

Research Article

Cite this article: Valera F, Veiga J, Sandoval A, Moreno E (2018). Coexistence, habitat associations and puparia description of three dipteran species of the Family Carnidae. *Parasitology Open* 4, e1, 1–9. <https://doi.org/10.1017/pao.2017.23>

Received: 17 August 2017
Revised: 29 November 2017
Accepted: 29 November 2017

Key words:

Carnus hemapterus; coexistence; ectoparasite; habitat selection; *Hemeromyia anthracina*; *Hemeromyia longirostris*; puparium

Author for correspondence:

Francisco Valera, E-mail: pvalera@eeza.csic.es

Coexistence, habitat associations and puparia description of three dipteran species of the Family Carnidae

Francisco Valera, Jesús Veiga, Alba Sandoval and Eulalia Moreno

Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (EEZA-CSIC), Ctra. de Sacramento s/n, La Cañada de San Urbano, Almería, E-04120, Spain

Abstract

Differentiation of niche by means of resource partitioning facilitates coexistence of species with similar requirements. Here we analyse the association between different habitats (i.e. nest types) and two Diptera species of the poorly known Family Carnidae that coexist during their larval and pupal stage in the nests of troglodytic bird species. We also describe for the first time the puparium of *Hemeromyia anthracina* and *Hemeromyia longirostris* and offer morphometric data of the puparia of these two species and of *Carnus hemapterus*. Both the smaller size and the occurrence of well-developed spiracles allow easy discrimination of the puparium of *C. hemapterus*. The puparia of both *Hemeromyia* species is very similar and only differ in the distance between the small spiracles. *Hemeromyia anthracina* and *C. hemapterus* coexisted in nest boxes but the former species did not occur in natural sandy cavities where, in turn, *C. hemapterus* was highly prevalent. *Carnus hemapterus* prevalence did not differ between nest boxes and natural cavities but its abundance was higher in the first type of nest. This study shows clear associations of the two dipteran species with specific types of nests. Yet, some conditions are seemingly acceptable for both species.

Introduction

The study of the factors that allow coexistence of closely related species has been an important subject in ecology for decades and various mechanisms (e.g. niche differentiation, temporal segregation) have been reported to prevent or reduce competitive exclusion (Tilman, 1982; Hairston *et al.* 1996; Leisham *et al.* 2014). Specifically, niche differentiation is a process by which competing species use the environment differently, therefore, facilitating coexistence. Niche differentiation can be achieved in different ways. For instance, differences in microhabitat selection criteria (even within the same general habitat type) may result in some spatial segregation that reduces interspecific competition. Studies based on interspecific comparisons among closely related species occurring in sympatry (Dearn, 1977; Dingle, 1978; Tauber and Tauber, 1981) can contribute to a better understanding of habitat selection criteria and stable coexistence by means of resource partitioning (Tauber and Tauber, 1981, 1982). Such studies can nonetheless be hampered by the lack of information on basic aspects of the study species, for example, the proper description and identification of different life stages of each species.

The Family Carnidae (Diptera, Schizophora) is a poorly investigated group of flies that includes parasitic species (genus *Carnus* Nitzsch 1818) as well as non-parasites belonging to the genus *Meoneura* (Rondani 1856) and *Hemeromyia* (Coquillet 1902) (Grimaldi, 1997; Brake, 2011; Stuke, 2016). Very little is known about the different species of the genus *Hemeromyia* and the available information is restricted to reports on their geographic distribution and to some notes on their biology (e.g. Papp, 1984, 1998; Carles-Tolrá, 2002). *Carnus hemapterus* has been studied in more detail (see, e.g., Capelle and Whitworth 1973; Guiguen *et al.* 1983; Dawson and Bortolotti 1997; Roulin 1998, 1999; Valera *et al.* 2004, 2006a, b; Václav *et al.* 2008; Valera and Zidková 2012; Amat-Valero *et al.* 2012), but several important aspects of its natural history are still unknown. Moreover, our knowledge is skewed since the most information available refers to the adult phase, although the requirements of other phases (e.g. larval and pupal stages), and therefore their biology and habitat preferences, may be very different. In fact, basic information, such as the description of the various life stages and of the puparium of many species of this family, is missing.

