



Record of *Leptometopa latipes* (Diptera: Milichiidae) from a human cadaver in the Mediterranean area

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ABSTRACT

In forensic entomology, insects are used mainly to obtain information about the time since death. This information is obtained studying the developmental rate of the first colonizers, principally species in the families Calliphoridae, Sarcophagidae, Muscidae, Stratiomyidae and Phoridae. However, species belonging to other families can provide information about body transfer or the season of the death. Among them Milichiidae are flies rarely reported from human cases despite the larvae of some species are known as saprophagous feeding on plant and animal decomposing matter. A potential cause of the lack of records of these species from forensic cases can be related with the paucity of descriptions and illustrations of the immature stages. In this article, the entomological samples collected from a human body found inside an apartment in a Maghreb country, in Northern Africa, is reported and *Leptometopa latipes* (Diptera: Milichiidae) is described in detail. Molecular analysis is also reported to confirm the morphological analysis.

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Introduction

Forensic entomology is a branch of forensic science in which insects are used as evidence in legal investigations relating to humans or wildlife [1]. The examination, identification and analysis of insects associated with human remains, combined with the knowledge of insect biology, distribution, phenology and ecology can provide a further level of detail in addition to medical and anthropological data in the reconstruction of events occurring close to the time of death. In particular, in forensic entomology, necrophagous insects are useful in estimating the minimum postmortem interval (mPMI), the movement of the cadaver after death (body transfer), the season of death, the presence of drugs or poisons [2] and, as reported in the last years, in the identification of the victim in cases of body removal before the investigators' arrival [3].

Flies are typically the first insects to colonize a dead body. The species of flies involved differ from one location to another, but several studies have indicated that the primary species involved belong to a relatively small number of families: Calliphoridae, Sarcophagidae and Muscidae [2]. Later during the decomposition other species belonging to the

Stratiomyidae, Fanniidae, Piophilidae, Phoridae families are often sampled from the body whereas species in the families of Syrphidae, Sphaeroceridae, Heleomyzidae, Sepsidae are only occasionally collected. Other species, in the families of Trichoceridae, Psychodidae, Milichiidae, Ulidiidae and Drosophilidae, are very rare and their presence depends on very specific seasonal, geographical or ecological contexts in addition to the decomposition stage of the body. These late species could provide a very detailed information about the perimortem events, as, for example, body transfer or concealment, despite generally they are useless for the mPMI estimation because the lack of developmental data and their unpredictable arrival on the body. Moreover, the paucity of records of these species can be related with the lack of a correct identification because no detailed identification keys are available for the morphological identification of their immature stages and the incompleteness of the molecular information about these "secondary" species in Genbank or in BOLD [4–6].

The case here reported concerns the finding on a human cadaver in advanced decay of several specimens of *Leptometopa latipes* (Meigen, 1830), a fly species in the family of Milichiidae. A detailed

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description of the puparium and the adult of the species are here presented in order to provide support for the identification of specimens from forensic cases. Molecular analysis is also presented and discussed.

Milichiidae sometimes called “free loader flies” from their kleptoparasitic habits are small little black acalyptrate flies with larvae generally saprophagous, usually developing in decomposing plants and animals but also collected from dung and human excrements or organic matters accumulated inside the nest of ants or other social insects [2].

Case description

In 2006, the corpse of an Italian young man was found inside an apartment in a Maghreb country, in Northern Africa. According to the first autopsy report, the cause of death was intoxication by carbon monoxide. The corpse was transferred to Italy, performing a second postmortem examination; however, the conditions of the remains made impossible determining the cause of death. Furthermore, the relatives asked for new research and analysis to confirm his identity. In 2013, the corpse was exhumed. Then, bone samples were collected, performing the kinship genetic tests. However, due to the poor conditions of the remains, these results were not successful on identity matching. The remains were heavily colonized by insects: large number of puparia and small flies were collected over the bone surface. Entomological samples were sampled, prepared and analysed following the standards and guidelines of the European Association for Forensic Entomology (EAFE) [7] and further analysed in order to obtain information useful for the investigation.

Materials and methods

All the samples were initially observed 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, Osaka, Japan). Puparia samples were carefully cleaned in a water and soap solution and air-dried. No sonication was needed for the observation of the diagnostic characters. Scanning electron microscopy (SEM) observations were performed only on puparia. Dried cleaned specimens were mounted on stubs with conductive adhesive tape and coated with Au-Pd in an SC7620 Mini Sputter Coater (Quorum Technologies, Lewes, UK) and observed with a FEI QUANTA 650 FEG SEM (Thermo Scientific, Waltham, MA, USA). Pictures were directly digitized from the SEM. Terminology of morphological characters in the puparia description follows the most recent works on the topic [8–11].

