First report of the dog louse fly *Hippobosca longipennis* in Romania

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Abstract. *Hippobosca longipennis* (Diptera: Hippoboscidae), the dog fly or dog louse fly, is an obligate blood-feeding ectoparasite of wild and domestic carnivores in Africa and the Middle East. Outside its typically known geographic range, *H. longipennis* has been reported occasionally on mainly domestic dogs in Asia and southern Europe, and infrequently in other areas (central Europe and the U.S.A.). This paper presents the first report of *H. longipennis* in Romania and the second record of *Lipoptena fortisetosa* (Diptera: Hippoboscidae), a potentially invasive species. *Hippobosca longipennis* was found on domestic dogs in two regions of the country (northern Romania in Maramures and southwestern Romania in Dobrogea) and on two road-killed wildcats in Maramures. *Lipoptena fortisetosa* was found on domestic dogs in Maramures. In both species identification was based on morphology and confirmed by barcoding of the cytochrome *c* oxidase subunit 1 gene. It is not clear for how long *H. longipennis* has been present in central Europe, nor if it was introduced (via the movement of domestic dogs or import of exotic carnivores) or present historically (Holocene remnants). This paper discusses the possible origins of *H. longipennis* in central Europe as its current distribution in the area is sparse and patchy.

Key words. *Hippobosca longipennis*, *Lipoptena fortisetosa*, dogs, Romania.

Introduction

Hippobosca longipennis, the dog fly or dog louse fly, is an obligate blood-feeding ectoparasite of wild and domestic carnivores including Canidae (jackals, foxes and domestic dogs), Viverridae (mongooses and civets), Hyaenidae (striped and spotted hyenas) and Felidae (cheetahs, servals, leopards, lions and domestic cats) (Bequaert, 1953; Sanborn & Hoogstraal, 1953; Maa, 1969; Lloyd, 2002). It has also been found on incidental hosts such as antelopes and birds, and it can occasionally attack humans (Bequaert, 1953; Maa, 1969). It is widely distributed in Africa and the Middle East (Bequaert, 1953) on both wild and domestic animals. Outside this geographic range, *H. longipennis* has been reported mainly on domestic dogs in warm parts of Asia,

North America and Europe (Bequaert, 1953). In the U.S.A., it was introduced through the import of cheetahs to the San Diego Zoo in California (Keh & Hawthorne, 1977) and later reported in various wild animals in California, Texas, Georgia and Oregon (Lloyd, 2002). In Europe, reports of *H. longipennis* come mainly from the Mediterranean countries and more rarely from central Europe (Bequaert, 1953). Occasional findings of imported cases have also been reported from the U.K. (O'Connor & Sleeman, 1987; Chandler, 1998). *Hippobosca longipennis* is considered to be the main vector of the vector-borne filarial nematode parasite *Acanthocheilonema dracunculoides* (Filarioidea: Onchocercidae) (Nelson, 1963; Anderson, 2000). Although *A. dracunculoides* has not yet been reported in Romania, it has been detected in Europe in red foxes from Italy (Magi *et al.*,

2008), in foxes, lynxes and domestic dogs and cats from Spain (Olmeda-Garcia *et al.*, 1993; Bolio *et al.*, 2004; Millán *et al.*, 2007), and in domestic dogs from Portugal (Alho *et al.*, 2014).

Deer keds of the genus *Lipoptena* are obligate blood-feeding ectoparasites that shed their wings after finding a suitable host. Deer keds are potential vectors of various pathogens, including *Anaplasma*, *Bartonella*, *Rickettsia* and *Trypanosoma*. Whereas *Lipoptena cervi* is a native European species, *Lipoptena fortisetosa* was first identified in Japan in the 1960s (Maa, 1965). This probably Asian species was also noted in Europe for the first time in the 1960s (Chalupský, 1980) and was later recorded in numerous countries.

This study presents the first report of *H. longipennis* and the second record of *L. fortisetosa* in Romania.

Materials and methods

During a routine epidemiologic survey for vector-borne diseases performed in September 2016, 73 domestic dogs (all with out-door housing and from rural environments) were examined for ectoparasites by visual inspection in several villages in south-western Maramures County, as follows: Buciumi (47.472055 N, 23.489352 W) (*n* = 2); Curtuiusu Mare (47.427404 N, 23.444610 W) (*n* = 4); Fericea (47.407208 N, 23.387726 W) (*n* = 21); Mesteacăn (47.381775 N, 23.518889 W) (*n* = 7); Valea Chioarului (47.427607 N, 23.483813 W) (*n* = 27), and Vărai (47.387184 N, 23.449287 W) (*n* = 12). Between September 2015 and November 2017, seven road-killed wildcats (*Felis silvestris*) were also collected in the same area as follows: Valea Chioarului (*n* = 3); Buciumi (*n* = 1); Mesteacăn (*n* = 1); Vărai (*n* = 1), and Fericea (*n* = 1). All data for positive animals are shown in Table S1. The sampled dogs were housed outdoors, in improvised shelters, together with other domestic livestock. Additionally, louse fly samples were occasionally found on dogs and collected using fine forceps during another study of dog ticks in the Dobrogea region (see Sándor *et al.*, 2014), in Iazurile (Tulcea County) (45.014040 N, 28.941084 N) during August 2013 and July 2015. These flies were collected from community dogs, which were living ferally and without shelter. The presence of louse flies in this area was occasional and no flies were found on later visits. Another louse fly was collected during a routine examination of a dog for ticks in Pestera (Constanta County) (44.188671 N, 28.111319 W) in June 2016. Indeed there has been a mistake. The dogs from Maramures county are housed outdoors in improvised shelters, while the dogs from Dobrogea region and Tulcea country were community dogs without shelter. All sampling locations are shown in Fig. 1.

All louse flies were collected alive directly from the respective hosts and placed in 70% ethanol and later examined under a stereomicroscope for morphological identification. Morphological species identification was performed using the available keys (Maa, 1962; Chalupský, 1980) and verified using molecular methods. Two randomly selected ethanol-stored specimens of each morphospecies were washed individually for 1 h in distilled water. Three legs of each specimen tested were removed and DNA extracted using a QIAamp DNA Mini Kit (Qiagen GmbH,

Hilden, Germany) according to the manufacturer's instructions and following the protocol for DNA purification from tissues. The barcoding involved the sequencing of the cytochrome *c* oxidase subunit 1 (cox1) gene using universal primers and conditions described by Hebert *et al.* (2003). Amplification reactions were performed in a total volume of 20 µL with EmeraldAmp GT polymerase chain reaction (PCR) master mix (Takara Bio Europe, Saint-Germain-en-Laye, France). Amplified products were visualized on 1% agarose gel, purified by QIAquick PCR Purification Kit (Qiagen GmbH), and directly sequenced in both directions using amplification primers (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit; Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.). The obtained sequences were viewed and aligned using the software GENEIOUS Version 10.0.6. The final sequences were compared with those available in the databases BOLD (http://www.boldsystems.org/) and Gen-Bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Collections from domestic dogs in Maramures County yielded 14 louse flies, all of which were found in one locality, the village of Fericea, on five dogs. Eleven specimens were morphologically identified as *H. longipennis* (Fig. 2) and three as *L. fortisetosa*. Of wildcats from the same area, two animals were found to be infested with louse flies, both with a single individual identified morphologically as *H. longipennis*. Collections from dogs in the Dobrogea region yielded three louse flies (two in Iazurile, one in Pestera), all of which were identified as *H. longipennis*. The louse flies on all dogs were found in the fur of the dorsal area of the body (i.e. the back, along the caudal part of the cervical spine and the thoracic area of the spine). The geographic distribution of findings is shown in the map in Fig. 1.

Comparisons of the newly obtained cox1 gene sequences with those available in the databases (BOLD and GenBank) confirmed (identity 98–99%) the morphological determination of both louse fly species. Two identical sequences of *H. longipennis* represent the same haplotype (GenBank accession no. of the specimen voucher HL_Ro_01: MK405667). However, two sequences of *L. fortisetosa* represent two different haplotypes (MK405668 for specimen voucher LF_Ro_01 and MK405669 for LF_Ro_02).