The only study about ecological aspects of *Hemeromyia* species is that by Valera *et al.* (2006b). These authors studied the coexistence of pupae and emergence phenology of imagoes of *C. hemapterus* and two species of *Hemeromyia* (*H. longirostris* and *H. anthracina*). Since all three species develop larval and pupal stages in birds' nests and in all cases the larvae feed on the organic matter that accumulates at the bottom of the nests (Grimaldi, 1997; Papp 1998), it has been hypothesized that coexistence within the same nest could result in competition among different species. Valera *et al.* (2006b) found no evidence of interspecific competition during the larval phase but they did find interspecific differences in habitat selection criteria: *C. hemapterus* appeared to avoid nests lined with plant material. They pointed out that more information about the occurrence of *Hemeromyia* species was necessary before drawing any

© Cambridge University Press 2018. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

conclusions about their habitat preferences. Valera *et al.* (2006b) also emphasised that knowledge of the natural history of these species is insufficient, what hinders addressing fundamental questions for this interesting study system (see, e.g., Soler *et al.* 1999, 2014; Martín-Vivaldi *et al.* 2006; Václav *et al.* 2008; Calero-Torralbo *et al.* 2013).

Here we intend to: (i) offer a complete description of the puparium of the above-mentioned species; (ii) study possible differences in habitat selection of two sympatric species of carnid flies, *C. hemapterus* and *H. anthracina*. Larvae of both species are saprophagous and they can be found in the same cavities, so that interspecific competition is likely. Our hypothesis is that competition during the larval stage may decrease if adults of the two carnid species prefer different types of avian nest substrates for egg laying. We predict that the prevalence and abundance of *C. hemapterus* and *H. anthracina*, calculated on the basis of pupae occurrence, in two different avian nest types (nest boxes and natural cavities in sandstone cliffs) will differ.

Materials and methods

Study species

Carnus hemapterus is a generalist ectoparasite about 2 mm in length, parasitizing nestlings of various species of birds (Grimaldi, 1997; Papp 1998; Brake, 2011). Its life cycle encompasses an adult (parasitic) phase, three larval stages and a pupal stage (Bequaert, 1942). Diapausing pupae are found in the nests of the host species. Imagoes, initially winged, emerge at the beginning of the spring and can remain in the nest where they emerged or disperse in search of hosts. Once these are located, adult flies lose their wings and feed on blood, epidermal cells and skin secretions. Mating occurs on the host and eggs are laid in the nest. After the larval stages, the pupa enters into diapause. A short diapause of a few weeks (Amat-Valero *et al.* 2012), a long diapause of some months (allowing it to hibernate in the nest, Guiguen *et al.* 1983) and a prolonged diapause of several years (Valera *et al.* 2006a) have been reported. The puparium of *C. hemapterus* has been described (Capelle and Whitworth, 1973; Sabrosky, 1987; Papp 1998) even though data on its morphometry is very scarce. Little is known about the dispersion of this parasite. It is considered that the flies are not transmitted by the host but colonize the nests actively during the winged phase of its life cycle (Grimaldi, 1997).

Very little is known about the species of the genus *Hemeromyia* and even their basic requirements are undetermined, which explains that *H. anthracina* and *H. longirostris* have seldom been collected. Valera *et al.* (2006b) found both species and *C. hemapterus* in nest boxes in Western Spain. It can, therefore, be deduced that they develop various stages of their life cycle (egg, larva and pupa) in the nest of various species of birds, mainly troglodytic ones, such as the European roller *Coracias garrulus*, Common kestrel *Falco tinnunculus*, Spotless starling *Sturnus unicolor* and Little owl *Athene noctua*. These bird species nest in natural hollows in trees and sandy slopes, nest boxes and in human constructions. All of these birds are regular breeders in our study areas.

Hemeromyia anthracina and *H. longirostris* are flies about 2–3 mm in length and their adult phases, unlike *C. hemapterus*, are not parasitic. Adults are suspected of feeding on flower nectar (Carles-Tolrá, 2002), while larvae appear to be saprophagous (Papp, 1998). To our knowledge, the puparium of these species has never been described.

The main morphological difference between *C. hemapterus* and *H. anthracina* imagoes lies in alar venation (Papp, 1998; Brake, 2011), whereas *H. longirostris* is easily distinguished by the presence of an elongated rostrum (Carles-Tolrá, 1992).

Study area and collection of nest material

The main study area (c. 50 km²) lies in the Desert of Tabernas (Almería, SE Spain, 37°05'N, 2°21'W). The climate in this area is semi-arid with high annual and seasonal rainfall variability (mean annual rainfall c. 218 mm), and strong thermal oscillations with interannual differences. Summers are long and hot and winters are usually mild.

Ten nest boxes and eight natural cavities used by birds were sampled in Almería on 4 February and 18 March 2016 with the aim of highlighting habitat associations for each carnid study species. Nest boxes were made of wood or cork and were prepared for rollers. Thus, they contained a layer of sand where birds laid their eggs. All nest boxes sampled were used the previous breeding season by rollers even though in some cases starlings and sparrows bred there before the arrival of rollers. The former bird species usually add vegetal matter in the nest box to build their nests and, once the rollers occupy the boxes, some of such vegetal matter remains there. Natural cavities sampled had been previously used by rollers (six cases) or little owls (two cases) and the substratum was entirely sandy. Five additional nest boxes were sampled in July 2017 in search of *C. hemapterus* pupae for morphometric studies.