To further confirm the morphological identification, a molecular DNA extraction from adult flies was performed. A 737 bp sequence of the cytochrome c oxidase subunit I (COI) mitochondrial gene was amplified and sequenced. The online system BLASTn[®] [12] was used for species identification based on the percentage of identity with those available on the online gene banks. The obtained sequence is deposited on GenBank (accession number MH069729). Sequences of some Milichiidae species were downloaded from GenBank and BOLD and included in the analysis (Supplementary material Table S1). In order to increase the number of species in the analysis a region of only 533 bp was considered. Sequences were aligned with Clustal Omega [13]. A phylogenetic tree was built using the Neighbour Joining method on MEGA version 7.0 [14]. A bootstrap of 1 000 replicates was used for testing the robustness of the phylogenetic reconstruction. The tree was visualized with iTOL [15]. In the phylogenetic reconstructions, sequences of *Drosophilidae* species were considered as outgroup.

Results

A large number of puparia and small flies were collected over the bone surfaces, identifying two different species: *Hydrotaea capensis* (Wiedemann, 1818) (Diptera: Muscidae), common species reported from decomposed and buried remains, and unexpectedly, *L. latipes* (Diptera: Milichiidae). The identification of the late species was confirmed as well by molecular analysis. The local alignment of the sequence belonging to adult flies from this case was performed using the online system BLAST[®] (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The output revealed a 99% and 97% identity, respectively, with two sequences coded in Genbank as *L. latipes* KR671912.1 and KR434028.1, with a 0.0 E value. A phylogenetic approach was used to verify the goodness of the molecular result. The tree based on 533 bp of the COI sequence (Figure 1) clearly confirms the morphological identification.

Hydrotaea capensis

The old world native synanthropic species *H. capensis* was recorded from different habitats [16] and its presence is mainly reported in warm seasons. Lefebvre and Pasquerault [17] calculated for this species a minimum developmental temperature of 12.8°C and a thermal constant expressed as Accumulated Degree Days (ADD) of (237.05±22.73) degree-days. *H. capensis* is reported to colonize exposed cadavers during the active decay stage of their decomposition [2,8] while it is also reported as one of the first colonizers in buried bodies [2,18–20].

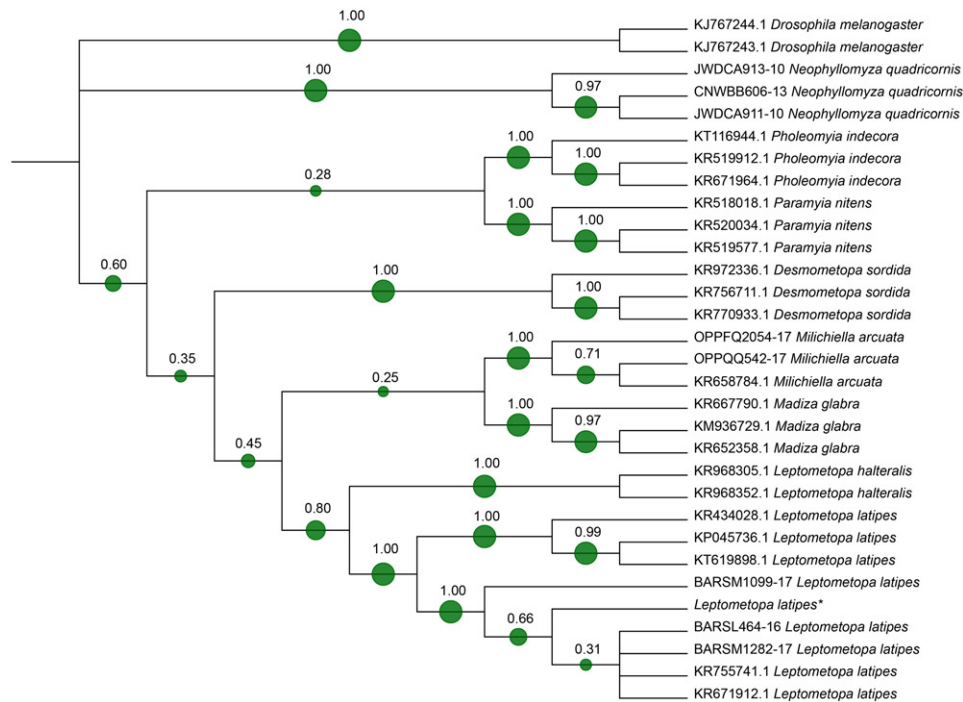


Figure 1. Phylogenetic tree based on Neighbour Joining method analysis of 533 bp sequence of the cytochrome c oxidase subunit I (*COI*) gene. The green spots and the number at each node indicate the bootstrap support. * indicates the sequence from this case (GenBank MH069729).

