The zoophilic fruitfly *Phortica variegata*: morphology, ecology and biological niche

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Abstract. Flies belonging to the subfamily Steganinae (Drosophilidae) display unusual zoophilic feeding habits at the adult and/or larval stage. *Phortica variegata* (Fallén) feeds on tears or eye liquid around the eyes of humans and carnivores. When feeding it is a potential vector of *Thelazia callipaeda* (Railliet and Henry) eyeworms. Adult and larval stages of this fly may be easily confused with other species belonging to the same genus, and little is known on the biology and ecology of *P. variegata*. In April–November 2005, a total of 969 *P. variegata* were collected in an area with a high prevalence of canine thelaziosis. The number of flies collected weekly was then related to climatic and environmental parameters (e.g. temperature, relative humidity and total rainfall) recorded daily at the collection site. The highest number of *Phortica* were collected during July−August. The sex ratio (number of males : females) rose from ~ 0.5 during May–July, to ~ 3.0 in August and 181 during September–October. Distributional data, representing 242 sites at which *P. variegata* has been collected in Europe, were analysed using a desktop implementation of the genetic algorithm for rule-set prediction (GARP) to model ecological requirements across Europe, as well as in Italy. *P. variegata* is shown to be mainly active at 20–25 °C and 50–75% RH. The ecological niche model suggests with a high degree of confidence that large areas of Europe are likely to represent suitable habitat for this species, mostly concentrated in central Europe. The results reported here contribute basic knowledge on the ecology and geographical distribution of *P. variegata* flies, which will be fundamental to gaining a better understanding of their role as vectors of human and animal pathogens.

Key words. *Phortica variegata*, *Thelazia callipaeda*, Drosophilidae, Steganinae, ecology, fruitfly, modelling, morphology, vector, zoophilic flies.

Introduction

Drosophilidae are probably among the best known insect taxa by virtue of their use in many areas of biological research, particularly in the field of genetics (Kwiatowski & Ayala, 1999). Drosophilidae comprises two recognized subfamilies, Drosophilinae and Steganinae, which together represent about 3800 species commonly known as 'fruitflies'. Whereas members of subfamily Drosophilinae feed and develop mostly on fruits and other vegetable matters (Bächli *et al.*, 2004), flies belonging to Steganinae display unusual feeding habits and their ecology is much less known. Steganinae larvae often exhibit zoophilic feeding habits (Ashburner, 1981), but in the adult stages this behaviour is confined to three genera: *Amiota* Loew, *Phortica* Schiner and the very rare extra-European genus *Apsiphortica* Okada (*Gitona distigma* Meigen was also mentioned by Kozlov [l963], but its zoophilic behaviour has never been confirmed). In fact, adults of these genera are reported as

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feeding on fermenting tree sap (Okada, 1962), as well as on rotten fungi and fox faeces (Papp, 2002). Interestingly, they are also attracted by the eye secretions of humans and animals such as dogs and other carnivores (Bächli *et al.*, 2004). *Phortica* flies in particular are recognized as a nuisance to animals and humans because of their attraction to the eyes and it has been demonstrated experimentally that they can act as vectors and intermediate hosts of the eyeworm *Thelazia callipaeda* Railliet and Henry (Otranto *et al.*, 2005). *T. callipaeda* parasitizes the conjunctival sac of dogs, cats, foxes, rabbits and humans, causing lacrimation, epiphora, conjunctivitis, keratitis and even corneal ulcers (reviewed in Otranto & Traversa, 2005). In Asian countries (e.g. Indonesia, Thailand, China, Korea, Myanmar, India and Japan), *T. callipaeda* is commonly known as 'oriental eyeworm' (Anderson, 2000). Recently, a number of reports of *T. callipaeda* from Europe (Otranto *et al.*, 2003; Chermette *et al.*, 2004; Hermosilla *et al.*, 2004) have increased the scientific interest in the vector of canine thelaziosis.