A second study area lies in Cáceres province (Western Spain, 39° 03'N, 5°14'W), where Valera *et al.* (2006b) reported the coexistence of the three carnid species. A sampling of detritus from nest boxes located in the area was carried out on 24 January 2016 (25 nest boxes) to find and describe the puparia of the three study species. Since only a single individual of *H. longirostris* emerged from such samples, we sampled 17 different nest boxes on 22–23 February 2017.

Sampling consisted of taking material (sand, detritus and organic matter – feces, insect remains, and vegetal material used for the elaboration of the nest –) from nests by hand or with the aid of a spoon tied to the end of a stick. In the nests from Extremadura most of the detritus was collected whereas in Almería only a fraction of the nests content was taken. The samples were placed in plastic bags and transferred to the Estación Experimental de Zonas Áridas (Almería).

Samples treatment

The samples were processed shortly after they were collected (on 28 January 2016 and on 27 February 2017 for samples from Extremadura; on 10 February 2016 and 21 March 2016 and on 2 August 2017 for samples from Almería). First, they were allowed to air-dry and then were put through a sieve column to obtain a sediment between 1 and 4 mm, thus ensuring that the pupae of *C. hemapterus*, about 2 mm in size (Capelle and Whitworth, 1973), were collected. It was assumed that the pupae of other Carnidae flies would have similar or slightly larger sizes, given the small difference in size between the imagoes of the species under consideration. The resulting material from each nest was weighed and stored in individualized and labelled transparent tubes.

The sieved samples collected from Extremadura in 2016 were observed periodically in order to detect the emergence of flies. Once emergence of the study species was detected (in just three samples), the whole material of such samples was examined with a Nikon SMZ645 binocular loupe to find any kind of pupae. From each of the Almería samples collected in 2016, subsamples of 8 g were selected at random and scrutinized in search of pupae. We also sought for pupae in two subsamples of 5 g from each of the samples collected in 2017 from Extremadura and Almería.

Identification of pupae

Apparently viable pupae (i.e. without external signs of breakage) were sorted in morphotypes according to size, presence/absence of spiracles and ornamentation of the latter.

Identification of the pupae of *C. hemapterus* was carried out according to Capelle and Whitworth (1973) and Papp (1998). Both the identified pupae of *C. hemapterus* and the remaining unidentified pupae were individually stored in Eppendorf tubes. Periodic monitoring (at least every 3 days) was done to check the emergence of the imagoes and, thus, identify the species with the aid of the binocular loupe. Whereas just one individual of *H. longirostris* was obtained from samples taken in 2016, several dozens of *H. anthracina* and *H. longirostris* emerged from samples taken in 2017. Pupae of identified imagoes were collected and measured with a micrometer and the binocular loupe. Measurements were taken of the maximum length, maximum width and the distance between spiracles of 30 pupae of *C. hemapterus* in 2016 and of 30 pupae of *C. hemapterus* in 2017, 23 pupae of *H. anthracina* and 68 pupae of *H. longirostris* in 2017. All *C. hemapterus* pupae used for the morphometric study come from Almería, whereas the ones of *Hemeromyia* spp. come from Extremadura.

Photographs of the pupae were taken with a Nikon SMZ1500 binocular loupe equipped with a digital viewfinder and the software NIS-Elements BR3.1.

For a more detailed description of the pupae, pictures were taken with the scanning electron microscope (SEM) of the University of Almería. The pupae were mounted on aluminium stubs and attached to them using double-sided graphite tape. They were coated with gold by the ion sputtering method using a BAL-TEC sputter coater, model SCD 005. The coating has a thickness of approximately 20 nm. Samples were visualized in high vacuum by the secondary electron signal (SE) with a HITACHI SEM, model S-3500N.

Statistical methods

Prevalence (percentage of infected nests among all examined) and 95% confidence intervals were calculated for each species. Two thousand replications were used for the estimation of confidence intervals. Median abundance (median number of pupae found in nests examined, whether or not they were parasitized) and median intensity (median number of pupae found in infected nests) and their respective quartiles were also calculated.

We used Fisher tests to compare prevalences, and median tests to compare medians of abundances and intensities. Statistical tests were performed with Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005) and STATISTICA (Dell Inc., 2016).