Puparia of this species were reported from forensic and archaeological contexts all over the Europe (Portugal, Spain, France, Italy, Germany, etc.) [2,18–22]. A detailed puparium description of this species is illustrated in a forthcoming paper by Giordani et al. [8].

Leptometopa latipes

This species has a widespread distribution in Europe, Asia, Africa and in the Nearctic and Neotropical bioregions [23–25]. Larvae belonging to this species are known to feed on decay organic matter (saprophagous) and to consume and re-digest the faeces of large animals (coprophagous) [24,25]. This species was reported from field traps and animal carrions [26,27] while, from archaeological contexts, puparia and an adult fragment of this species were reported only in the sarcophagus of Federico II in Palermo, Italy [25] (Supplementary material Table S2). In this article, we report the first record of a high number of adults and puparia (>100) in association to a human corpse.

Furthermore, because of the lack of description, we report here some illustrations and morphological description of the species focusing our attention mainly on the puparium.

Puparia of *L. latipes* are yellow to light brown (Figure 2A–C). In the analysed samples, the length of the close puparium is (0.32±0.05) mm long ($N=5$). The posterior anal region is covered by several excrescences surrounding a smooth anal plate with no expansions (wings). All the anal papillae are absent or not discernable (Figures 2D, E and 3A). The

intersegmental spines of ventral welt of abdominal segment 7 are small and differently oriented, with the top half directed towards the anal plate and the bottom half directed on the opposite direction. Spines in the external lines are smaller and closer than the central ones and ending with sharp tips (Figures 2F and 3B).

Posterior spiracles are situated on two strongly projected processes. The three slits are themselves allocated each one on a different expansion kept closed to the puparium by filaments emanating from perispiracular glands (Figures 2G, H and 3C–E). In the analysed samples, star-shape anterior spiracles showed five to six prospiracular lobes (Figures 2I and 3F).

Adults are shiny black flies and their diagnostic features are mainly associated with the frons, the thorax and the legs. In fact, *L. latipes* is a small species showing reddish margin frons, silvery microtomentose median longitudinal stripe and a bare mesopleuron. Male and female fore and mid tibia show yellow basal and medial rings. Male hind tibia is enlarged. Female body length is (1.68±0.06) mm ($N=3$) while male body length is (1.22±0.07) mm ($N=5$). Wings are (1.74±0.14) mm long for female and (1.31±0.25) mm long for male. On the head, the light grey ocellar triangle shows a distinctive microtomentum, two ocellar setae and three to five short central setae. The frons is rectangular, longer than wider, with anterior margin red and silvery microtomentose longitudinal stripes next to eye margin. The front presents four orbital and one lower fronto-orbital pairs of setae. Lunula is yellow

and bare triangular-shaped. Antennae are dark with the first flagellomere irregularly rounded and a pubescent arista.

Para-facial is yellow with microtomentum, gena are thick, 1/4 of eye height and yellowish. Strong vibrissae are present, located at level of lower eye

margin. Palpi are yellow, slightly sickle-shaped in lateral view with black setulae in the ventral margin. The proboscis is geniculate, darkish brown, with sparse setulae in the margin (Figure 4).

The convex thorax shows one postpronotal, one notopleural, one posterior dorsocentral, one supra-