The importance of identifying *Phortica* to species level in terms of their role as vectors of *T. callipaeda* deserves further explanation. *Amiota variegata* (Fallén) and *Amiota magna* Okada were recorded as vectors in Japan by Nagata (1959, 1960), both species are now considered to belong to the genus *Phortica* (Máca, 2003). Further papers listing *Phortica variegata* as the vector in the Siberian Far East were published by Kozlov (1961, 1963). The determination of *Phortica magna* appears to be correct, but the occurrence of *P. variegata* in these countries now seems improbable, as the papers were published prior to taxonomic revisions showing *P. variegata* as a European species (Máca, 1977). The only records of this species from Asia that seem to be reliable are from the Caucasian region (Gornostaev, 1996) and Turkey (Máca, 1987). Later *Phortica okadai* (Máca), distributed in Japan, Korea, eastern Siberia and across the majority of China, was quoted as the vector of *T. callipaeda* in China (e.g. Wang *et al.*, 2002; specimens examined by J. Máca, 2005). In some Asian countries (i.e. Thailand, Myanmar, Indonesia and India), where none of the three above-mentioned *Phortica* species are known, the vector of *T. callipaeda* is unknown; however, tens of other *Phortica* spp. occur in South-East Asia.

P. variegata flies may be easily confused in the adult and larval stages with other species such as *Phortica semivirgo* (Máca) and *P. okadai*. Again, the biology and ecology of *P. variegata* have been investigated little and knowledge on these topics is sparse (Okada, 1956; Máca, 1977, 2003; Bächli *et al.*, 2004). Indeed, it is only very recently that *P. variegata* has been, for the first time, reliably proven to act as a vector of *T. callipaeda* in experimental conditions (Otranto *et al.*, 2005). This gap constitutes an obstacle to the investigation of the vectorial capacity of *P. variegata* flies for *T. callipaeda*, as well as for other pathogens and to the control of eyeworm infection (Otranto *et al.*, 2005).

As it is often difficult for non-dipterologists to use the keys to Diptera, the present paper illustrates the key morphological characteristics for the straightforward identification of adult stages of *P. variegata.* Further aims of this work are to provide insights into the biology and ecology of the adult flies in their natural environment and to estimate the potential ecological niches for *P. variegata* in Europe.

Materials and methods

Fly collection and morphological identification

From April to November 2005, *Phortica* flies were collected weekly in an area characterized by a high level of relative humidity and thick undergrowth (municipality of Oliveto Lucano, Potenza province, Italy, 16°15′ E, 40°54′ N). The area is 800−1000 m above sea level (a.s.l.). Vegetation characteristics are dependent on altitude, exposure and relative humidity. In particular, oaks are common, with *Quercus cerris* being the most frequent species and holly (*Ilex* spp.) found in the undergrowth. The site for capturing flies was chosen primarily on the basis of the high prevalence of dogs infected with *T. callipaeda*. These dogs usually accompanied sheep and cattle at least once a day during summer (Otranto *et al.*, 2005). The temperature, relative humidity (RH), total rainfall (mm) and number of rainy days were monitored and recorded daily by a local meteorological station (Metapontum Agrobios; agro-meteorological system).

Flies were collected by capturing insects with a hard net from around bait or the eyes of collectors. The bait consisted of a white cloth containing cut fruit (about 2 kg of mixed apples, pears and bananas) fermented at 25 °C for 48 h. The bait was fastened to the north side of a tree with string. Flies were captured during a period lasting about 4 h in each collection, usually in the morning and afternoon (09.00–11.00 hours and 15.00–17.00 hours). Once captured, flies were transferred in a transparent plastic bag for a preliminary screening (under a field stereomicroscope) for some morphological characteristics indicative of the genus *Phortica*, including the presence of three dark bands on the legs, pale rings around the eyes and characteristic colouring of the abdomen. *Phortica* flies were counted (trapped flies of other genera were discarded), then transferred to a cage (26 × 21 × 21 cm, 1-mm diameter mesh) and transported to the laboratory at the Parasitological Unit, Faculty of Veterinary Medicine, University of Bari. Pieces of fresh (not fermented) fruit were put on the roof of the cage as food and covered with wet cloths to maintain high RH.