We used linear mixed effect models (LME) to study: (i) inter-annual differences in the size of the puparium of *C. hemapterus*, and (ii) interspecific differences in the size of the puparium of *Hemeromyia* spp. The dependent variables were maximum length, maximum width and the distance between spiracles. Fixed factors were the year for the first aim and species for the second one. In both cases, we considered nest as a random effect and examined and accounted for the correlation structure among dependent samples. The interaction between the factors was not studied because: (i) in the case of *C. hemapterus*, the nests sampled were different each year; (ii) we did not find pupae of both *Hemeromyia* species in each nest and in some nests the number of pupae found for one or both species was low. Normality of residuals was met. In cases with heteroscedasticity we used the varIdent function in nlme 3.1-131 package (Pinheiro *et al.* 2017). These tests were carried out with R software, version 3.4. (R Development Core Team, 2017).

Results

Description of the puparia of the Family Carnidae

The puparium of three species (*C. hemapterus*, *H. anthracina* and *H. longirostris*) was identified after the emergence of the

corresponding imagoes (more than 100 individuals for each of the first two species and several dozens for the third one).

The puparium of *C. hemapterus* is reddish-brown in colour, cylindrical or barrel-shaped, and has annular ornamentations distributed over most of its length (Fig. 1). Its main feature is the presence of two very notorious divergent spiracles, each with three digitiform extensions, at the caudal end (Fig. 2).

The puparium of *H. anthracina* is light brown, with marked annular ornamentations, larger, and wider than the one of *C. hemapterus* (see below) (Fig. 3). It also has two spiracles, but unlike the ones of *C. hemapterus*, they are quite small and without prolongations (Fig. 4). There is some variability in the size of the spiracles: some pupae have small but perceptible spiracles with the binocular loupe, whereas the spiracles can hardly be seen with the loupe in other individuals. All the pupae observed show, at the caudal end, a depression bounded by marked rims (Fig. 4).

The puparium of *H. longirostris* is very similar to the one of *H. anthracina* (Fig. 5), with short, non-ornamented spiracles at the caudal end and a conspicuous depression close to the spiracle (Fig. 6).

The identification by external characters examined with a binocular loupe is possible for *C. hemapterus* but not for both *Hemeromyia* species (Figs 7 and 8).

Morphometry of the puparia of the Family Carnidae

None of the dimensions of the puparium of *C. hemapterus* varied between years (LME model, year: $P > 0.20$ in all cases; Estimate \pm

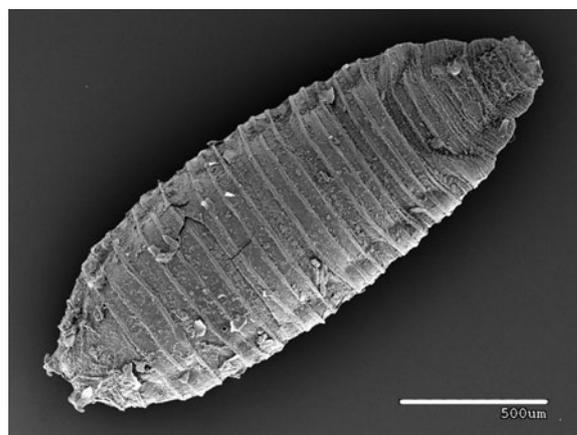


Fig. 1. Puparium of *Carnus hemapterus*.

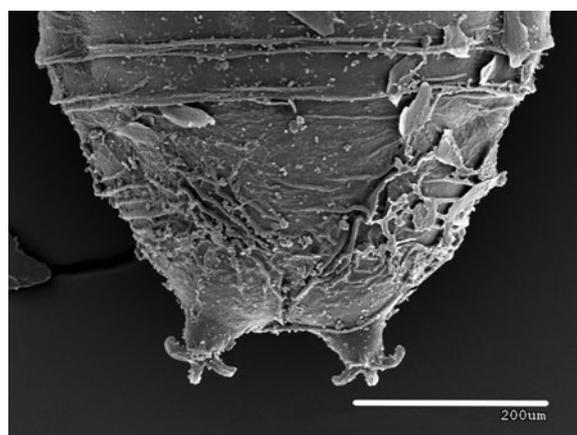


Fig. 2. Puparium of *Carnus hemapterus*. Spiracles with short curved finger-like projections are evident.

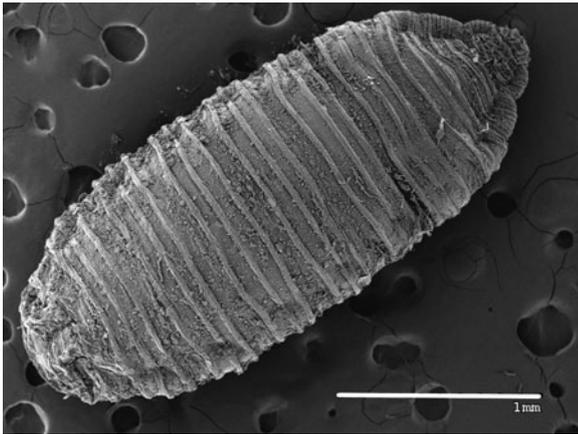


Fig. 3. Puparium of *Hemeromyia anthracina*.