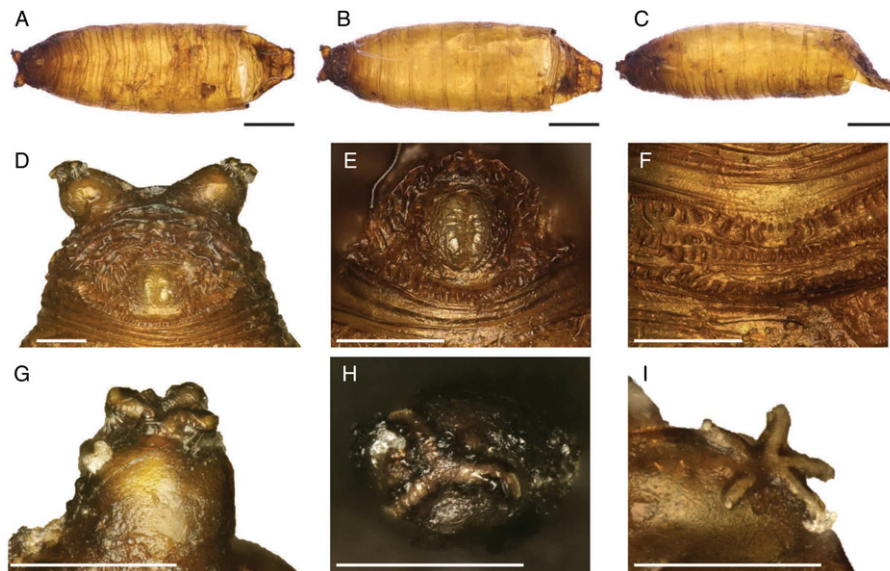


Figure 2. *Leptometopa latipes* puparium in ventral (A), dorsal (B) and lateral (C) view (scale bar 500 µm). Puparium details: Posterior anal region (D), anal plate (E), intersegmental spicules (F), posterior spiracle (G, H) and anterior spiracle (I) (scale bar 100 µm).

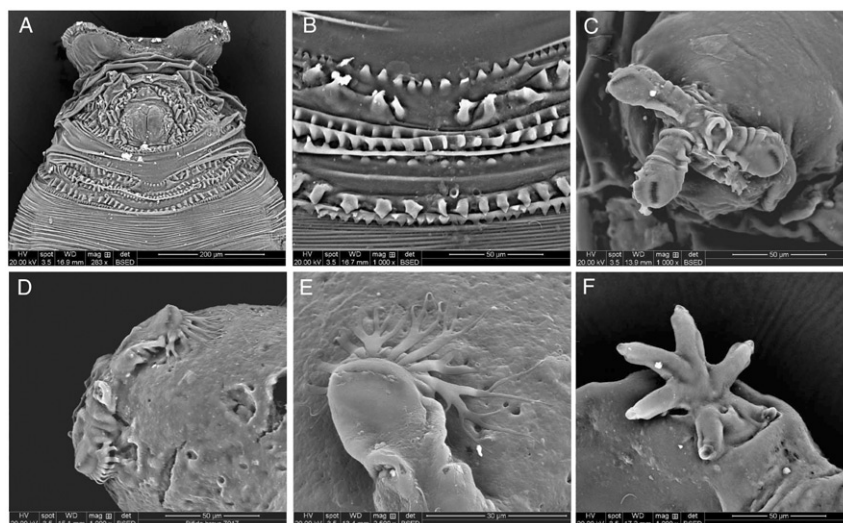


Figure 3. *Leptometopa latipes* puparium details: anal plate (A), intersegmental spicules (B), posterior spiracle (C, D) and filaments emanating from perispiracular glands (E), anterior spiracle (F).

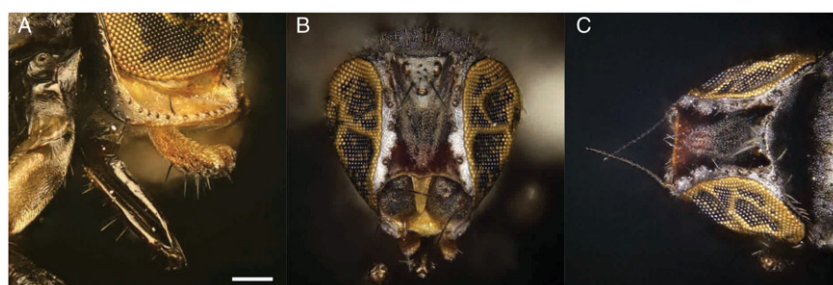


Figure 4. *Leptometopa latipes* adults head details. Female oral region (A), male bristles (B) and male antennae (C) (scale bar 100 µm).

and two post-alar (in intra-alar position) pairs of setae. The scutellum presents two pairs of marginal bristles. No apical scutellar bristles are present (Figure 5A). As in other Milichiidae, the wing has two costal breaks, once near humeral cross-vein and once near apex of vein R_1 . Anal vein is extremely reduced, perceptible only as shadow (Figure 5B). A yellowish ring in the foreleg and midleg and light colour tarsi are present. Tibiae are without dorsal preapical bristle. In the male, the particular shape of the hind tibia, strongly broaden and flat, is a diagnostic character of the species (Figure 6).