All *Phortica* flies captured were identified to species level using the identification keys proposed by Máca (1977) and Bächli *et al.* (2004). To confirm the morphological identification, the terminalia of dead males were processed as suggested by Bächli *et al.* (2004). Briefly, the terminalia were left to clear for 3 h in individual vials containing 10% KOH dissolved in hot water and, after removing the last tergite, the terminalia were coloured using Gage's stain mix (0.5 mL acid fuchsin, 25 mL 10% HCl and 300 mL distilled water). The preparation (in glycerine) was then observed under a stereomicroscope for analysis of the terminalia.

Data on fly distribution and ecological niche modelling

For the ecological niche modelling, distributional data were drawn representing 242 sites in Europe at which *P. variegata* have been collected (at longitudes between 8° W and 48° E and latitudes between 38° N and 62° N); 240 sites came from published records (http://taxodros.unizh.ch) and two from personal collections in the Oliveto Lucano, Basilicata region (16°15′ E, 40°54′ N) and Perticaro, Calabria region (16°88′ E, 39°34′ N) in Italy (D. Otranto, unpublished data). Several approaches (Walker & Cocks, 1991; Scott *et al.*, 1993, 1996, 2002) were used to approximate ecological niches of *P. variegata*. Of these, the most robust appears to be the genetic algorithm for rule-set prediction (GARP), which includes numerous inferential approaches in an interactive, artificial intelligence-based approach (Stockwell & Peters, 1999). A desktop implementation of GARP (http://www.lifemapper.org/desktopgarp/) was used to model a suitable environment for *P. variegata* in Europe and, specifically, in Italy. In the model used for Europe, the GARP datasets included 14 electronic maps summarizing aspects of topography (e.g. elevation, slope, aspect and topographic index) and aspects of climate (annual means), including diurnal temperature range, precipitation, temperatures, solar radiation, wet days and vapour pressure, for a time period from 1960 to 1990 (Intergovernmental Panel on Climate Change, http://www.ipcc.ch/). In the model for Europe the datasets were generalized to a pixel resolution of 10 × 10 km, whereas in the model for Italy a customized dataset was used with a high pixel resolution (1 × 1 km), including elevation, slope and land cover from CORINE (Co-ordination of Information on the Environment) as well as climate data from the *Italian Agroclimatic Atlas* Version 1.0 (Perini *et al.*, 2004). The model for Europe was calculated using all 242 points of *P. variegata* presence. Only 17 points of fly occurrence were available for Italy. To increase the number of sites, the presence of *P. variegata* was postulated for 12 sites in which thelaziosis was reported in dogs (Otranto *et al.*, 2003). In this manner a total of 29 points were eventually used in the model for Italy. Statistical parameters set for both models were 100 runs, 0.01 coverage and 1000 of maximum interactions; a combination of negated range and logistic regression was set as the rule type. To choose the best subsets of models, all models that had non-zero omission error based on independent test points were eliminated and the median area predicted to be present among these zero-omission points was calculated (Peterson & Shaw, 2003). Finally, a map combining the 10 models closest to the overall median area predicted was used to identify the most suitable areas for *P. variegata* in both Europe and Italy.

Results

A total of 969 *P. variegata* flies (557 males and 412 females) were collected during the entire observation period (Table 1). Of these, 523 flies were collected from around the eyes of the collectors and 446 by netting insects around the fermented fruit bait. Average daily values from the recorded meteorological data (i.e. temperatures, RH, total rainfall and number of rainy days) are reported in Table 1, along with the numbers of *Phortica* flies collected monthly.

Phortica flies were first collected in the first week of May and disappeared in the first week of November 2005. The minimum number of *Phortica* flies collected ($n = 20$) was recorded in October (12.3 °C, 78.7% RH), whereas the highest number ($n = 291$) was recorded in August (20.2 °C, 53.6% RH). A high number of *Phortica* flies ($n = 216$) were also collected in July (22.3 °C, 48.9% RH). It is worth mentioning that the highest number of rainy days was registered in October (21 days, total rainfall 74.8 mm), whereas low numbers of rainy days were recorded in July and August (3 and 8 days, total rainfall 8.4 and 77.8 mm, respectively). Striking changes in sex ratio (male : female) were observed during the season. In May, June and July females prevailed (*c.* 1 : 2). In August, males began to prevail (*c.* 3 : 1) and in September–October, males prevailed very strongly (181 : 1).

