coll 1999, de Jong et al. 2007). Furthermore, species of Tipuloidea play important ecological roles in several ecosystems being well known components of bird and bat diets (e.g. Alford 2012, Alford 2014, Buchanan et al. 2006, Krüger et al. 2013, Rhymer et al. 2012, Vaughan 1997, Wilson et al. 1999).

In Portugal, the knowledge on Tipuloidea is still very incomplete. Of the four families that compose the Tipuloidea, only the Cylindrotomidae has not so far been recorded for the country, although it is known from Spain (Oosterbroek et al. 2020, Oosterbroek 2021). Recently, 33 new species were added to the Portuguese species list, raising its total to 149 (Eiroa and Báez 2002a, Eiroa and Báez 2002b, Oosterbroek et al. 2020, Oosterbroek 2021, Kolcsár et al. 2021). This is certainly an underestimate as 376 species are already known from the Iberian Peninsula (Oosterbroek et al. 2020). Furthermore, the distribution and ecology of the Portuguese crane flies are also poorly known.

The IBI Diptera 02 dataset contains records of 412 specimens of crane flies collected in Portugal, all morphologically identified to species level, for a total of 83 species, two of which were further identified to subspecies level. This dataset is part of the ongoing IBI database public releases in both the Global Biodiversity Information Facility (GBIF) and the Barcode of Life Data System (BOLD) (e.g. Ferreira et al. 2020a, Ferreira et al. 2020b).
General description

**Purpose:** This dataset aims to provide the second contribution to a DNA barcode sequences library for Portuguese Diptera. It covers the three families of Tipuloidea known from Portugal. This library aims to advance DNA-based species identification from regular molecular studies and new DNA-metabarcoding studies. It presents also an important resource for taxonomic research on Portuguese crane flies and its distribution.

**Additional information:** A total of 412 specimens of crane flies were collected and DNA barcoded (Suppl. materials 1, 2). Fig. 1 illustrates examples of the diversity of species that are part of the dataset of distribution data and DNA barcodes of Portuguese Diptera 02. All sequences of cytochrome c oxidase I (COI) DNA barcodes are 658 bp long. This dataset contributes significantly to the representation of both species and genetic diversity of Tipuloidea in public libraries. Of the 83 taxa barcoded, 53 (64%) are new to the DNA barcode database BOLD at the moment of the release (marked with * in Taxa field of Table 1). Moreover, the species *Gonomyia tenella* (Meigen, 1818) is recorded for the first time for Portugal. Eighty-six species in the dataset (22%) were previously represented in BOLD with less than 10 publicly available DNA barcode sequences at the moment of the release (marked with " in Species field of Table 1). The submitted sequences were found to cluster in 88 BINs in BOLD (Barcode Index Number, Ratnasingham and Hebert 2013), 54 of which were new to BOLD. Of the submitted sequences, *Tipula intermedia* Eiroa, 1990 and *Tipula lateralis* Meigen, 1804 share the same BIN in BOLD. Moreover, the generated sequence of *Nephrotoma suturalis wulpiana* (Bergroth, 1888) shared BIN with sequences of *Nephrotoma ferruginea* (Fabricius, 1805) from the United States of America, where both species are native. These two species are very closely related and are frequently misidentified. Differences between the two species have been outlined in the revision of the Nearctic non-dorsalis species (Oosterbroek 1984). The two former records from Portugal are discussed in detail and figured in Hancock et al. 2016. The generated sequence of *Molophilus testaceus* Lackschewitz, 1940 shares the BIN with *Molophilus griseus* (Meigen, 1804) from Finland and with a sequence of a specimen identified as *Molophilus cf. ochraceus* (Meigen, 1818) from Norway. The generated sequences of *Pseudolimnophila ebulata* Stary, 1982 share the BIN with a sequence of *Pseudolimnophila sepium* (Verrall, 1886) from Estonia. In addition, the generated sequences of *Nephrotoma submaculosa* Edwards, 1928 clustered in two BINs, one of which also harbours sequences of *Nephrotoma flavescens* (Linnaeus, 1758) from Germany and Finland. The identifications of *Nephrotoma suturalis wulpiana* and the *Nephrotoma submaculosa* specimens clustering with *Nephrotoma flavescens* were double-checked by Herman de Jong (Naturalis Biodiversity Center, Leiden, The Netherlands).
Table 1.
List of taxa that were collected and DNA barcoded within this project. In column Taxa: * - Indicates taxa without a DNA barcode prior to this study; " " indicates taxa with less than 10 sequences available prior to this study.