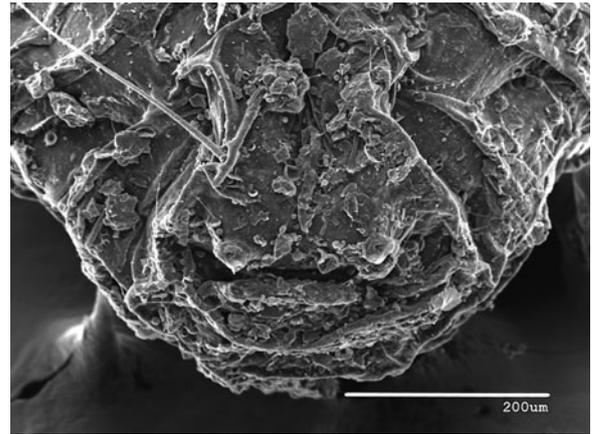


Fig. 6. Spiracles of the puparium of *Hemeromyia longirostris* and the depression close to them.

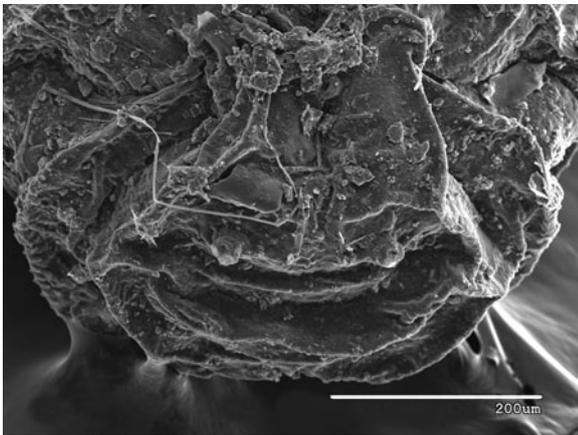


Fig. 4. Puparium of *Hemeromyia anthracina* with small, non-ornamented spiracles and the depression bounded by marked rims.



Fig. 7. Puparium of *Carnus hemapterus* (left), *Hemeromyia longirostris* (middle) and *H. anthracina* (right).

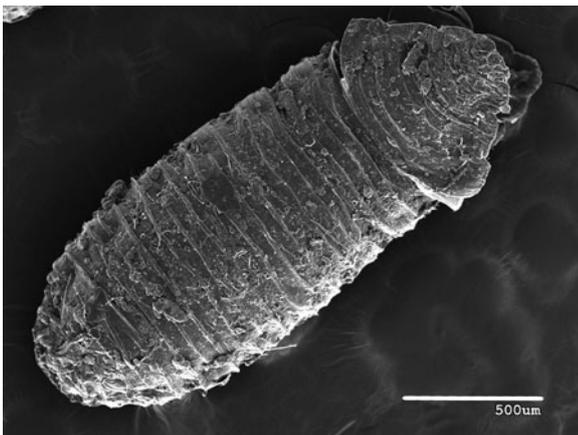


Fig. 5. Puparium of *Hemeromyia longirostris*.



Fig. 8. Detail of the caudal end of the puparia of *Carnus hemapterus* (left), *Hemeromyia longirostris* (middle) and *H. anthracina* (right).

s.e.: length 2016: 1.80 ± 0.04 , length 2017: 1.77 ± 0.04 ; width 2016: 0.63 ± 0.01 , width 2017: 0.60 ± 0.02 ; distance between spiracles 2016: 0.09 ± 0.003 , distance between spiracles 2017: 0.09 ± 0.003 , $n = 30$ for 2016 and 30 for 2017; Fig. 9).

The maximum length and width of the puparium of *H. anthracina* and *H. longirostris* did not differ (LME model, species: $P > 0.05$ in both cases; Estimate \pm s.e.: length *H. anthracina*: 2.13 ± 0.05 , length *H. longirostris*: 2.06 ± 0.04 ; width *H. anthracina*: 0.79 ± 0.03 , width *H. longirostris*: 0.76 ± 0.01 , $n = 23$ for *H. anthracina* and 68 for *H. longirostris*; Fig. 10). However, the

distance between spiracles was significantly longer for *H. anthracina* (LME model, $F = 20.3$, $P < 0.001$, Estimate \pm s.e.: *H. anthracina*: 0.12 ± 0.003 , *H. longirostris*: 0.10 ± 0.002 , $n = 23$ for *H. anthracina* and 68 for *H. longirostris*; Fig. 10).