Key characteristics for morphological identification of adults

All the 969 *Phortica* flies captured were identified as *P. variegata* using the identification keys for Diptera families (Oldroyd, 1970) and then the keys for Drosophilidae proposed by Bächli *et al.* (2004).

The males, about 3.5–4 mm in length, are generally dark brown flies (Fig. 1a). The scutum is short and plump compared with other Drosophilidae, presenting numerous, partially confluent greyish spots (Fig. 1a, b) and eight irregular rows of acrostichal setae. The scutellum is dark at the broadest part, with greyish spots (Fig. 1a). The legs are characterized by a dark coxa and dark femur with a yellow base and apex, and by three conspicuous dark bands around the tibia (Fig. 1b). The tarsus is generally yellow; the apical part of the last segment is darker

Table 1. Average temperature, relative humidity, total rainfall, number of rainy days, number of flies captured and sex ratio registered by month at the site of fly collection from April to November 2005.

Month	Average temperature (°C)	Relative humidity (%)	Total rainfall (mm)	No. of rainy days	No. of flies captured	Sex ratio (M : F)
April	9.4	65.8	28	8	–	–
May	15.5	58	29.2	5	152	38 : 114
June	17.9	62.7	64.8	9	128	45 : 83
July	22.3	48.9	8.4	3	216	68 : 148
August	20.2	53.6	77.8	8	291	225 : 66
September	16.8	73.3	83.4	17	162	162 : 0
October	12.3	78.7	74.8	21	20	19 : 1
November*	9.8	82.4	22.2	13	–	–
Total	–	–	–	–	969	557 : 412

*Data for November refer to the first 20 days of the month only.

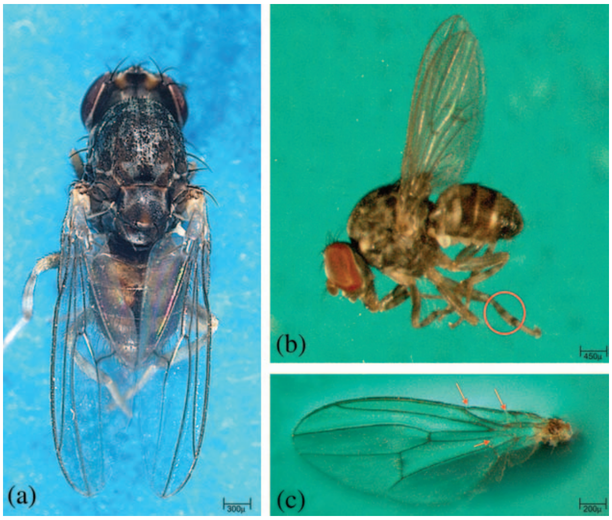

Fig. 1. (a) Male *Phortica variegata* in dorsal view. The scutum presents partially confluent greyish spots, whereas the scutellum is almost totally dark. (b) Male *P. variegata*, in lateral view showing three black bands around the tibia (circled), a dark coxa, a dark femur with yellow base and apex, and a totally yellow tarsus. (c) Wing of *P. variegata*. Note the cross-veins (shaded), the discal and second basal cells separated by an additional cross-vein and the costal vein with two interruptions (the last characteristic being typical of the Drosophilidae family).

(Fig. 1b). The wing is hyaline, but the two cross-veins are shaded and thus clearly visible (Fig. 1c). There are two interruptions of the costal vein (a taxonomically important characteristic of several fly families, particularly Drosophilidae) (Fig. 1c). The discal and second basal cells are separated by an additional cross-vein, characteristic of several genera of Steganinae (Fig. 1c). The eye is red with a pale ring around it (Fig. 2a). In some dark males, the upper half of the eye margin is dark and the lower half yellowish. The antennae are golden yellow (Figs 1b, 2a). The arista has three to six short dorsal branches decreasing in length towards the tip (Fig. 2b). The abdomen has a yellow and dark brown pattern consisting (in dorsal aspect) of roughly three transversal and one longitudinal dark band on a pale subcolour (Fig. 2c); the last tergite (epandrium with cerci) terminates bluntly and covers the characteristically shaped inner genital structures (Fig. 2d). Clarification of the male terminalia allows observation of three ‘teeth’ (sensilla) on the medial branch and one sensillum on the dorsal branch of the outer paraphysis (anterior paramere). The inner paraphysis of the aedeagus extends anteriorly, beyond the tip of the aedeagus and, near the level of the aedeagal tip, bears a slightly sinuate dorsal branch (Fig. 2e, f).