| Family (Subfamily) | Taxa                                         | IBI code A | BOLD code                     | BOLD BIN            | GenBank          |
|--------------------|----------------------------------------------|------------|-------------------------------|---------------------|------------------|
| Limoniidae (Chioneinae) | *Erioconopa diuturna* (Walker, 1848) *         | INV06209, INV08150 | IBIIDP258-19, IBIIDP446-19   | BOLD:ADZ3321       | MZ196613, MZ196520 |
| Limoniidae (Chioneinae) | *Erioptera fuscipennis* Meigen, 1818 *       | INV08142    | IBIIDP438-19                  | BOLD:AEC7071       | MZ196632         |
| Limoniidae (Chioneinae) | *Gonomyia tenella* (Meigen, 1818) *          | INV08655    | IBIIDP644-20                  | BOLD:ABV4194       | MZ196605         |
| Limoniidae (Chioneinae) | *Idiocera sziladyi* (Lackschewitz, 1940) *   | INV07222, INV07671, INV07679, INV08144, INV08393, INV08394, INV08395, INV08396 | IBIIDP331-19, IBIIDP338-19, IBIIDP339-19, IBIIDP440-19, IBIIDP474-19, IBIIDP475-19, IBIIDP476-19, IBIIDP477-19 | BOLD:AEC9216 | MZ196587, MZ196919, MZ196788, MZ196902, MZ196747, MZ196607, MZ196872, MZ196585 |
| Limoniidae (Chioneinae) | *Molophilus baezi* Theowald, 1981 *          | INV08355    | IBIIDP450-19                  | BOLD:AEC9561       | MZ196837         |
| Limoniidae (Chioneinae) | *Molophilus flavus* Goetghueber, 1920 "      | INV06375    | IBIIDP283-19                  | BOLD:AEC9823       | MZ196640         |
| Limoniidae (Chioneinae) | *Molophilus testaceus* Lackschewitz, 1940 * | INV10269    | IBIIDP810-20                  | BOLD:AVB9683       | MZ196639         |
| Limoniidae (Chioneinae) | *Symplecta hybrida* (Meigen, 1804)           | INV07037, INV08356 | IBIIDP323-19, IBIIDP451-19   | BOLD:AAZ4292       | MZ196555, MZ196715 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|-------------------|------|------------|-----------|----------|---------|
| **Limoniidae**    | **Symplecta pilipes** (Fabricius, 1787) | INV06218, INV06499, INV06992, INV08153, INV08592, INV08597, INV08599, INV09687, INV09693, INV09695, INV09709, INV10185, INV10192, INV10310 | IBIDP260-19, IBIDP294-19, IBIDP321-19, IBIDP449-19, IBIDP636-20, IBIDP637-20, IBIDP639-20, IBIDP766-20, IBIDP767-20, IBIDP768-20, IBIDP769-20, IBIDP783-20, IBIDP785-20, IBIDP830-20 | BOLD:ACO3207 | MZ196843, MZ196778, MZ196859, MZ196685, MZ196601, MZ196804, MZ196593, MZ196776, MZ196833, MZ196539, MZ196525, MZ196787, MZ196858, MZ196908 |
| **Limoniidae**    | **Symplecta stictica** (Meigen, 1818) | INV06513, INV08125 | IBIDP297-19, IBIDP421-19 | BOLD:ADN4631 | MZ196807, MZ196840 |
| **Limoniidae**    | **Dactylolabis sexmaculata** (Macquart, 1826) * | INV04587 | IBIDP193-19 | BOLD:ADW0650 | MZ196744 |
| **Limoniidae**    | **Australolinophila analis** (Santos Abreu, 1923) * | INV08357 | IBIDP452-19 | BOLD:AEC6671 | MZ196681 |
| **Limoniidae**    | **Australolinophila latistyla** Starý, 1977 * | INV07797, INV08145, INV08440, INV08507, INV08531, INV08535, INV08538, INV08542, INV08549, INV08551, INV08554, INV08556, INV08557, INV08608, INV08661, INV08775, INV08817, INV09017, INV10347 | IBIDP415-19, IBIDP441-19, IBIDP594-20, IBIDP619-20, IBIDP622-20, IBIDP624-20, IBIDP625-20, IBIDP626-20, IBIDP628-20, IBIDP629-20, IBIDP630-20, IBIDP632-20, IBIDP633-20, IBIDP641-20 | BOLD:AEC7677 | MZ196916, MZ196669, MZ196789, MZ196718, MZ196618, MZ196809, MZ196523, MZ196875, MZ196752, MZ196604, MZ196732, MZ196719, MZ196750, MZ196647 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|-------------------|------|------------|-----------|----------|---------|
| **Limoniidae** (Limnophilinae) | *Dicranophragma nemorale* (Meigen, 1818) | INV06199, INV06201 | IBIDP256-19, IBIDP257-19 | BOLD:AEC9486 | MZ196708, MZ196735 |
| **Limoniidae** (Limnophilinae) | *Hexatoma nigra* Latreille, 1809 | INV06356, INV06477, INV06478, INV06479, INV09307 | IBIDP257-19, IBIDP279-19, IBIDP286-19, IBIDP287-19, IBIDP750-20 | BOLD:AEC8914 | MZ196712, MZ196815, MZ196814, MZ196563, MZ196799 |
| **Limoniidae** (Limnophilinae) | *Hexatoma obscura* (Meigen, 1818) | INV08133 | IBIDP429-19 | BOLD:AEC6596 | MZ196917 |
| **Limoniidae** (Limnophilinae) | *Paradelphomyia senilis* (Haliday, 1833) | INV08136 | IBIDP432-19 | BOLD:ADZ6740 | MZ196564 |
| **Limoniidae** (Limnophilinae) | *Euphytidorea aperta* (Verrall, 1887) | INV08529 | IBIDP621-20 | BOLD:AEE5475 | MZ196717 |
| **Limoniidae** (Limnophilinae) | *Phylidorea ferruginea* (Meigen, 1818) | INV06899, INV08137, INV08651, INV08998, INV09310, INV10294 | IBIDP318-19, IBIDP433-19, IBIDP643-20, IBIDP707-20, IBIDP751-20, IBIDP819-20 | BOLD:ABW4832 | MZ196891, MZ196720, MZ196541, MZ196856, MZ196713, MZ196805 |
| **Limoniidae** (Limnophilinae) | *Pseudolimnophila ebullata* Starý, 1982 | INV07686, INV08122 | IBIDP340-19, IBIDP418-19 | BOLD:ABW9444 | MZ196734, MZ196583 |
| **Limoniidae** (Limnophilinae) | *Pseudolimnophila lucorum* (Meigen, 1818) | INV07149, INV07150, INV07162, INV07309, INV09780, INV10281 | IBIDP324-19, IBIDP325-19, IBIDP328-19, IBIDP333-19, IBIDP777-20, IBIDP816-20 | BOLD:ABA4227 | MZ196655, MZ196721, MZ196876, MZ196559, MZ196635, MZ196580 |
| Family (Subfamily) | Taxa                                                       | IBI code A          | BOLD code       | BOLD BIN         | GenBank        |
|-------------------|-----------------------------------------------------------|---------------------|-----------------|------------------|----------------|
| Limoniidae (Limoniinae) | * Achyrolimonia decemmaculata (Loew, 1873) | INV08126           | IBIDP422-19     | BOLD:ABU8822     | MZ196691       |
|                   | * Dicranomyia affinis (Staeger, 1840)                     | INV08387, INV08388, INV08389, INV08390 | IBIDP366-19, IBIDP367-19, IBIDP368-19, IBIDP369-19 | BOLD:AEC8483 | MZ196877, MZ196751, MZ196808, MZ196561 |
|                   | * Dicranomyia chorea (Meigen, 1818)                       | INV08131           | IBIDP427-19     | BOLD:ABU9992     | MZ196706       |