Habitat associations of two sympatric carnid flies, *C. hemapterus* and *H. anthracina*

Only two species, *H. anthracina* and *C. hemapterus*, were found in Almeria.

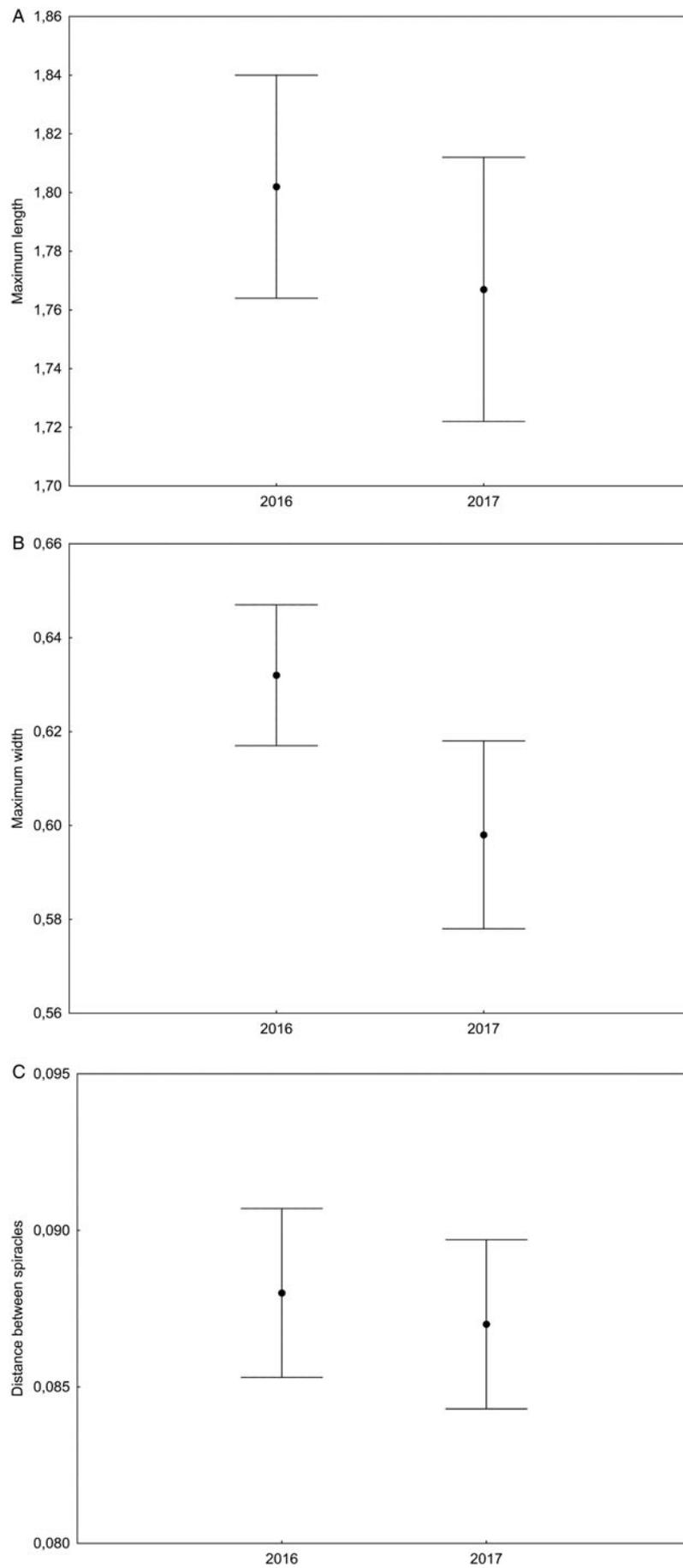


Fig. 9. Interannual differences in (A) maximum length, (B) maximum width and (C) distance between spiracles (estimated values \pm s.e.) of the puparium of *Carnus hemapterus* in 2016 and 2017.