The females, about 3.5–5 mm in length, look generally paler than males because of their more voluminous bicoloured abdomens. The ring around the eye is almost always pale. The last tergite is conical and the epiproct and hypoproct bear several short hairs (Fig. 2g). The cerci are not sclerotized, are hairy and practically separated at the base; the vagina has a pair of oval sclerites situated basolaterally, but no sclerite medioventrally (Máca, 1977; Fig. 2g). Other morphological characteristics of females are identical to those of males (see above).

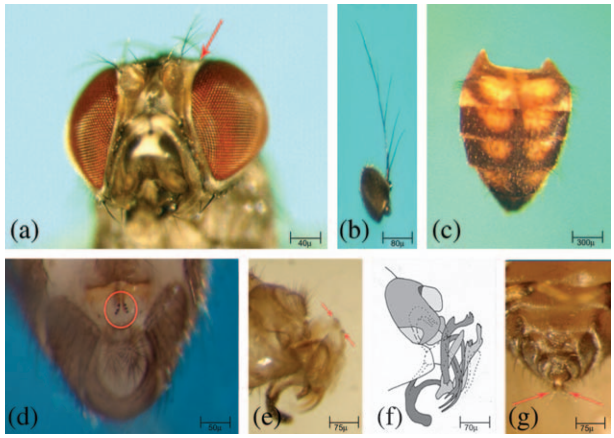

Fig. 2. (a) Head of *Phortica variegata*. A pale ring (arrow) is seen around the red eye. (b) Antenna of *P. variegata*. The arista has three to six short dorsal branches progressively decreasing in length towards the tip. (c) Abdomen of *P. variegata* showing dark bands in characteristic configuration. (d) Terminalia of male *P. variegata* before clarification. The last tergite is sufficiently large to cover the complex genital structure. Note the presence of three ‘teeth’ (sensilla) on the medial branch of the outer paraphysis (circled). (e) Phallic organs partially detached from the last tergite: terminalia of a male *P. variegata* after clarification, with three sensilla on the medial branch of the outer paraphysis (arrow). (f) Outline of the male terminalia of *P. variegata*, including the structures out of focus in (e). (g) Terminalia of female *P. variegata*. The last tergite is conical, epiproct with several short hairs. The cerci are non-sclerotized, hairy and confluent on the base (arrow).

Ecological niche model

The ecological niche models showed widespread potential distribution of *P. variegata* in both study areas. The model for Europe (Fig. 3) predicted vast suitable areas, mostly in central Europe (i.e. France, Germany, Poland, the Czech Republic and Hungary). This model showed good predictive value, as 193 (79.7%) of 242 points in which the occurrence of *P. variegata* has been recorded fell inside the area indicated by the model ($\chi^2 = 159.3$, $P < 0.001$). In the model for Italy (Fig. 4), 26 (89.6%) of the 29 points used to test the model fell inside the predicted area ($\chi^2 = 258.1$, $P < 0.001$). Suitable areas in the Italian model were mostly concentrated in mountainous areas at 600−1200 m a.s.l. Excluding some isolated spots, a very low potential distribution of *P. variegata* was registered along the Po plain in northern Italy, as well as in the Puglia and Sicily regions in southern Italy. Only two of the 12 points of dog thelaziosis used in the model for Italy fell outside the predicted area, although they were only 0.2 km and 1.4 km distant from the nearest predicted area of distribution.