|                   | * Dicranomyia didyma (Meigen, 1804)                       | INV04553, INV04580, INV04591, INV04593, INV04661, INV06485, INV08015, INV08392, INV09304 | IBIDP191-19, IBIDP192-19, IBIDP194-19, IBIDP196-19, IBIDP215-19, IBIDP288-19, IBIDP416-19, IBIDP473-19, IBIDP748-20 | BOLD:ADW2241 | MZ196627, MZ196883, MZ196661, MZ196551, MZ196683, MZ196608, MZ196748, MZ196783, MZ196745 |
|                   | * Dicranomyia eulaliae Geiger & Starý, 1994               | INV10336           | IBIDP839-20     | BOLD:AEE5290     | MZ196702       |
|                   | * Dicranomyia hamata Becker, 1908                         | INV04550, INV04592, INV08135 | IBIDP190-19, IBIDP195-19, IBIDP431-19 | BOLD:ADW6546 | MZ196736, MZ196576, MZ196652 |
|                   | * Dicranomyia longicollis (Macquart, 1846)               | INV08149, INV08391 | IBIDP445-19, IBIDP370-19 | BOLD:AEC9918     | MZ196909, MZ196631 |
|                   | * Dicranomyia maderensis (Wollaston, 1858)               | INV08359           | IBIDP454-19     | BOLD:AED0520     | MZ196841       |
|                   | * Dicranomyia modesta (Meigen, 1818)                     | INV07031, INV08130, INV08555, INV08582, INV09011, INV09013 | IBIDP322-19, IBIDP426-19, IBIDP631-20, IBIDP635-20, IBIDP708-20, IBIDP709-20 | BOLD:AAI1352 | MZ196599, MZ196769, MZ196664, MZ196558, MZ196621, MZ196714 |
| Family (Subfamily) | Taxa                        | IBI code A          | BOLD code         | BOLD BIN       | GenBank          |
|-------------------|-----------------------------|---------------------|-------------------|----------------|-----------------|
| Limoniidae (Limoniinae) | *Dicranomyia novemmaculata* (Strobl, 1906) | INV08124 | IBIDP420-19 | BOLD:AEC9490 | MZ196578        |
|                   | *Dicranomyia patricia* Starý, 1982 | INV08129 | IBIDP425-19 | BOLD:AEC7553 | MZ196826        |
|                   | *Dicranomyia pauli* Geiger, 1983 | INV06870, INV06967, INV07596, INV07772, INV07809, INV09763, INV09764, INV09858, INV10264 | IBIDP408-19, IBIDP320-19, IBIDP337-19, IBIDP348-19, IBIDP414-19, IBIDP775-20, IBIDP776-20, IBIDP782-20, IBIDP809-20 | BOLD:AEC9051 | MZ196577, MZ196625, MZ196741, MZ196629, MZ196739, MZ196630, MZ196553, MZ196662, MZ196864 |
|                   | *Dicranomyia sericata* (Meigen, 1830) | INV08001, INV08140 | IBIDP350-19, IBIDP436-19 | BOLD:ACR3244 | MZ196537, MZ196913 |
|                   | *Dicranomyia vicina* (Macquart, 1838) | INV08360 | IBIDP455-19 | BOLD:AEC9533 | MZ196758        |
|                   | *Dicranoptycha fuscescens* (Schummel, 1829) | INV06696, INV06698, INV08007, INV08152, INV08500, INV08505, INV08606, INV08858, INV08917, INV08923, INV08927, INV08929, INV08933, INV08940, INV08944, INV08948, INV08951 | IBIDP309-19, IBIDP311-19, IBIDP355-19, IBIDP448-19, IBIDP614-20, IBIDP617-20, IBIDP640-20, IBIDP694-20, IBIDP697-20, IBIDP698-20, IBIDP699-20, IBIDP700-20, IBIDP701-20, IBIDP702-20, IBIDP703-20, IBIDP704-20, IBIDP706-20 | BOLD:ADZ3003 | MZ196545, MZ196836, MZ196673, MZ196549, MZ196824, MZ196690, MZ196692, MZ196663, MZ196526, MZ196803, MZ196865, MZ196737, MZ196733, MZ196862, MZ196660, MZ196651, MZ196674 |
| Family (Subfamily) | Taxa                          | IBI code A | BOLD code          | BOLD BIN   | GenBank   |
|-------------------|-------------------------------|------------|--------------------|------------|-----------|
| Limoniidae (Limoniinae) | *Geranomyia bivittata Becker, 1908* | INV08362   | IBIDP457-19        | BOLD:AEC9740 | MZ196781  |
| Limoniidae (Limoniinae) | *Geranomyia unicolor Haliday, 1833* | INV07561, INV07806 | IBIDP336-19, IBIDP347-19 | BOLD:AED0251 | MZ196884, MZ196762 |
| Limoniidae (Limoniinae) | *Helius calviensis Edwards, 1928* | INV08123, INV08581 | IBIDP419-19, IBIDP634-20 | BOLD:AEC6944 | MZ196624, MZ196588 |
| Limoniidae (Limoniinae) | *Helius hispanicus Lackschewitz, 1928* | INV08141, INV08687, INV08808, INV08809 | IBIDP437-19, IBIDP648-20, IBIDP849-20, IBIDP850-20 | BOLD:AEC9798 | MZ196677, MZ196592, MZ196772, MZ196586 |
| Limoniidae (Limoniinae) | *Limonia hercegovinae (Strobl, 1898)* | INV06357, INV06369, INV08132, INV09306, INV09721, INV09826, INV10280 | IBIDP280-19, IBIDP282-19, IBIDP428-19, IBIDP749-20, IBIDP770-20, IBIDP779-20, IBIDP815-20 | BOLD:AEC9714 | MZ196911, MZ196845, MZ196903, MZ196726, MZ196589, MZ196609, MZ196623 |
| Limoniidae (Limoniinae) | *Limonia maculipennis (Meigen & Wiedemann, 1818)* | INV06022, INV06181, INV06287 | IBIDP235-19, IBIDP251-19, IBIDP276-19 | BOLD:ADX7619 | MZ196823, MZ196860, MZ196835 |
| Limoniidae (Limoniinae) | *Limonia nubeculosa Meigen, 1804* | INV07774, INV07775, INV07776, INV08134, INV08430, INV08446, INV08488, INV08498, INV08503, INV08506, INV08534, INV08662, INV08698, INV08699, INV08705, INV08706, INV08707, INV08708, INV08770, INV08772, INV09038, INV09270, INV10295, INV10340 | IBIDP341-19, IBIDP342-19, IBIDP343-19, IBIDP430-19, IBIDP592-20, IBIDP599-20, IBIDP611-20, IBIDP613-20, IBIDP615-20, IBIDP618-20, IBIDP623-20, IBIDP646-20, IBIDP651-20, IBIDP652-20, IBIDP655-20 | BOLD:AAG8508 | MZ196646, MZ196573, MZ196728, MZ196777, MZ196831, MZ196682, MZ196671, MZ196716, MZ196672, MZ196528, MZ196894, MZ196633, MZ196641, MZ196616, MZ196648, MZ196781 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|--------------------|------|------------|-----------|----------|---------|
| **Limoniidae (Limoniinae)** | *Limonia phragmitidis* (Schrank, 1781) | INV08441, INV08442, INV08452, INV08487, INV08738 | IBIDP595-20, IBIDP596-20, IBIDP600-20, IBIDP610-20, IBIDP665-20 | BOLD:ABV3744 | MZ196700, MZ196699, MZ196700, MZ196880, MZ196878, MZ196544, MZ196581, MZ196870 |
| **Limoniidae (Limoniinae)** | *Neolimonia dumetorum* (Meigen, 1804) " | INV08127, INV08472, INV08483, INV08485, INV08509, INV08547, INV08663, INV08697, INV08701, INV09018, INV09034, INV10221 | IBIDP423-19, IBIDP605-20, IBIDP608-20, IBIDP609-20, IBIDP620-20, IBIDP627-20, IBIDP647-20, IBIDP650-20, IBIDP654-20, IBIDP711-20, IBIDP716-20, IBIDP792-20 | BOLD:ABV5347 | MZ196882, MZ196518, MZ196790, MZ196802, MZ196594, MZ196597, MZ196753, MZ196926, MZ196890, MZ196659, MZ196707, MZ196560 |
| **Pedicidae (Pediciniae)** | *Pedicia occulta* (Meigen, 1830) | INV08003, INV08013 | IBIDP590-20, IBIDP591-20 | BOLD: AEE4508 | MZ196888, MZ196725 |