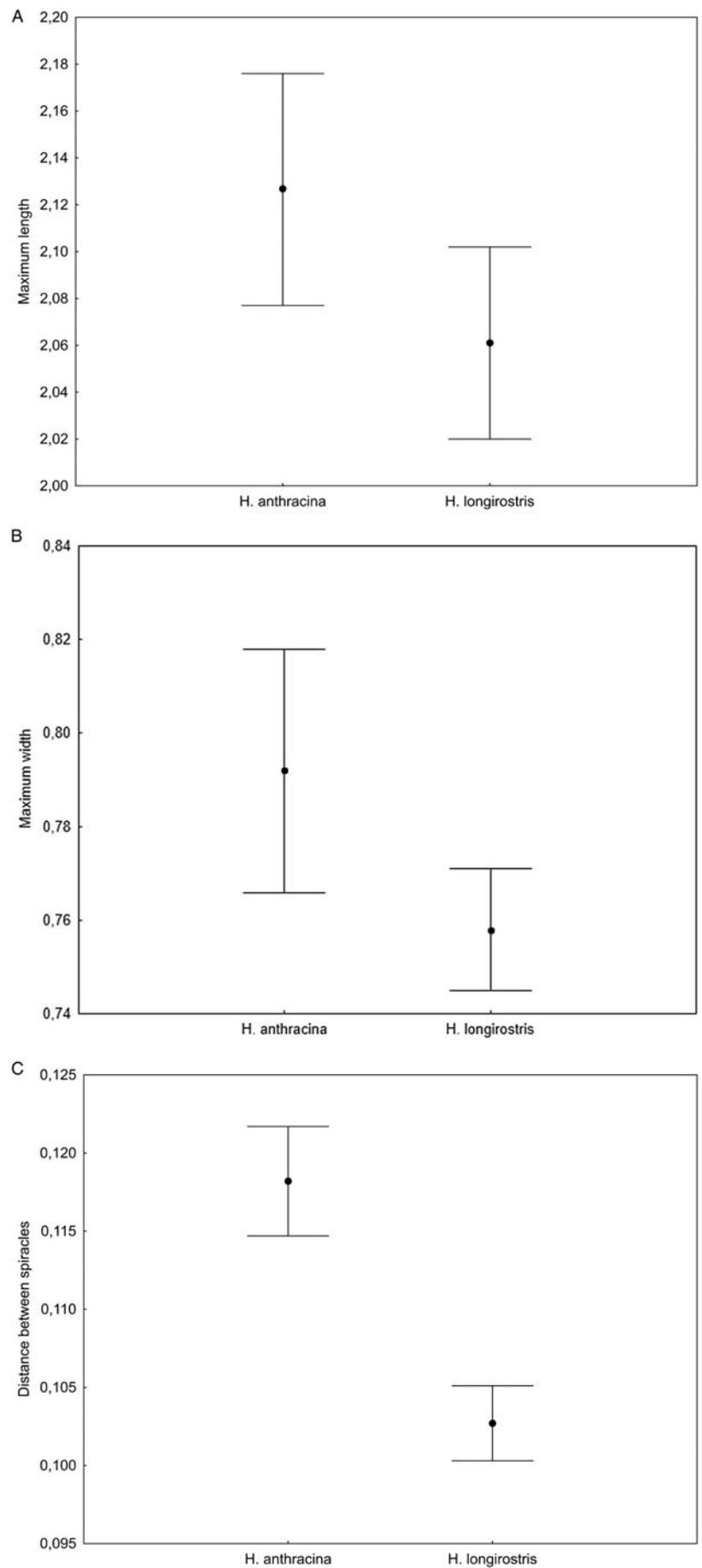


Fig. 10. Differences in (A) maximum length, (B) maximum width and (C) distance between spiracles (estimated values \pm s.e.) of the puparia of *Hemeromyia anthracina* and *H. longirostris*.

Table 1. Prevalence, median abundance and median intensity of viable pupae of *Carnus hemapterus* and *Hemeromyia anthracina* in 8 g of detritus taken from nest boxes and nests in natural cavities sampled in Almería (southeast Spain)

	<i>Carnus hemapterus</i>		<i>Hemeromyia anthracina</i>	
	Nest box (10)	Natural cavity (8)	Nest box (10)	Natural cavity (8)
Prevalence	80.0 [44.4–97.5]	75.0 [34.9–96.8]	50.0 [18.7–81.3]	0.0
Median abundance	17.5 [3.0–35.0]	3.50 [0.5–7.0]	0.5 [0.0–3.0]	–
Median intensity	21.5 [14.0–51.5]	5.5 [3.0–7.0]	3.0 [2.0–17.0]	–

Sample size (in brackets), 95% confidence intervals for prevalence and quartiles for median abundance and intensity [in square brackets] are shown.

The prevalence of viable pupae of *C. hemapterus* and *H. anthracina* in nest boxes did not differ significantly (80.0 vs 50.0%, respectively; two-tailed Fisher's test, $P = 0.35$, Table 1). Pupae of both species were found in 50% (five out of ten) of the nest boxes. The median abundance of *C. hemapterus* pupae in nest boxes was significantly higher than that of *H. anthracina* (Median test, $P = 0.02$). The median intensity of *C. hemapterus* pupae in nest boxes also tended to be higher than that of *H. anthracina* (Table 1), although the differences were not significant (Median test, $P > 0.10$).

In contrast, *H. anthracina* pupae were not found in natural cavities, whereas viable pupae of *C. hemapterus* were found in 75% of the cavities sampled (two-tailed Fisher's test, $P = 0.009$) (Table 1).

The prevalence of *C. hemapterus* did not differ between the two nest types (two-tailed Fisher's test, $P = 1.0$). However, both the median pupae abundance and the median pupae intensity were significantly higher in the nest boxes than in the natural cavities (Median test, abundance: $P < 0.05$; intensity: $P < 0.01$).

Discussion

This paper provides a full description, including morphometrics, of the puparium of *C. hemapterus*, *H. anthracina* and *H. longirostris* after unequivocally verifying the emergence of adults of the collected pupae, being the most complete report until the date for *C. hemapterus* and the first one for the two later species. We also describe patterns in habitat associations of *C. hemapterus* and *H. anthracina* that can reflect partial niche segregation.

Description of the pupae of the Family Carnidae

The determination of key traits for the identification of various stages of closely related species is important because it enables further studies on significant processes occurring at these phases (e.g. diapause during the pupal phase, see Amat-Valero *et al.* 2013 for *C. hemapterus*) that are frequently longer than the adult phase for many insect species. Moreover, the possibility of identifying sister taxa facilitates comparative studies on relevant topics such as coexistence, niche partitioning or the evolution of life histories (Tauber and Tauber, 1981, 1982).

Our results show that the puparium of *C. hemapterus* that we describe coincides with the description provided elsewhere (Capelle and Whitworth, 1973; Sabrosky, 1987; Papp 1998). Both the occurrence of two obvious spiracles and its morphometry (much smaller size than both *Hemeromyia* species, Fig. 7) distinguish the puparium of this parasite from that of *H. anthracina* and *H. longirostris*. In contrast, distinguishing the puparium of the two *Hemeromyia* species is not straightforward. Pupae of both species obtained from the same location and year did not differ in length and width and we only found significant differences in the distance between spiracles (larger in *H. anthracina*). Even though significant, such differences are small and could

depend on factors such as food availability or seasonality that are known to influence larval and pupal mass and size (Williams and Richardson, 1983; Tsuda and Takagi, 2001; Temeyer, 2009). We did not find interannual differences in puparium size of *C. hemapterus* but this can be different for *Hemeromyia* spp. or for other study years. Therefore, caution about the reliability of the distance between spiracles for distinguishing between both *Hemeromyia* spp. is necessary.

Differences in habitat selection and coexistence

This study is based on the absence/occurrence and abundance of pupae of two dipteran species in two different cavity types used by birds. We assume that the presence of larvae and pupae of a given species in a cavity reflects the choice of such cavity by the adult phase as an appropriate habitat for subsequent developmental stages. In contrast, the absence of pupae of a given species in a cavity is more difficult to interpret. It could be that our study area is not within the range of the species or that the cavity is not selected by the imago because it is considered unsuitable for larval or pupal development. Other factors, such as larval/pupal predation or fungal infection, could also account for the absence of pupae of a given species in a cavity.

Coexistence of the three studied species in the same cavities has been cited by Valera *et al.* (2006b) in western and south Spain (ca. 40 km far from our study area). *Hemeromyia longirostris* was not detected in this study and, contrary to the two other species, has not been found in the study area in spite of intensive sampling of imagoes in nest boxes in several years (personal observation). Our study area lays in the most arid region of continental Europe and is quite different from the areas where this species was found, so that it could be that *H. longirostris* is not distributed along the arid southeastern Spain. Alternatively, other reasons, like the ones mentioned above, may account for the absence of the species in the cavities sampled.

We recorded the occurrence of *H. anthracina* in nest boxes but not in natural cavities. The latter could be explained by several non-mutually exclusive factors: (i) larvae/pupae may have been differentially preyed/infected in natural cavities. However, given the similarities in the biology and morphology of the larval and pupal stages of *C. hemapterus* and *Hemeromyia* it seems unlikely that differential predation or infection occurs in a given cavity type for a given species; (ii) emergence of *H. anthracina* imagoes could pass unnoticed to us. Still, Valera *et al.* (2006b) described a similar emergence phenology for both species and we did not find open pupae of *H. anthracina* in the material from natural cavities neither in this study nor in previous ones; (iii) this cavity type is not selected by *H. anthracina*. The substratum of natural cavities in sandstone cliffs is essentially sandy, whereas in nest boxes (where the species is found) it may include plant material (depending on the bird species using it). Valera *et al.* (2006b) did not find any of the *Hemeromyia* species in nests of birds

breeding in the sandy substratum. These results suggest that *H. anthracina* could avoid nests with such substratum.

Concerning *C. hemapterus*, it was highly prevalent in both cavity types. The prevalence and abundance of this parasite are known to depend on innate host features such as ontogeny or immune capacity (Valera *et al.* 2004; