First record of *Physyphora alceae* (Preyssler, 1791) (Diptera, Ulidiidae) from a forensic case in Northern Italy: description of immature stages, DNA barcoding and phylogenetic analysis

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Abstract

In forensic entomology the estimation of the minimum postmortem interval and any other evaluation depend on the accuracy of the species identification. Despite the most common species received a lot of attention in the last years, other species belonging to less common families still remain poorly studied and sometimes their immature stages are not described. The finding of *Physyphora alceae* (Preyssler, 1791) (Diptera, Ulidiidae) in a decapitated cadaver in Northern Italy allowed the description of the III instar larvae and of the puparium of this species. Further molecular analysis allowed the confirmation of the correct species identification and the monophyly of the genus. In general, phylogenetic analysis confirmed the monophyly of the family, however some issues arose on the real monophyly of the genus *Homalocephala*. This paper highlights the need of a combination of morphological and molecular identification of the species especially when immatures are collected.

Keywords: Forensic entomology, body colonization, burial, Diptera

Introduction

In forensic entomology a correct species identification is the *conditio sine qua non* to answer the investigative questions concerning the peri and post mortem events. To achieve this goal, the morphological approach involving the use of dichotomous keys is the traditional and most used method among the taxonomists. However, sometimes it can be obstructed by the lack of experts and by the limited information concerning the immature stages (i.e. larvae and puparia) (Giordani et al. 2018, 2019; Giordani & Vanin 2020). Nevertheless, even if specific identification keys are available, a poor state of preservation of the specimens may negatively affect the morphological analysis thus preventing the accurate identification of the samples (Bortolini et al. 2018; Pradelli et al. 2019).

To overcome such circumstances, from the second half of the 1980s, molecular biology has become a support tool for species identification based on the sequencing of genetic targets proved to be conserved enough to allow the design of universal and specific primers (Folmer et al. 1994), and diverse enough to allow species discrimination.

It is worth mentioning that much research has been dedicated to the most common taxa of forensic interest, especially in the families Calliphoridae, Sarcophagidae and Muscidae, whereas little effort has been directed towards less common taxa (Tuccia et al. 2016a). These “secondary taxa”
collected from a corpse could as well provide important information (Smith 1986; Sessa et al. 2019; Giordani et al. 2019b; Giordani & Vanin 2020).

This paper provides the description of the immature stages of the “picture-winged fly” Physiphora alcea (Preysler, 1791) (Diptera: Ulidiidae) collected from a human cadaver found in Northern Italy. This work wants to contribute in reducing the risk of misidentification of this species that has been reported from carcasses from all over the world (Figure 1) but has been poorly investigated in its forensic applications. The phylogenetic reconstruction for identification purposes based on molecular analysis is also provided.  

Case description

At the beginning of September 2017, the decapitated body of a woman, that was reported missing two months before, was found in a small town within the metropolitan area of Milan (Northern Italy) (Figure 2) in a urban context surrounded by forest and agricultural areas. The body was beheaded, buried in an artificial grave and covered with plastic sheets. A few days later, the missing skull was found in a plastic bag a few kilometers far from the body. Both parts of the cadaver were found in advance decay and partially skeletonized (Caccianiga et al. 2020; Mazzarelli et al. 2021). The analysis of the insect communities revealed two different colonisation patterns, one for the head composed by species in the families Calliphoridae, Muscidae, Fanniidae and Phoridae whereas the body was colonised by species belonging to the Muscidae, Stratiomyidae, Phoridae and Ulidiidae families.

The absence of Calliphoridae, first colonisers of exposed cadavers, suggested the hypothesis of the concealing of the cadaver after the death. Based on the temperature and on the developmental rates of the necrofagous insects collected, an estimation of a minimum Post Mortem Interval (minPMI) in the range of 35–46 days was provided.

Materials and methods

Sample preparation and morphological identification

The adults and the immature stages of the flies (Diptera) were analysed using a Keyence VHX-S90BE digital microscope, equipped with Keyence VH-Z250R and VH-Z20R lens and VHX-2000 Ver. 2.2.3.2 software (Keyence, Japan).