| **Pedicidae (Pediciniae)** | *Tricyphona immaculata* (Meigen, 1804) | INV08154, INV09303 | IBIDP478-19, IBIDP747-20 | BOLD: ADZ1801 | MZ196759, MZ196524 |
| **Tipulidae (Ctenophorinae)** | *Ctenophora ornata* Meigen & Wiedemann, 1818 " | INV05306, INV05449 | IBIDP219-19, IBIDP222-19 | BOLD: ADX2561 | MZ196521, MZ196570 |
| **Tipulidae (Dolichopezinae)** | *Dolichocheza albipes* (Strom, 1768) " | INV00683 | IBIDP587-20 | BOLD: ACB7905 | MZ196554 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|-------------------|------|------------|------------|----------|---------|
| Tipulidae (Tipulinae) | *Nephrotoma appendiculata pertenua* Oosterbroek, 1978 | INV04605, INV04614, INV06007, INV06172, INV06173, INV06177, INV06178, INV06192, INV06193, INV06275, INV06282, INV09272 | IBIDP199-19, IBIDP208-19, IBIDP225-19, IBIDP246-19, IBIDP247-19, IBIDP248-19, IBIDP249-19, IBIDP254-19, IBIDP255-19, IBIDP267-19, IBIDP273-19, IBIDP738-20 | BOLD-ADX1669 | MZ196729, MZ196638, MZ196565, MZ196598, MZ196600, MZ196819, MZ196773, MZ196653, MZ196527, MZ196724, MZ196861, MZ196855 |
| Tipulidae (Tipulinae) | *Nephrotoma flavipalpis* (Meigen, 1830) | INV04862, INV05975, INV08700 | IBIDP217-19, IBIDP392-19, IBIDP653-20 | | MZ196770, MZ196606, MZ196571 |
| Tipulidae (Tipulinae) | *Nephrotoma guestfalica* (Westhoff, 1879) | INV07242, INV08005, INV08713 | IBIDP332-19, IBIDP353-19, IBIDP660-20 | | MZ196857, MZ196821, MZ196895 |
| Tipulidae (Tipulinae) | *Nephrotoma luteata* (Meigen, 1818) | INV04296, INV04861, INV06546, INV09313, INV09314 | IBIDP186-19, IBIDP216-19, IBIDP301-19, IBIDP752-20, IBIDP753-20 | | MZ196522, MZ196574, MZ196536, MZ196649, MZ196800 |
| Tipulidae (Tipulinae) | *Nephrotoma submaculosa* Edwards, 1928 | INV04526, INV04608, INV06006, INV06008, INV06009, INV06035, INV06171, INV06182, INV06272, INV08002, INV08379, INV08380, INV08480, INV08495, INV08504 | IBIDP187-19, IBIDP202-19, IBIDP224-19, IBIDP226-19, IBIDP227-19, IBIDP243-19, IBIDP245-19, IBIDP252-19, IBIDP264-19, IBIDP351-19, IBIDP363-19, IBIDP364-19, IBIDP607-20, IBIDP612-20, IBIDP616-20 | | MZ196620, MZ196693, MZ196686, MZ196615, MZ196829, MZ196763, MZ196812, MZ196786, MZ196642, MZ196844, MZ196754, MZ196617, MZ196710, MZ196899, MZ196887 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|-------------------|------|------------|-----------|----------|---------|
| Tipulidae (Tipulinae) | Nephrotoma submaculosa Edwards, 1928 * | INV06021, INV06025, INV06170, INV06276 | IBIDP234-19, IBIDP238-19, IBIDP244-19, IBIDP268-19 | BOLD:ABW3498 | MZ196552, MZ196667, MZ196816, MZ196588 |
| Tipulidae (Tipulinae) | Nephrotoma sullingtonensis Edwards, 1938 * | INV06270, INV06489, INV06497, INV09279, INV09295 | IBIDP262-19, IBIDP290-19, IBIDP292-19, IBIDP742-20, IBIDP851-20 | BOLD:ADX5268 | MZ196668, MZ196595, MZ196534, MZ196839, MZ196704 |
| Tipulidae (Tipulinae) | Nephrotoma suturalis wulpiana (Bergroth, 1888) * | INV07541 | IBIDP335-19 | BOLD:ABZ0908 | MZ196922 |
| Tipulidae (Tipulinae) | Tipula cava Riedel, 1913 * | INV06028, INV06030, INV09319, INV09320, INV09324 | IBIDP239-19, IBIDP241-19, IBIDP757-20, IBIDP758-20, IBIDP762-20 | BOLD:ADX7095 | MZ196722, MZ196928, MZ196853, MZ196866, MZ196792 |
| Tipulidae (Tipulinae) | Tipula confusa van der Wulp, 1883 * | INV09075, INV09076, INV10230, INV10245, INV10274, INV10333, INV10337, INV10343, INV10344, INV10349 | IBIDP735-20, IBIDP736-20, IBIDP798-20, IBIDP803-20, IBIDP812-20, IBIDP838-20, IBIDP840-20, IBIDP843-20, IBIDP844-20, IBIDP846-20 | BOLD:ABV4653 | MZ196579, MZ196746, MZ196634, MZ196542, MZ196727, MZ196590, MZ196794, MZ196912, MZ196603, MZ196885 |
| Tipulidae (Tipulinae) | Tipula fabiola Mannheims, 1968 * | INV08004, INV08764 | IBIDP352-19, IBIDP678-20 | BOLD:AEC9463 | MZ196873, MZ196723 |
| Tipulidae (Tipulinae) | Tipula helvola Loew, 1873 * | INV08443 | IBIDP597-20 | BOLD:AAK1647 | MZ196636 |
| Tipulidae (Tipulinae) | Tipula hispanolivida Mannheims, 1968 * | INV05330 | IBIDP220-19 | BOLD:ADW8816 | MZ196896 |
| Tipulidae (Tipulinae) | Tipula iberica Mannheims, 1963 * | INV04609, INV06278, INV06358 | IBIDP203-19, IBIDP270-19, IBIDP281-19 | BOLD:ADX7515 | MZ196795, MZ196675, MZ196854 |
| Family (Subfamily) | Taxa                       | IBI code A                                                                 | BOLD code                                                                 | BOLD BIN                      | GenBank       |
|-------------------|----------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|-------------------------------|--------------|
| Tipulidae (Tipulinae) | *Tipula intermedia*        | INV09057, INV10229, INV10254, INV10255, INV10259, INV10270, INV10287, INV10297, INV10309, INV10313 | BOLD:ABZ5659, IBIDP732-20, IBIDP797-20, IBIDP805-20, IBIDP806-20, IBIDP808-20, IBIDP811-20, IBIDP818-20, IBIDP822-20, IBIDP829-20, IBIDP832-20 | MZ196889, MZ196614, MZ196756, MZ196830, MZ196676, MZ196665, MZ196851, MZ196743, MZ196811, MZ196731 |
| Tipulidae (Tipulinae) | *Tipula lateralis*         | INVO4630                                                                  | IBIDP212-19                                                                | BOLD:ADK4356                  | MZ196575     |
| Tipulidae (Tipulinae) | *Tipula lateralis*         | INV05431, INV09056, INV10278, INV10286, INV10296, INV10300, INV10303, INV10316 | IBIDP221-19, IBIDP731-20, IBIDP813-20, IBIDP817-20, IBIDP821-20, IBIDP824-20, IBIDP826-20, IBIDP834-20 | BOLD:ABZ5659                  | MZ196678, MZ196897, MZ196904, MZ196801, MZ196550, MZ196847, MZ196567, MZ196879 |
| Tipulidae (Tipulinae) | *Tipula maxima*            | INV05298, INV06522, INV06532                                              | IBIDP218-19, IBIDP299-19, IBIDP300-19                                       | BOLD:AAD6106                  | MZ196923, MZ196622, MZ196687 |
| Tipulidae (Tipulinae) | *Tipula mediterranea*      | INV04294, INV04613, INV04637, INV04660, INV06879, INV10187, INV10321     | IBIDP185-19, IBIDP207-19, IBIDP213-19, IBIDP214-19, IBIDP314-19, IBIDP784-20, IBIDP835-20 | BOLD:ADX2493                  | MZ196832, MZ196548, MZ196628, MZ196612, MZ196842, MZ196779, MZ196738 |