Discussion

Until now, information on the biology, ecology and zoophilic habits of *P. variegata* has been scanty, probably because of its apparently limited importance in the field of medical and veterinary entomology. Recently, the assessment of *P. variegata* as

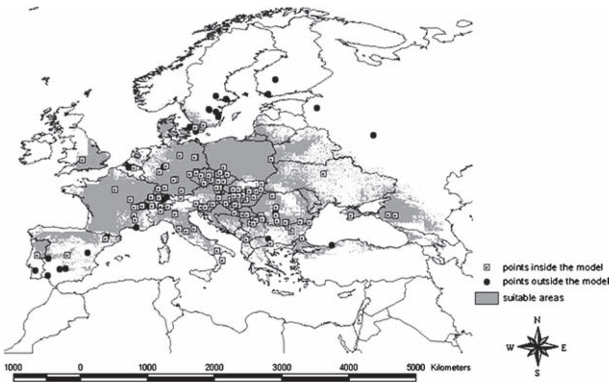

Fig. 3. Predicted geographical distribution of *Phortica variegata* in Europe: grey areas indicate suitable areas identified by the GARP model. Known occurrence points of *P. variegata* that fall inside the areas indicated by the model are shown as dotted squares and those outside are shown as black dots.

a/the vector of *T. callipaeda* to humans and animals has encouraged interest in the zoophilic habits of this drosophilid (Otranto *et al.*, 2005). The results presented here provide information on the biology and ecology of *P. variegata* flies in natural conditions and contribute morphological data to the rapid identification of *P. variegata* adults.

Of the four species ranked within the genus *Phortica* that have been reported in Europe, *P. variegata* and *P. semivirgo* are widely distributed. Conversely, the third species *Phortica erinacea* (Máca) (distinguishable by the pale colouring of its scutellum) was recorded only from type locality in Bulgaria and the last, *Phortica oldenbergi* Duda (a member of the subgenus *Allophortica* Máca), was probably transiently introduced to Europe in the first half of the 20th century (Máca, 1977, 2003). The difference in tarsal colouring can be used to distinguish

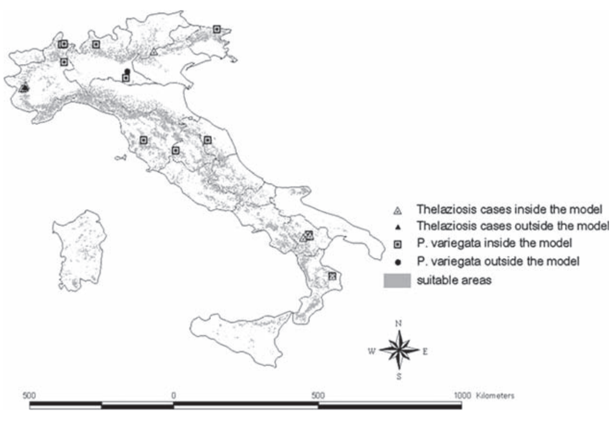

Fig. 4. Predicted geographical distribution of *Phortica variegata* in Italy: grey areas indicate suitable areas identified by the GARP model. Known occurrence points of *P. variegata* that fall inside the areas indicated by the model are shown as dotted squares and those outside are shown as black dots. Known occurrence points at which dog thelaziosis has been registered inside the area indicated by the model are shown as dotted triangles and those outside as black triangles.

P. variegata from *P. semivirgo*, as the whole of the last segment, at least half of the penultimate (fourth) segment and often the apex of the third segment are darker in the latter species. In all cases, identification to species level should be confirmed by clarification of the terminalia of males (see above). In particular, whereas the terminalia of male *P. variegata* is characterized by the presence of three 'teeth' (sensilla) on the medial branch and one on the dorsal branch of the outer paraphysis (Fig. 2d, e), the medial branch of male *P. semivirgo* presents usually four, but sometimes three or five sensilla (Bächli *et al.*, 2004). Whereas the inner paraphysis extends anteriorly beyond the tip of the aedeagus and possesses a slightly sinuate dorsal branch in *P. variegata*, the inner paraphysis does not reach the tip of the aedeagus in *P. semivirgo* (Bächli *et al.*, 2004). The females of the two species can be distinguished according to the structure of the vaginal sclerites (Máca, 1977). The habitat in which *Phortica* specimens were collected was an oak wood (*Q. cerris*), sited at about 850 m a.s.l., characterized by high RH and undergrowth constituted mainly of holly. The area surrounding the site of capture was characterized by the presence of rivers that further increase the RH. From the results of this study it appears that the biological activity of *Phortica* flies is highest when temperature and RH are 20–25 °C and 50–75%, respectively. Nevertheless, the number of *Phortica* flies collected in May and September at lower temperatures and higher RH suggests that the latter parameter plays an important role in the maintenance of their biological activity. In fact, based on the results obtained by modelling the potential ecological niches of *P. variegata* in southern Europe, it seems that its distribution is limited to hilly areas with relatively high precipitation and continental temperatures. In southern Italy, the biological activity of *Phortica* flies occurs from May to October. A previously unpublished finding of one male *P. variegata* (together with five female *Drosophila kuntzei* Duda) in Slovakia (at Zikmundova cave, 5 km north of Margecany, 15 m from the cave entrance, 14/10/2002, collected by A. Mock, identification by J. Máca) suggests that *Phortica* flies may be able to use caves as winter shelters, a habitat they are presumably also able to exploit in southern Italy.

The change in sex ratio during the year is of particular interest. Previous findings suggest that flies of the genus *Phortica* collected in flight around the eyes of humans and animals are mostly males, whereas flies collected on baits have a relatively balanced sex ratio. This was determined by comparing Taiwanese specimens (Máca & Lin, 1993), Bulgarian specimens (Máca, 1977), and specimens from the Russian Far East (Kozlov, 1963), all of which were collected from around eyes, with Czech specimens (Máca, 1977), which were collected in traps. The predominance of males around human eyes in late summer/early autumn may be due to dietary needs (e.g. protein supplementation) or possibly to environmental and biological factors that remain to be elucidated.

During trapping, *P. variegata* was observed to fly particularly slowly in small vertical circles around the bait (presumed to represent 'searching' behaviour) before landing on it. This observation accords with the findings of Okada (1962), who observed the same behaviour in the genus *Amiota*, of which *Phortica* was previously considered a subgenus (Duda, 1934). Unlike the flight pattern described above, however, other drosophilids fly straight onto bait without hesitating around it (Okada, 1962).

The modeling of the potential ecological niches of this fly represents a new tool for the prediction of sites in which *P. variegata* might occur. The maps presented here were drawn on the basis of the climatic and environmental characteristics of the areas in which both *P. variegata* and *T. callipaeda* and/or *P. variegata* only have been reported. This approach offers intriguing perspectives for understanding the geographical and ecological distributions of flies involved in the transmission of *T. callipaeda* eyeworm. Nevertheless, a major limitation of this method is the crude spatiotemporal resolution of the climate and environment projections (Peterson & Shaw, 2003). In fact, if we compare the modeled areas of distribution of *P. variegata* in Italy and Europe, the former appears to be the more accurate, probably due to the use of a regional climate dataset at high spatiotemporal resolution as well as the CORINE land cover dataset. As the biology and distribution of insects are highly dependent on microhabitat and climate, the accuracy of prediction models is not easy to determine. For this reason climate datasets often represent the limiting variable for the modeling of insect distribution (Peterson & Shaw, 2003). Further aspects of the biological activity of *P. variegata* adults (e.g. radius flight range and longevity of adults) would probably help to increase the reliability and sensitivity of such a model and thus our understanding of the dynamics of human and canine thelaziosis in other areas. Although the reports of *P. variegata* in the literature as well as its potential distribution estimated in the models cover a vast area of Europe, reports of cases of canine thelaziosis refer to only limited and well defined areas. This could be explained by the fact that this disease is confined to rural environments with a high presence of hunting and/or sheep dogs that have frequent access to the areas constituting the natural habitat of *P. variegata*. In these conditions, a relatively low number of heavily affected dogs might be considered the source of transmission of thelaziosis in strictly defined areas. However, it is possible that animal and human clinicians have been aware of canine thelaziosis for a relatively short time and for this reason reports are scant. This hypothesis is supported by the fact that since the first report of canine thelaziosis in Italy (Rossi & Bertaglia, 1989), its occurrence has quickly been registered in other Italian as well as European areas (Otranto *et al.*, 2003; Chermette *et al.*, 2004; Hermosilla *et al.*, 2004), whereas fox thelaziosis has been largely neglected.

P. variegata undoubtedly constitutes an interesting model with which to explore the evolution of a non-parasitic insect toward a parasitic behaviour, which is, in this case, connected with the transmission of nematodes and, probably, of several other pathogens.

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