Identification keys from Kameneva and Korneyev (2010) were used to investigate the morphology of the Ulidiidae adults’ specimens, further confirmed

Figure 1. Records of P. alcea and other Ulidiidae sampled during experiments carried out for forensic purposes (world map from © 2007–2018 d-maps.com).
by comparison with the collection of the NHM of London and through molecular analysis.

In order to better visualise the diagnostic characters, larvae were diaphanised using a NaOH solution (Tuccia et al. 2016b). As no DNA analysis were intended to be performed on puparia, they were carefully cleaned in acetic acid solution and air-dried at room temperature (Pradelli et al. 2021). The description of the external characters follows the nomenclature as in Giordani et al. (2018) while the morphology of the cephalopharyngeal skeleton follows McAlpine et al. (1987) and Bjerke et al. (1992).

Molecular identification

Total DNA was extracted from larval and adult specimens using the DNA Investigator Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Prior to extraction, the larva was processed as described in Tuccia et al. (2016b) and incubated overnight in a solution of Proteinase K (0.1 mg/ml final concentration) (Promega, Madison, Wisconsin, USA) and ATL Buffer. A region of the standard barcoding region of the mitochondrial COI gene (658 bp) was amplified using universal LCO-1490 and HCO-2198 primers (Folmer et al. 1994). A total reaction volume of 20 μl was prepared using Promega GoTaq® Flexi Polymerase protocol: 4 μl of Colourless GoTaq Flexi Buffer (5×), 2 μl of MgCl2 (25 mM), 0.5 μl of each primer (10 pmol/μl), 0.5 μl of dNTPs Mix (10 mM), 0.25 μl GoTaq DNA Polymerase (5 u/μl) and 2 μl of DNA template. The reactions, including positive and negative controls, were assembled under a Purair PCR-36 laminar flow cabinet (AirScience®, Florida, USA) in order to prevent cross contaminations between samples.

The thermal cycler BioRad C1000 (Bio-Rad Laboratories, Inc.) was used to perform the amplification setting the following program: initial heat activation step at 95°C for 10 min, 35 cycles of 95°C for 1 min, 49.8°C for 1 min, 72°C for 1 min and a final extension step at 72°C for 10 min. Positive and negative DNA templates were used as controls for the
reaction. The quality of the reactions was qualitatively assessed by 1.5% (w/v) agarose gel electrophoresis stained with Modi Green DNA Stain (Geneflow Ltd., Lichfield, UK). Amplicons were purified using QIAquick PCR Purification Kit® (Qiagen, Hilden, Germany) following the manufacturer’s instructions, eluted in 40 μl of EB and sequenced by Eurofins Genomics (Ebersberg, Germany). The identity of the sequences was searched by BLAST® (Altschul et al. 1990). The nucleotide sequences generated have been uploaded on GenBank [access number: MH686505, MH686506].

**Phylogenetic analysis**

Fifty-four mtCOI sequences of Ulidiidae (Diptera) with a length over 580 bp were downloaded from BOLD and GenBank databases including 20 sequences of the subfamily Ulidiinae and 34 sequences of the subfamily Otitinae. The same species analysed by Galinskaya and collaborators (2014) in their study of the phylogeny of the family were chosen. Within Ulidiinae, 4 species belonging to Physiphora Fallén, 1810, 2 species belonging to Ulidia Meigen, 1826, 3 species belonging to Timia Wiedemann, 1824, and 3 species belonging to Homalocephala were selected (Tables I – III). Within Otitinae, 3 species belonging to Ceromyia Macquart 1835, 2 species of Otites Latreille, 1804, 2 species belonging to Herina Robineau-Desvoidy, 1830, 4 species belonging to Meliera Becker in 1903, and the species Myennis octopunctata (Coquebert, 1798), Pseudonephris corticalis (Loew, 1873), Seioptera Vibrans (Linnaeus, 1758), and Tetanops Sinensis Becker, 1909 were chosen (Table I). In addition, Psila Fimetaria (Linnaeus, 1761) and three species of Drosophila Fallén, 1823 (Drosophila melanogaster Meigen, 1830, Drosophila simulans Sturtevant, 1919, and Drosophila viriles Sturtevant, 1916) were chosen as outgroups (Galinskaya et al. 2014).

Two sequences of P. alceae obtained from this work were included to create a final dataset of 61 sequences (Table II) that were aligned using the ClustalW software in MEGA7 (Kumar et al. 2016). The GTR+Γ + I G4 model was chosen as substitution model among 286 implementing the ModelFinder tool on IQ-TREE v.1.6.10 (Nguyen et al. 2015). The best-fit model was chosen based on the highest w-AICc (+0.9647) and on the lowest BIC (13107.8044) values and used to build a Maximum Likelihood phylogenetic reconstruction. One thousand bootstrap replicates were inferred setting the ultrafast bootstrap parameter (Hoang et al. 2018).

**Results**

Six dipteran species were identified within the entomological assemblage collected from the cadaver of the woman (Table III), however, according to the purposes of this article only Physiphora alceae (Peyssler, 1791) (Ulidiinae) is described in detail being recorded from a cadaver in Italy for the first time. The other taxa are already known from cadavers and reported from Italian forensic cases or archaeological contexts (Turchetto et al. 2001; Turchetto & Vanin 2004; Bugelli et al. 2015; Giordani et al. 2018, 2019a, 2019b; Giordani & Vanin 2020).

**Adult description**

The adults of this species were described in detail by Kameneva and Korneyev (2010). Adult flies can be easily distinguished from the other species because of the colour of their legs, the wing shape and the

| Table I. Species of Ulidiidae used in the phylogenetic analysis. |
|---------------------------------|
| **Subfamily Ulidiinae** | **Subfamily Otitinae** |
| Physiphora alceae | Ceromyia urticae (Linnaeus, 1758) |
| Physiphora aenea (Fabricius, 1794) | Ceromyia mundula (Loew, 1868) |
| Physiphora clausa (Macquart, 1843) | Ceromyia cinfera (Loew, 1846) |
| Physiphora demandata (Fabricius, 1798) | Otites laudi (Schrank, 1781) |
| Ulidia megacephala Loew, 1845 | Otites formosa (Panzer, 1798) |
| Ulidia rugicaps Becker, 1913 | Herina lugubris (Meigen, 1826) |
| Timia monticola Becker, 1906 | Herina nigribasis McAlpine, 1951 |
| Timia erythrocephala Wiedemann, 1824 | Meliera cana (Loew, 1858) |
| Timia libani Gregor, 1970 | Meliera omisa (Meigen, 1826) |
| Homalocephala angustata (Wahlberg, 1838) | Meliera acuticorinis (Loew, 1854) |
| Homalocephala apicalis (Wahlberg, 1838) | Meliera crasipennis (Fabricius, 1794) |
| Homalocephala albitarsis Zetterstedt, 1838 | |
Table II. mtCOI barcoding sequences used for phylogenetic analysis. Sequences are listed per species, BOLD/GenBank accession number, and geographical origin. * = sequences from this study.

| Subfamily | Species                     | BOLD accession number | GenBank accession number | Origin          |
|-----------|-----------------------------|-----------------------|--------------------------|-----------------|
| Ulidiinae | Physiphora alceae*          | MH686505*             | Northern Italy           |
| Ulidiinae | Physiphora aenea*           | MH686506*             | Northern Italy           |
| Ulidiinae | Physiphora aenea            | DIQT470-09            | Australia                |
| Ulidiinae | Physiphora aenea            | DIQT341-11            | Australia                |
| Ulidiinae | Physiphora aenea            | DIQT344-11            | Australia                |
| Ulidiinae | Physiphora clausa           | GBDP15226-14          | India                    |
| Ulidiinae | Physiphora clausa           | GBDP15297-14          | Russia                   |
| Ulidiinae | Physiphora clausa           | TGS PA085-07          | Russia                   |
| Ulidiinae | Physiphora demandata        | JSDIS042-11           | Canada                   |
| Ulidiinae | Ulidia megacephala          | GBDPT992-14           | Russia                   |
| Ulidiinae | Ulidia ruficeps             | GBDPT993-14           | Russia                   |
| Ulidiinae | Timia monticola             | GBDP15299-14          | Russia                   |
| Ulidiinae | Timia erythrocephala        | TGS PA80-07           | Russia                   |
| Ulidiinae | Timia erythrocephala        | TGS PA81-07           | Russia                   |
| Ulidiinae | Timia libani                | GBDP15298-14          | Russia                   |
| Ulidiinae | Homalocephala angustata     | TGS PA066-07          | Russia                   |
| Ulidiinae | Homalocephala angustata     | TGS PA067-07          | Russia                   |
| Ulidiinae | Homalocephala angustata     | TGS PA068-07          | Russia                   |
| Ulidiinae | Homalocephala angustata     | TGS PA069-07          | Russia                   |
| Ulidiinae | Homalocephala apicalis      | TGS PA062-07          | Russia                   |
| Ulidiinae | Homalocephala albitarsis    | TGS PA069-07          | Russia                   |
| Otitinae  | Cercoxys urticae            | TGS PA011-07          | Russia                   |
| Otitinae  | Cercoxys urticae            | TGS PA012-07          | Russia                   |
| Otitinae  | Cercoxys urticae            | TGS PA022-07          | Russia                   |
| Otitinae  | Cercoxys mundae             | TGS PA016-07          | Moldova                  |
| Otitinae  | Cercoxys cinifera           | TGS PA018-07          | Kyrgyzstan               |
| Otitinae  | Cercoxys cinifera           | TGS PA019-07          | Kyrgyzstan               |
| Otitinae  | Otitites lamed              | TGS PA008-07          | Ukraine                  |
| Otitinae  | Otitites lamed              | TGS PA010-07          | Ukraine                  |
| Otitinae  | Otitites formosa            | GGM GI330-14          | Germany                  |
| Otitinae  | Otitites formosa            | GGM GI1243-14         | Germany                  |
| Otitinae  | Otitites formosa            | TGS PA007-07          | Russia                   |
| Otitinae  | Otitites formosa            | GGM GI005-07          | Russia                   |
| Otitinae  | Herina nigribiasis          | BARSC297-16           | Canada                   |
| Otitinae  | Herina nigribiasis          | JECDG252-10           | Canada                   |
| Otitinae  | Herina lagubris             | TGS PA053-07          | Russia                   |
| Otitinae  | Herina lagubris             | TGS PA054-07          | Russia                   |
| Otitinae  | Herina lagubris             | TGS PA056-07          | Russia                   |
| Otitinae  | Maliera crusipennis         | TGS PA041-07          | Russia                   |
| Otitinae  | Maliera crusipennis         | TGS PA042-07          | Russia                   |
| Otitinae  | Maliera cana                | CNWBG2660-13          | Canada                   |
| Otitinae  | Maliera cana                | TGS PA038-07          | Kyrgyzstan               |
| Otitinae  | Maliera cana                | TGS PA039-07          | Russia                   |
| Otitinae  | Maliera omissa              | TGS PA031-07          | Turkmenistan             |
| Otitinae  | Maliera acuticornis         | TGS PA043-07          | Russia                   |
| Otitinae  | Maliera acuticornis         | TGS PA045-07          | Russia                   |
| Otitinae  | Myennis octopunctata        | TGS PA028-07          | Russia                   |
| Otitinae  | Myennis octopunctata        | TGS PA029-07          | Russia                   |
| Otitinae  | Pseudopenis corticis        | TGS PA046-07          | Russia                   |
| Otitinae  | Seioptera vibrans           | JSDIS277-11           | Canada                   |
| Otitinae  | Seioptera vibrans           | OPPF A325-17          | Canada                   |
| Otitinae  | Tetanops sintensis          | TGS PA073-07          | Russia                   |
| Otitinae  | Tetanops sintensis          | TGS PA074-07          | Russia                   |
| Outgroup  | Drosophila melanogaster     | DIQT B5104-12         | Australia                |
| Outgroup  | Drosophila simulans         | BBDCQ289-10           | Canada                   |
| Outgroup  | Drosophila virilis          | GBDP2524-06           | United Kingdom           |
| Outgroup  | Phila fimentaria            | TGS PA086-07          | Russia                   |
| Outgroup  | Phila fimentaria            | TGS PA087-07          | Russia                   |
| Outgroup  | Phila fimentaria            | TGS PA088-07          | Russia                   |
| Outgroup  | Phila fimentaria            | TGS PA089-07          | Russia                   |
Table III. Diptera species from the crime scenes. Pc = puparium closed, Po = puparium open, LIII = third instar larva, A = adult.

| family        | species                  | body               | head               |
|---------------|--------------------------|--------------------|--------------------|
| Fanniidae     | *Fannia scalaris*        | Pc, Po             |                    |
| Calliphoridae | *Chrysomyia albiceps*    |                    | Pc, Po             |
| Muscidae      | *Hydrotæa capensis*      |                    | LIII, Pc           |
|               | *Muscina sp.*            |                    | Po                 |
| Phoridae      | Gen. sp.                 |                    | L, Pc, Po          |
| Stratiomyidae | *Hermetia illucens*      |                    | L, Pc, Po          |
| Ulidiidae     | *Physiphora alceae*      |                    | L, Pc, Po, A       |

microtrichose pattern on the frons (Figure 3(a–c)). The legs are black except for the foreleg with creamy-yellow metatarsus (Figure 3(b)), midtarsus and hindtarsus (Figure 3(c)).

Wings are totally hyaline with the cell r4 + 5 almost closed similar to *Physiphora longicornis* (Hendel, 1909) (Chen & Kameneva 2007) (Figure 4(a)). Two narrow microtrichose patches on each side of the frons represent a diagnostic character of the species (Figure 4(b)).

III instar larva description

The body length of third instar larva in the analysed sample was 8.2 ± 0.3 mm (N = 3) (Figure 5(a–d)). Anterior spiracle is composed by short, flattened stalk bearing 13–15 nodular branches ranged fan-wise with in some specimens a slight median introflexion (Figure 5(a)). The posterior spiracles, not protruding from the spiracular disc, are very close to each other and show an ovoid-like shape with a thin, dark-brown and close peritreme (difficult to see in the diaphanised specimens) (Figure 5(c)). The spiracular scar is not included in the peritreme and is located in the dorsal side of each spiracle. The three respiratory slits have an arcuate radial arrangement and converge towards the spiracular scar. Slits one and two are specular and slightly curving one towards the other at the lower extremity (Figure 5(c,d)).

The cephalopharyngeal skeleton varies from light to dark brown in colour and measures, in the analysed specimens 0.85 and 0.28 mm, as maximum length and maximum width respectively (Figures 5(b), 6). The mandibles, well sclerotised, have two sharply hooked teeth and a sharp robust dental sclerite. The basal part of the mandible has a robust process postero-dorsally oriented. The parastomal bar and hypopharyngeal sclerite are elongated and slender. Dorsal cornu of the tentoropharyngeal sclerite is arched, slender, large 1/3 of the ventral cornu, without window. The dorsal (anterior) bridge is robust and well sclerotised. The ventral part of the ventral cornu is straight (Figure 6).

Figure 3. *Physiphora alceae* adult. (a) dorsal, (b) lateral, (c) ventral view. The black arrow indicates the foreleg with the creamy-yellow metatarsus. Scale bar: 1 mm.

Figure 4. *Physiphora alceae* adult details. (a) Wing showing the r4 + 5 cell almost closed and (b) frontal view head. The white arrow indicates the typical narrow white microtrichose patches. Scale bar: (a) 500 μm, (b) 100 μm.
Puparium description

Puparium. Ochre in colour, measures 4–5 mm in length (Figure 7(a–c)). The anterior extremity of the puparium seen from a lateral view shows a tapered shape (Figure 7(c)). Posterior spiracles are very close (Figure 7(d)). The same characters observed for the III instar larva are also well conserved in the puparium morphology: oval-rounded posterior spiracles, spiracle scar in the dorsal side and excluded by the peritreme which is complete.
thick and varies from dark-brown to black in colour, radial oriented respiratory slits (Figure 7(d)). The anterior spiracles are composed of twelve yellowish finger-like lobes. The intersegmental spines of ventral well of the seventh abdominal segment are distributed in six ordinate and continuous rows except for the third one where spines are grouped by five or six (Figure 7(e)). The majority of the spines extend forward towards the anal plate but some others are back oriented. In the two rows closest to the anal division spines are large, generally with single rounded tip while finer, shorter and sharp tipped spines form the remaining rows (Figure 7(f)).

Molecular analysis and phylogenetic reconstruction of the Ulidiidae

The morphological identification was confirmed by the sequencing of a region of the COI barcoding target. The ClustalW alignment produced a dataset of 61 sequences of equal length (520 nucleotide positions) and counting 307 constant sites and 205 informative sites. The ML tree reported in Figure 8 has a Log-likelihood of −6098.9844. The distinction between the two subfamilies Ulidiinae and Otitinae is well supported by the molecular phylogensis (BS: 98). The node between the groups Timia-Ulidia and Physiphora is weakly supported by a BS of 48, and both of them are monophyletic. In the first group, no further separation was observed in regards to superior taxonomic levels (genus, species). Within the subfamily Otitinae, the monophyly of the genera is generally well supported while no further well supported clusters distinction can be argued. Additionally, the genus Homalocephala shows an unresolved position. In fact, sequences of species within this genus cluster both in the Ulidiinae (H. apalis) and in the Otitinae (H. albitarsis and H. angustata) subfamilies, thus showing that the taxonomic resolution of this genus remains unresolved (Figure 8).

Discussions

Ecological and forensic observations

The analysis of the necrophagous insect’s community assemblage found on the cadaver in the metropolitan area of Milan (Italy) revealed the presence of the “picture-winged” fly P. alceae, belonging to the subfamily Ulidiinae, which is mainly a tropical taxon (Marshall 2012).

The genus Physiphora Fallén, 1810 includes 16 species widespread in the tropical, subtropical and temperate areas, with the majority of the species inhabiting the Afrotropical regions (Chen & Kameneva 2007). In particular, P. alceae is distributed in all the biogeographical regions (Kameneva & Korneyev 2010) and in Europe, based on the Fauna Europaea data (https://fauna-eu.org/), it is widespread in the majority of the countries with the exception of the Northern countries such as Iceland, Ireland, Norway, Baltic regions, and the west coast of the Balkanic region (Figure 9). Likely, the absence of P. alceae in the Southern Balkan regions is potentially due to missing records, as it often occurs.

In the last 10 years, P. alceae has been found in association with animal carcasses during experiments conducted on field for forensic purposes, while the Italian record here presented seems to be the first case ever related to a human cadaver (Table IV).

The finding of P. alceae on decomposing corpses is in line with the fact that the larvae develop in dung, decaying vegetable matter and compost
Figure 8. Maximum Likelihood phylogenetic tree. The phylogenetic tree was derived by the alignment of 61 mtCOI barcoding sequences of equal length (520 bp). The displayed topology was obtained upon the selection the best substitution model (Log-likelihood $=-6098.9844$). The evolutionary distance between Ulidiinae and Otitinae is strongly supported (BS = 98). Red arrows point out to the Homalocephala spp.
heaps (Ebejer 2015). Most of the details of the biology of this species, however, still remains unclear. **Physiphora alceae** as member of the family Ulidiidae can be considered as a taxon that is occasionally present in the crime scenes, together with

![Figure 9. European distribution of *P. alceae*. Green: present, white: absent. (Data from Fauna Europaea, https://fauna-eu.org/; figure modified from © 2007–2018 d-maps.com).](image)

Table IV. Records of *P. alceae* and other Ulidiidae sampled during experiments carried out for forensic purposes.

| species          | country    | source                          | references                                             |
|------------------|------------|---------------------------------|--------------------------------------------------------|
| *Physiphora alceae* | Malaysia   | monkey carcasses                | (Rumiza et al. 2010)                                   |
|                  | Egypt/Kuwait | rabbit carcasses                | (Al-Mesbah et al. 2012, Sawaby et al. 2018)            |
| *Physiphora sp.*  | Spain      | squat-baited traps              | (Martin-Vega & Baz 2013, Baz et al. 2015)              |
|                  | Malaysia   | composting piles                | (Morales & Wolff 2010)                                 |
|                  |            | horse dung                      | (Heo et al. 2015)                                      |
| *Pseudexesta prima* | Island of Guam | traps carion baited human faeces | (Bohart & Gressitt 1951)                               |
| *Seiptera sp.*   | Portugal   | traps                            | (Prado e Castro et al. 2011)                          |
| *Ulidiidae*      | Brazil     | decomposing rats                | (Monteiro-Filho & Penereiro 1987)                      |
|                  | Portugal   | swine                           | (Prado e Castro et al. 2012)                          |
other Diptera families, such as Heleomyzidae, Trichoceridae, Psycodidae, Drosophilidae, and Milichiidae (Sessa et al. 2019; Giordani et al. 2019b).

Based on the most recent literature (Table IV), it seems that this fly is becoming more and more interesting in forensic entomology, but its potential role is still under investigation. On one hand the morphology of the adults can deceive, as some of the morphological characters are similar to other close-related species (e.g. Physphora longicornis (Hendel, 1909) (Chen & Kameneva 2007)). However, there are no available descriptions of the immature stages that are typically found on a crime scene or on the body. Only a few descriptions of larvae and puparia of Ulidiidae have been published (Bohart & Glossitt 1951) but none concerns any species of potential forensic interest or anyway associated with carcasses and cadavers.

In light of these observations, the finding of immature stages of *P. alceae* in this case offered the opportunity to accurately describe the morphology of the third instar larva and the puparium. In addition, as completion of this investigation, a molecular approach was used to confirm the morphological identification, as a standard practice during the forensic entomology analysis, and to enrich the public database (the mtCOI barcoding sequences are now available on GenBank with the accession numbers MH686505 and MH686506).

**Phylogenetic analysis**

The molecular results were further corroborated through the phylogenetic approach, mainly referring to the work of Galinskaya et al. (2014) who exhaustively outlined the DNA barcoding of Palearctic Ulidiidae and made a comparison with the morphological data of the family. In contrast to their dataset, a smaller dataset of COI sequences (61) was used, but it was specifically composed of sequences of equal length in order to provide sufficient information for the phylogenetic reconstruction and reduce any potential length bias. In addition, the nucleotide substitution model has been automatically selected using ModelFinder tool on IQ-Tree v.1.6.10 (Nguyen et al. 2015), and, unlike the above mentioned authors, testing different substitutions models fell out of the purposes of this work. Our results strongly support the monophyly of the groups Ulidiinae and Otitinae (BS 98, Figure 8). The observations related to the species separation are in line with what has been already underlined as well as for the genus *Homalocephala*. So far, the taxonomic resolution of this genus has not been well defined (Galinskaya et al. 2014) due to the high similarity of its morphological features shared between both Ulidiinae and Otitinae species. In addition, the phylogenesis based on the barcoding target seems not to resolve this debate among taxonomists, suggesting that other DNA targets should be tested to increase the resolution power of this analysis. According to our results the species *H. angustata* and *H. albitarsis* fall in the cluster of Otitinae while *H. apicalis* is included in the Ulidiinae cluster. However, this cannot be further elucidated due to the limited number of species of *Homalocephala* genus and species sequences (3 and 5, respectively) used in this analysis and available in the databases.

**Conclusions**

The collection of *P. alceae* from a human cadaver in Northern Italy and its findings on animal carcasses used for decomposition experiments support the hypothesis of a role of this species also as carrion-feeders and open a new prospective of its implication for forensic purposes despite further research about its developmental rate and arrive time on a body are needed.

This work highlights also that the identification of flies of forensic interest necessarily requires the morphological analysis of the specimens which can be confirmed and highly supported by the genetic analysis. Also, when dealing with immatures, none of the two approaches is sufficiently reliable to be used independently by excluding the other a priori, therefore it is highly recommended to use them in combination as previous proposed also for other species (Tuccia et al. 2016b).

**Disclosure statement**

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