| Tipulidae (Tipulinae) | *Tipula morenae*           | INV06024, INV08010, INV08381                                              | IBIDP237-19, IBIDP358-19, IBIDP365-19                                       | BOLD:ADW6590                  | MZ196596, MZ196768, MZ196774 |
| Tipulidae (Tipulinae) | *Tipula morenae*           | INV06504, INV08014                                                        | IBIDP296-19, IBIDP361-19                                                   | BOLD:AEC7892                  | MZ196626, MZ196680 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|--------------------|------|------------|-----------|----------|---------|
| Tipulidae (Tipulinae) | *Tipula oleracea* Linnaeus, 1758 | INV06514, INV06929, INV07536, INV08461, INV08720, INV08727, INV08728, INV08742, INV08743, INV08744, INV08745, INV08749, INV08787, INV08788, INV08791, INV08792, INV08795, INV08796, INV09032, INV09039, INV09043, INV09050, INV09051, INV09052, INV09053, INV09055, INV10209 | IBIDP298-19, IBIDP319-19, IBIDP334-19, IBIDP602-20, IBIDP661-20, IBIDP663-20, IBIDP664-20, IBIDP666-20, IBIDP667-20, IBIDP668-20, IBIDP669-20, IBIDP670-20, IBIDP684-20, IBIDP685-20, IBIDP686-20, IBIDP687-20, IBIDP688-20, IBIDP689-20, IBIDP715-20, IBIDP719-20, IBIDP722-20, IBIDP726-20, IBIDP727-20, IBIDP728-20, IBIDP729-20, IBIDP730-20, IBIDP786-20 | **BOLD:AAF9041** | MZ196810, MZ196915, MZ196644, MZ196658, MZ196688, MZ196771, MZ196793, MZ196780, MZ196834, MZ196611, MZ196532, MZ196705, MZ196924, MZ196868, MZ196817, MZ196893, MZ196907, MZ196852, MZ196766, MZ196519, MZ196867, MZ196703, MZ196740, MZ196886, MZ196767, MZ196798, MZ196921 |
| Tipulidae (Tipulinae) | *Tipula paludosa* (Meigen, 1830) | INV06872, INV06885, INV06889, INV09761, INV10219, INV10223, INV10332, INV10341 | IBIDP313-19, IBIDP316-19, IBIDP317-19, IBIDP774-20, IBIDP790-20, IBIDP794-20, IBIDP837-20, IBIDP842-20 | **BOLD:ADZ7173** | MZ196701, MZ196828, MZ196547, MZ196697, MZ196910, MZ196755, MZ196619, MZ196530 |
| Family (Subfamily) | Taxa | IBI code A | BOLD code | BOLD BIN | GenBank |
|-------------------|------|------------|-----------|----------|---------|
| Tipulidae (Tipulinae) | *Tipula parallela* Theischinger, 1977 | INV04544, INV04615, INV06011, INV06029, INV06033, INV06273, INV06274, INV06277, INV09019, INV09273, INV09282, INV09293 | IBIDP189-19, IBIDP209-19, IBIDP229-19, IBIDP240-19, IBIDP242-19, IBIDP265-19, IBIDP266-19, IBIDP269-19, IBIDP712-20, IBIDP739-20, IBIDP740-20, IBIDP741-20 | BOLD:ADV9772 | MZ196929, MZ196699, MZ196925, MZ196584, MZ196914, MZ196645, MZ196920, MZ196749, MZ196694, MZ196898, MZ196905, MZ196569 |
| Tipulidae (Tipulinae) | *Tipula pilicauda* Pierre, 1922 | INV06010, INV08012, INV08763 | IBIDP228-19, IBIDP360-19, IBIDP677-20 | BOLD:ADW9682 | MZ196918, MZ196757, MZ196760 |
| Tipulidae (Tipulinae) | *Tipula pseudocinerascens* Strobl, 1906 | INV04527, INV04600, INV04606, INV04607, INV04610, INV04611, INV04616 | IBIDP188-19, IBIDP197-19, IBIDP200-19, IBIDP201-19, IBIDP204-19, IBIDP205-19, IBIDP210-19 | BOLD:ADX3916 | MZ196871, MZ196742, MZ196863, MZ196848, MZ196849, MZ196813, MZ196695 |
| Tipulidae (Tipulinae) | *Tipula pustulata* Pierre, 1920 | INV08008 | IBIDP356-19 | BOLD:AEC8062 | MZ196900 |
| Tipulidae (Tipulinae) | *Tipula repanda* Loew, 1864 | INV06880, INV07163, INV07164, INV10210, INV10217, INV10218, INV10220, INV10224, INV10235, INV10251, INV10298 | IBIDP315-19, IBIDP329-19, IBIDP330-19, IBIDP787-20, IBIDP788-20, IBIDP789-20, IBIDP791-20, IBIDP795-20, IBIDP801-20, IBIDP804-20, IBIDP823-20 | BOLD:AEC7761 | MZ196591, MZ196892, MZ196689, MZ196709, MZ196822, MZ196657, MZ196874, MZ196543, MZ196838, MZ196901, MZ196827 |
| Tipulidae (Tipulinae) | *Tipula rufina* Meigen, 1818 | INV10228 | IBIDP796-20 | BOLD:ACR4602 | MZ196796 |
| Tipulidae (Tipulinae) | *Tipula intermixta* Riedel, 1913 | INV06863 | IBIDP312-19 | BOLD:AEC7837 | MZ196825 |
| Family (Subfamily) | Taxa             | IBI code A                                                                 | BOLD code   | BOLD BIN       | GenBank     |
|-------------------|------------------|----------------------------------------------------------------------------|-------------|----------------|-------------|
| Tipulidae (Tipulinae) | *Tipula serrulifera* Alexander, 1962 | INV09722, INV08000, INV08378                                              | IBIDP771-20 | BOLD: AEE5789 | MZ196765    |
| Tipulidae (Tipulinae) | *Tipula trifasciculata* Strobl, 1900 | INV06279, INV08006, INV08445, INV08469, INV08462, INV08463, INV08726, INV08755, INV08757, INV08758, INV08760, INV08761, INV09027, INV09047 | IBIDP271-19, IBIDP349-19, IBIDP362-19 | BOLD: ADX0913 | MZ196557, MZ196906, MZ196670 |
| Tipulidae (Tipulinae) | *Tipula trigona* Mannheims, 1966 | INV06345, INV06347, INV08009, INV08111, INV08438, INV08445, INV08460, INV08462, INV08463, INV08726, INV08755, INV08757, INV08758, INV08760, INV08761, INV09027, INV09047 | IBIDP354-19, IBIDP357-19, IBIDP359-19, IBIDP593-20, IBIDP598-20, IBIDP601-20, IBIDP603-20, IBIDP604-20, IBIDP662-20, IBIDP672-20, IBIDP673-20, IBIDP674-20, IBIDP675-20, IBIDP676-20, IBIDP714-20, IBIDP723-20 | BOLD: AE C7552 | MZ196610, MZ196538, MZ196806, MZ196927, MZ196529, MZ196566, MZ196684, MZ196764, MZ196656, MZ196643, MZ196818, MZ196533, MZ196791, MZ196546, MZ196582, MZ196820 |
| Tipulidae (Tipulinae) | *Tipula vernalis* Meigen, 1804 | INV06345, INV06347                                                        | IBIDP277-19, IBIDP278-19 | BOLD: AAD2491 | MZ196540, MZ196666 |
| Tipulidae (Tipulinae) | *Tipula vittata* Meigen, 1804 | INV09301                                                                  | IBIDP745-20   | BOLD: AEE0656 | MZ196679    |
| Tipulidae (Tipulinae) | *Tipula yerburyi* Edwards, 1924 | INV09325                                                                  | IBIDP763-20   | BOLD: AEE8951 | MZ196848    |
| Tipulidae (Tipulinae) | *Tipula zarcoi* Mannheims, 1967 | INV04603, INV06020, INV06023, INV06269, INV06271, INV06280, INV06283, INV06284, INV08477 | IBIDP198-19, IBIDP233-19, IBIDP236-19, IBIDP261-19, IBIDP263-19, IBIDP272-19, IBIDP274-19, IBIDP275-19, IBIDP606-20 | BOLD: ADX0912 | MZ196784, MZ196654, MZ196846, MZ196711, MZ196637, MZ196785, MZ196602, MZ196535, MZ196650 |
Project description

**Title:** The InBIO Barcoding Initiative Database: DNA barcodes of Portuguese Diptera 02 - Limoniidae, Pediciidae and Tipulidae

**Personnel:** Pedro Beja (project coordinator), Sónia Ferreira (taxonomist and IBI manager), Joana Paupério (IBI manager), Pedro Sousa (project technician), Vanessa Mata (contributor) and Luis P da Silva (contributor), all affiliated to CIBIO-InBIO; Pjotr Oosterbroek (taxonomist), affiliated to Naturalis and Jaroslav Starý (taxonomist), affiliated to Olomouc-Nedvězí & Silesian Museum.

**Study area description:** Portugal, including the Autonomous Regions of the Azores and of Madeira (Fig. 2).

![Figure 1. Examples of the diversity of species that are part of the dataset of distribution data and DNA barcodes of Portuguese Diptera 02. All photos by Rui Andrade.](image)

- **a:** Phylidorea ferruginea (Meigen, 1818) - BIN URI [BOLD:ABW4832](https://www.boldsystems.org/bold/?r=S5QXOOGQH5) doi
- **b:** Symplecta stictica (Meigen, 1818) - BIN URI [BOLD:ADN4631](https://www.boldsystems.org/bold/?r=95E929PS51) doi
- **c:** Nephrotoma luteata (Meigen, 1818) - BIN URI: [BOLD:ADW1410](https://www.boldsystems.org/bold/?r=8538C9Q921) doi
- **d:** Tipula repanda Loew, 1864 - BIN URI: [BOLD:AEC7761](https://www.boldsystems.org/bold/?r=798229Q041) doi
**Design description:** Tipuloidea specimens were collected in the field, morphologically identified and DNA barcoded.

**Funding:** This project was funded by European Union’s Horizon 2020 Research and Innovation Programme under grant agreement No 668981 and by project PORBIOTA - Portuguese E-Infrastructure for Information and Research on Biodiversity (POCI-01-0145-FEDER-022127), supported by Operational Thematic Program for Competitiveness and Internationalization (POCI), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FEDER). The fieldwork benefited from EDP Biodiversity Chair, the project “Promoção dos serviços de ecossistemas no Parque Natural Regional do Vale do Tua: Controlo de Pragas Agrícolas e Florestais por Morcegos”, funded by the Agência de Desenvolvimento Regional do Vale do Tua and includes research conducted at the Long Term Research Site of Baixo Sabor (LTER_EU_PT_002). LPdS and SF were supported by individual research contracts (CEECIND/02064/2017, 2020.03526.CEECIND), funded by FCT. The work of JS was funded by the Ministry of Culture of the Czech Republic through institutional financing of long-term conceptual development of the Silesian Museum Research Institution (MK000100595).

**Sampling methods**

**Study extent:** Portugal, including the Autonomous Regions of the Azores and of Madeira.

**Sampling description:** The studied material was collected in 83 different localities from Portugal, 77 from continental Portugal and six from the Autonomous Regions of the Azores and of Madeira. The Bragança District was the most heavily sampled (21% of total specimens) and where most species were recorded, with almost half of the species (41%) in the dataset found there (Fig. 2, Table 2). Sampling was conducted between 2003 and
2019, although the vast majority of specimens were collected in 2018 (32%) and 2019 (50%). Specimens were collected by direct search and individual netting of specimens, by sweeping the vegetation or were directly collected at light traps (using both UV and mercury vapour lights) and stored in 96% ethanol for downstream molecular analysis, unless stated otherwise.

| District or Region | Specimens (n) | Specimens (%) | Taxa (n) | Taxa (%) |
|--------------------|---------------|---------------|----------|----------|
| Bragança           | 88            | 21.4          | 35       | 42.2     |
| Faro               | 41            | 10.0          | 29       | 34.9     |
| Castelo Branco     | 38            | 9.2           | 19       | 22.9     |
| Porto              | 35            | 8.5           | 15       | 18.1     |
| Vila Real          | 33            | 8.0           | 14       | 16.9     |
| Guarda             | 32            | 7.8           | 20       | 24.1     |
| Setúbal            | 27            | 6.6           | 10       | 12.0     |
| Santarém           | 23            | 5.6           | 11       | 13.3     |
| Beja               | 20            | 4.9           | 9        | 10.8     |
| Leiria             | 17            | 4.1           | 9        | 10.8     |
| Coimbra            | 16            | 3.9           | 9        | 10.8     |
| Lisboa             | 16            | 3.9           | 5        | 6.0      |
| Viana do Castelo   | 12            | 2.9           | 7        | 8.4      |
| Aveiro             | 7             | 1.7           | 4        | 4.8      |
| Madeira            | 5             | 1.2           | 5        | 6.0      |
| Azores             | 1             | 0.2           | 1        | 1.2      |
| No data            | 1             | 0.2           | 1        | 1.2      |
| **TOTAL**          | **412**       |               | **83**   |          |

Table 2.
Number of specimens and species collected per Portuguese District or Autonomous Region and corresponding percentage.

DNA extraction and sequencing followed the general pipeline in use by the IBI. Genomic DNA was extracted from leg tissue using the EasySpin Genomic DNA Tissue Kit (Citomed) according to the manufacturer’s protocol. The cytochrome c oxidase I (COI) barcoding fragment was then amplified as two overlapping fragments (LC and BH), using two sets of primers: LCO1490 (Folmer et al. 1994) + III_C_R (Shokralla et al. 2015) and III_B_F (Shokralla et al. 2015) + HCO2198 (Folmer et al. 1994), respectively. The COI barcode (Folmer region) was then sequenced in a MiSeq benchtop system. OBITools (https://
git.metabarcoding.org/obitools/obitools) was used to process the initial sequences which were then assembled into a single 658 bp fragment using Geneious 9.1.8. (https://www.geneious.com).

**Quality control:** All DNA barcodes sequences were compared against the BOLD database and the top 99 hits were inspected to detect possible problems arising from contaminations or misidentifications. The data were checked for errors and inconsistencies with OpenRefine 3.4 (http://openrefine.org) before submission to GBIF.

**Step description:**

1. Specimens were collected in 83 different Portuguese localities. Fieldwork was carried out between 2003 and 2019, with 82% of the records made in the years 2018 and 2019.
2. Specimens were collected during fieldwork by direct search and individual netting of specimens, by sweeping the vegetation or were directly collected at light traps (using both UV and mercury vapour lights) and preserved in 96% alcohol. The majority of captured specimens were deposited in the IBI reference collection at CIBIO (Research Center in Biodiversity and Genetic Resources).
3. All specimens were morphologically identified using the available literature, except seven that were identified using the BOLD Identification Engine. For some specimens, it was necessary to prepare and then exam their terminalia.
4. All specimens were DNA barcoded. To sequence the 658 bp COI DNA barcode fragment, one leg was removed from each individual, DNA was extracted and then amplified. All DNA extracts were deposited in the IBI collection.
5. All sequences in the dataset were submitted to BOLD and GenBank databases and, to each sequenced specimen, the morphological identification was contrasted with the results of the BLAST of the newly-generated DNA barcodes in the BOLD Identification Engine.
6. Prior submission to GBIF, data were checked for errors and inconsistencies with OpenRefine 3.4 (http://openrefine.org/).

**Geographic coverage**

**Description:** Continental Portugal, Autonomous Regions of the Azores and of Madeira.

**Coordinates:** 32.65 and 41.97 Latitude; -25.51 and -6.34 Longitude.

**Taxonomic coverage**

**Description:** The dataset is composed of data relating to 412 specimens of Diptera, all from the Tipuloidea superfamily. All specimens were morphologically identified to species or subspecies level by Pjotr Oosterbroek and/or Jaroslav Starý, except for seven specimens identified using the BOLD Identification Engine. In total, 83 species and two subspecies are represented in the dataset. These species belong to three families,
Limoniidae, Pediciidae and Tipulidae. Limoniidae and Tipulidae account for similar numbers of collected specimens, 197 (48%) and 211 (51%) (Fig. 3A), respectively, although Limoniidae have a higher proportion of recorded species in the dataset (45 species - 54% of the total) when compared with Tipulidae (36 species - 43%) (Fig. 3B). At the subfamily level, Tipulinae and Limoniinae represented the most collected specimens (50% and 29%, Fig. 3A) and also the highest number of recorded species in the dataset (41% and 29%, Fig. 3B). The species, represented in this dataset, correspond to about 55% of all the crane fly species known from Portugal (52% Limoniidae, 66% Pediciidae and 57% Tipulidae) and 22% of crane fly species known from the Iberian Peninsula.

![Figure 3.](image_url)

Number of specimens (A) and species (B), per Tipuloidea (Diptera) family and subfamily present in the dataset. Pie chart depicts family only data.