Discussion and conclusions

After death, body goes through a series of physical and biochemical transformations that create the conditions for insect colonization. The first colonizers are usually species of flies in the families

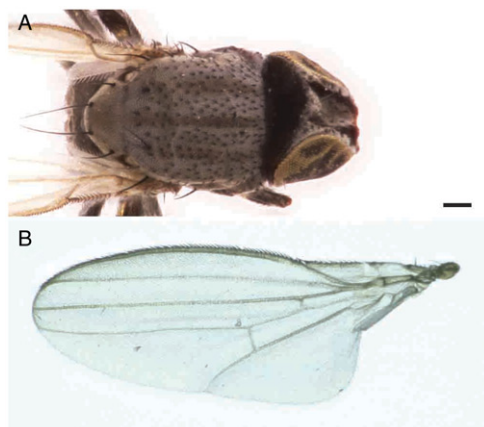


Figure 5. *Leptometopa latipes* adults details: thorax (A) and wing (B) (scale bar 100 μ m).

Calliphoridae, Muscidae and Sarcophagidae followed by other Diptera taxa and then by Coleoptera and Lepidoptera. Hymenoptera can be present on the body as scavenger, predators or parasitoids of other insects. In the Mediterranean area, blowflies (Calliphoridae) in the genera *Calliphora*, *Chrysomya* and *Lucilia* are the most common taxa among the first exposed bodies colonizers, mainly depending on the season of the death [28,29]. In this case, the absence of blowflies can be explained with the finding of the body few hours after the death however the understanding of the time when the colonization happened in this case is very difficult because of the repeated autopsies and exhumations of the body and the initial storage in a very warm country.

Fly in the family of Muscidae are very common in body found in urban area [2,30] with some species typically found indoor as well in crypts and other hypogean sites [31]. The Muscidae species, *H. capensis*, found on the corpse is typical of bodies that are stored in crypts or buried [2,18–20] but also it is reported from active decomposing exposed bodies [2,11]. For these reasons, its presence in this case is not surprising.

The Milichiidae species, *L. latipes*, is reported from decomposing matter, excrements and insect nests and, as other species of the same family, it is only rarely mentioned from decomposing human bodies [2]. The finding of the species in this case is very interesting and could be related to specific condition of the initial storage of the body (e.g. morgue, repeated autopsies, etc.).

From a geographical point of view both species, *H. capensis* and *L. latipes*, are present in the

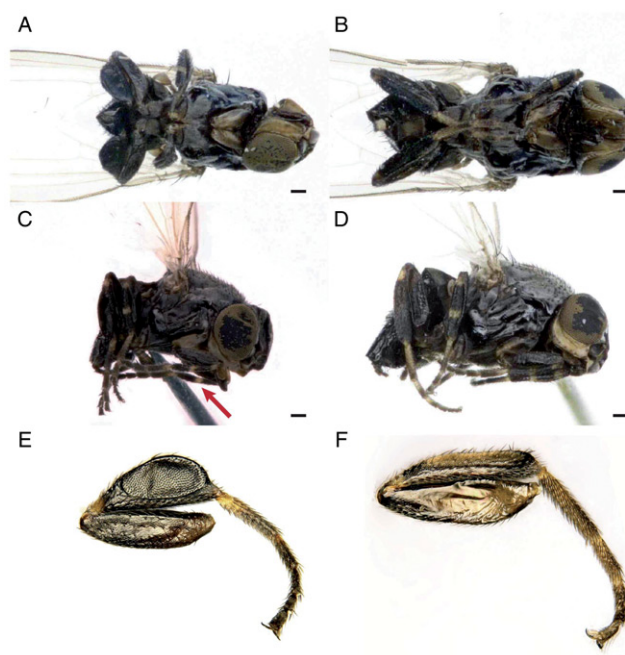


Figure 6. *Leptometopa latipes* adults. Male in ventral (A) and lateral (C) view. The arrow shows the yellowish ring in the foreleg. Female in ventral (B) and lateral (D) view. *L. latipes* hind tibia: male (E) and female (F) (scale bar 100 μ m).

Mediterranean basin, a datum that is not in disagreement with the declared origin of the body, a Maghreb country. As in other cases, only an improvement in the knowledge about the species ecology and distribution would allow a better application of the entomological analysis that can complement data from other forensic disciplines like forensic pathology and anthropology.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors. All the procedures in the cases were performed in accordance with the national legislation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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