In the survey in Maramures County, a total of five dogs from four households were found to be infested with *H. longipennis* and/or *L. fortisetosa*. In the first household, two of three dogs were infested. Both were mongrels that lived outdoors and were used for security purposes; they were aged 1 year and 4 years, respectively. The second household had one infected mongrel dog aged 5 years. The last two positive households were situated in close proximity to one another (next-door neighbours) and had one dog each. The dogs were aged 7 years and 10 years, respectively. The samples collected from Dobrogea region originated from two different locations. In Iazurile the same household provided two samples in two different years (in both cases from one young mongrel that was kept outdoors), whereas the sample from Pestera was collected from one of two mongrels examined, both of which were used as sheep dogs.
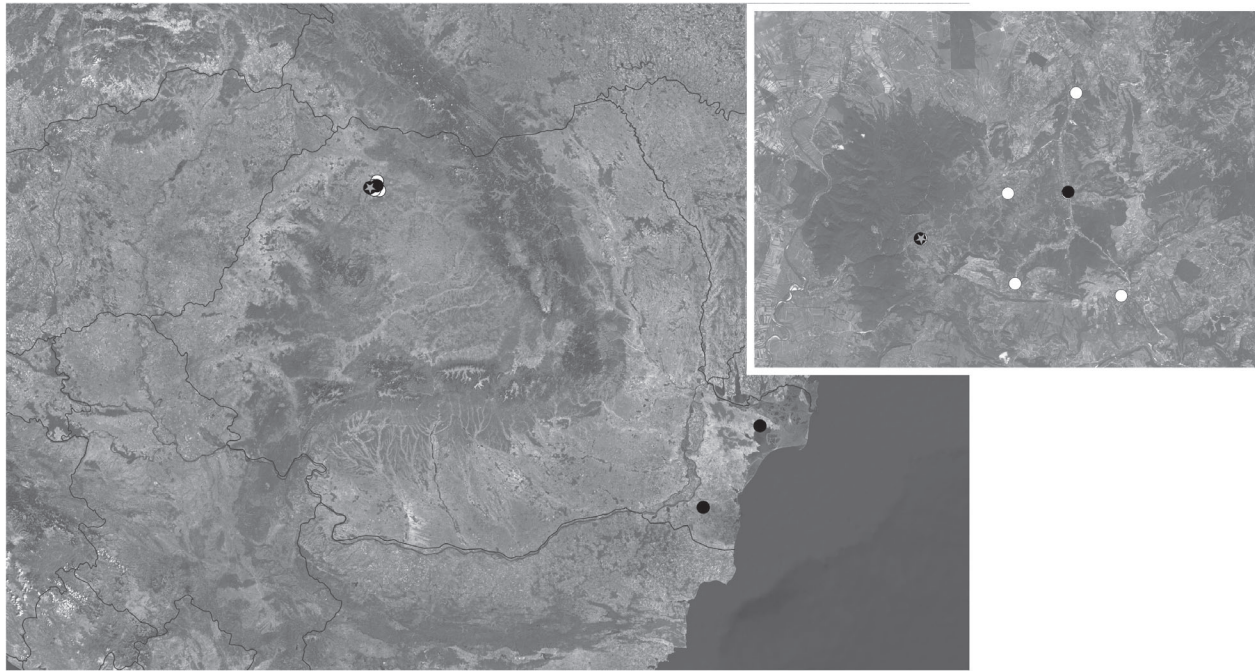
Fig. 1. Positive and negative locations of *Hippobosca longipennis* and *Lipoptena fortisetosa* in Romania.

Fig. 2. *Hippobosca longipennis* identified on one of the dogs examined in the present study. [Colour figure can be viewed at wileyonlinelibrary.com].

Discussion

This work represents the first record of the dog louse fly (*H. longipennis*) and the second record of the deer louse fly (*L. fortisetosa*) in Romania. *Lipoptena fortisetosa* has been identified in several European countries, including the Czech Republic (Chalupský, 1980; Barták, 1995), Germany (Schumann & Messner, 1993), Poland (Kowal *et al*., 2009), Lithuania (Lithuanian Entomological Society, 2006) and Moldavia (Metelitsa & Veselkin, 1989). This species is certainly more widespread in Europe; however, it is probably overlooked and misidentified as *L. cervi* in many cases. If the Asian origin of *L. fortisetosa* is accepted, its most probable route of introduction to Europe was translocation via its host, probably the sika deer (*Cervus nippon*). However, there is also the possibility that this species has been overlooked for a long time and misidentified

as *L. cervi* or has naturally dispersed throughout Eurasia. In Romania, *L. fortisetosa* was recorded only once, in 2004, in two neighbouring localities (Lung Albina islets) on the lower Danube (Pârvu, 2005). Thus, the present finding represents the second piece of evidence for the presence of the species in Romania and refers to a locality geographically distant from that cited in the previous record. Typical hosts of *L. fortisetosa* are deer, antelopes, cattle, goats and sheep; however, similarly to the present findings, the species has also been captured on dogs (Sokół & Gałecki, 2017).

Although, *H. longipennis* is a very common parasite of domestic and wild carnivores, particularly in East Africa, North Africa and the Middle East (Bequaert, 1953), the species has been reported occasionally in many other countries, mainly on domestic dogs. Most of the European records originate from regions with a warm Mediterranean climate, such as Spain, Italy including Sicily and Sardinia, Greece, Cyprus and Bulgaria (Bequaert, 1953). Wild carnivores are also important hosts in southern Europe. Lariviere & Calzada (2001) reported an unspecified *Hippobosca* (with high probability of being *H. longipennis*) in genets (*Genetta genetta*) in southern Spain. In another study from southern Spain, Millán *et al*. (2007) found prevalence rates of 31% in the endangered Iberian lynx (*Lynx pardinus*), 26% in red foxes (*Vulpes vulpes*) and 2% in domestic cats. Cordero *et al*. (1994) reported *H. longipennis* in domestic dogs from central Spain (Salamanca) and Portugal.

Records in central Europe are far less common. *Hippobosca longipennis* has been sporadically collected from domestic dogs in Hungary, Ukraine (Crimea), Germany, Poland and Slovakia (Bequaert, 1953; Straka & Majzlan, 2010; Sokół & Gałecki, 2017). Straka & Majzlan (2010) trapped *H. longipennis* in southern Slovakia, although the first finding dates back to 1953 (Chalupský, 1980). More recently, an extensive study was performed in urban dogs from central Poland and four species of louse fly were identified, with *H. longipennis* being the most dominant (Sokół & Gałecki, 2017). Furthermore, *H. longipennis* was imported to the U.K. and Ireland with wild carnivores from Africa (O'Connor & Sleeman, 1987; Chandler, 1998). However, there is no evidence of its permanent establishment. Moreover, none of the previous studies of ectoparasites of domestic and wild carnivores in Romania reported the presence of *H. longipennis* (Dumitrache *et al*., 2011; D'Amico *et al*., 2017a, 2017b; Foley *et al*., 2017; Sándor *et al*., 2017). It should be noted that *Hippobosca equina* has also been reported in dogs from Albania (Danielova, 1960) and Poland (Sokół & Gałecki, 2017).

Similarly to *L. fortisetosa*, it is not clear how long *H. longipennis* has been present in Europe nor whether it was introduced or present historically, particularly in southern Europe (Bequaert, 1953), as its main African hosts were present in some of these areas until the Holocene (i.e. lions as recently as 1000 BC in Greece) (Kingdon & Hoffmann, 2013). Bequaert (1953) mentioned *H. longipennis* in dogs from ancient Greece (*c*. 800 BC) and Huchet *et al*. (2013) found a single specimen on the mummy of a dog from ancient Egypt. In central Europe, it is not clear if the reported populations are Holocene remnants or if *H. longipennis* has been introduced through the movement of domestic dogs, resident wild carnivores or importation of African wildlife to zoos, as in the U.K., Ireland

and the U.S.A. (Keh & Hawthorne, 1977; O'Connor & Sleeman, 1987; Chandler, 1998). Other authors have hypothesized that *H. longipennis* is under geographic expansion (Sokół & Gałecki, 2017) as a result of climate change and increases in mean temperatures. However, there is no direct evidence for such a claim. It is interesting that, as was highlighted by Bequaert (1953), the distributions of most other canine parasites are more or less worldwide, whereas *H. longipennis* has a very patchy distribution outside its typical range which does not seem to be related to climatic factors. The factors determining its patchy distribution in non-Mediterranean regions of Europe remain unknown.

The importance of the presence of *H. longipennis* resides in its vectorial capacity, so far clearly demonstrated only for the filarial nematode *A. dracunculoides* (Nelson, 1963). Although the presence of *A. dracunculoides* in central and eastern Europe has not yet been reported despite systematic surveillance in both domestic and wild carnivores (Ionică *et al*., 2015, 2016, 2017a, 2017b), the presence of its vectors should raise awareness. However, the louse flies (Hippoboscidae) are not the sole vectors of this parasite as ticks [*Rhipicephalus sanguineus sensu lato* (Ixodida: Ixodidae)] have also been incriminated (Olmeda-Garcia *et al*., 1993; Olmeda-Garcia & Rodriguez-Rodriguez, 1994; Mihalca *et al*., 2012; Dantas-Torres & Otranto, 2015). Hence, the importance of this finding, together with the underlying factors determining the distribution of *H. longipennis*, require further investigation.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Records of louse flies collected in Romania, including collection date, locality, coordinates (N, latitude; E, longitude), host species, sex, age and origin.

Acknowledgements

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