**Taxa included:**

| Rank         | Scientific Name  |
|--------------|------------------|
| kingdom      | Animalia         |
| phylum       | Arthropoda       |
| class        | Insecta          |
| order        | Diptera          |
| superfamily  | Tipuloidea       |
| family       | Limoniidae       |
| family       | Pediciidae       |
| family       | Tipulidae        |
| subfamily    | Chioneinae       |
subfamily Ctenophorinae
subfamily Dactylolabinae
subfamily Dolichopezinae
subfamily Limnophilinae
subfamily Limoniinae
subfamily Pediicininae
subfamily Tipulinae

Temporal coverage

Data range: 2003-9-08 - 2019-10-04.

Notes: The sampled material was collected in the period from 8 September 2003 to 14 October 2019.

Usage licence

Usage licence: Other

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Data resources

Data package title: The InBIO Barcoding Initiative Database: DNA barcodes of Portuguese Diptera 02 - Limoniidae, Pediicidae and Tipulidae

Resource link: dx.doi.org/10.5883/DS-IBIDP02

Number of data sets: 1

Data set name: DS-IBIDP02 IBI Diptera 02

Download URL: http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-IBIDP02

Data format: dwc, xml, tsv, fasta

Description: The InBIO Barcoding Initiative Database: DNA barcodes of Portuguese Diptera 02 - Limoniidae, Pediciidae and Tipulidae dataset can be downloaded from the Public Data Portal of BOLD (dx.doi.org/10.5883/DS-IBIDP02) in different formats (data as dwc, xml or tsv and sequences as fasta files). BOLD users can also log-in and access the dataset through the Workbench platform of BOLD. All records are also discoverable within BOLD, using the platform search function.
The InBIO Barcoding Initiative will continue to sequence crane flies and other Diptera for the BOLD database, with the ultimate objective of achieving a comprehensive coverage of the Portuguese fauna. The version of the dataset, at the time of writing the manuscript, is included as Suppl. materials 1, 2, 3 in the form of two text files with specimen data, as downloaded from BOLD and from GBIF (the latter in Darwin Core Standard format) and one fasta file containing all sequences as downloaded from BOLD.

The BOLD database is not completely compliant with the Darwin Core Standard (DCS) format and, as such, the Darwin Core formatted file (dwc) downloaded from the BOLD platform is not strictly DCS formatted. For a correctly DCS formatted file, see http://ipt.gbif.pt/ipt/resource?r=ibi_diptera_02&v=1.6 (Suppl. material 2).

Column labels below follow the labels downloaded in the tsv file downloaded from BOLD. Columns with no content in our dataset are left out in the list below.

| Column label     | Column description                                      |
|------------------|---------------------------------------------------------|
| processid        | Unique identifier for the sample                        |
| sampleid         | Identifier for the sample being sequenced, i.e. IBI catalogue number at Cibio-InBIO, Porto University. Often identical to the "Field ID" or "Museum ID" |
| recordID         | Identifier for specimen assigned in the field           |
| catalognum       | Catalogue number                                        |
| fieldnum         | Field number                                            |
| institution_storing | The full name of the institution that has physical possession of the voucher specimen |
| bin_uri          | Barcode Index Number system identifier                  |
| phylum_taxID     | Phylum taxonomic numeric code                           |
| phylum_name      | Phylum name                                              |
| class_taxID      | Class taxonomic numeric code                             |
| class_name       | Class name                                               |
| order_taxID      | Order taxonomic numeric code                             |
| order_name       | Order name                                               |
| family_taxID     | Family taxonomic numeric code                            |
| family_name      | Family name                                              |
| subfamily_taxID  | Subfamily taxonomic numeric code                         |
| subfamily_name   | Subfamily name                                           |
| genus_taxID      | Genus taxonomic numeric code                             |
| genus_name       | Genus name                                               |
| species_taxID | Species taxonomic numeric code |
|--------------|-------------------------------|
| species_name | Species name                  |
| subspecies_taxID | Subspecies taxonomic numeric code |
| subspecies_name | Subspecies name               |
| identification_provided_by | Full name of primary individual who assigned the specimen to a taxonomic group |
| identification_method | The method used to identify the specimen |
| voucher_status | Status of the specimen in an accessioning process (BOLD controlled vocabulary) |
| tissue_type | A brief description of the type of tissue or material analysed |
| collectors | The full or abbreviated names of the individuals or team responsible for collecting the sample in the field |
| lifestage | The age class or life stage of the specimen at the time of sampling |
| sex | The sex of the specimen |
| lat | The geographical latitude (in decimal degrees) of the geographic centre of a location |
| lon | The geographical longitude (in decimal degrees) of the geographic centre of a location |
| elev | Elevation of sampling site (in metres above sea level) |
| country | The full, unabbreviated name of the country where the organism was collected |
| province_state | The full, unabbreviated name of the Province ("Distrito" in Portugal) where the organism was collected |
| region | The full, unabbreviated name of the Municipality ("Concelho" in Portugal) where the organism was collected |
| exactsite | Additional name/text description regarding the exact location of the collection site relative to a geographic relevant landmark |

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Supplementary materials

**Suppl. material 1: IBI - Diptera 02 library - Specimen details**

**Authors:** Pedro Sousa, Pjotr Oosterbroek, Jaroslav Stary, Vanessa A Mata, Luis P. da Silva, Pedro Beja, Sónia Ferreira  
**Data type:** Specimen data records  
**Brief description:** The file includes information about all records in BOLD for the IBI - Diptera 02 library. It contains collecting and identification data. The data are as downloaded from BOLD in the tsv format, without further processing.  
[Download file](174.12 kb)

**Suppl. material 2: IBI - Diptera 02 library - Specimen details - Darwin Core Standard**

**Authors:** Pedro Sousa, Pjotr Oosterbroek, Jaroslav Stary, Vanessa A Mata, Luis P. da Silva, Pedro Beja, Sónia Ferreira  
**Data type:** Specimen data records in the Darwin Core Standard format  
**Brief description:** The file includes information about all records in GBIF for the IBI - Diptera 02 library. It contains collecting and identification data. The data are as downloaded from GBIF, without further processing.  
[Download file](439.79 kb)

**Suppl. material 3: IBI- Diptera 02 library - DNA sequences**

**Authors:** Pedro Sousa, Joana Paupério, Pedro Beja, Sónia Ferreira  
**Data type:** Specimen genomic data, DNA sequences  
**Brief description:** COI sequences in fasta format. Each sequence is identified by the BOLD ProcessID, species name, genetic marker name and GenBank accession number, all separated by a vertical bar. The data are as downloaded from BOLD.  
[Download file](284.76 kb)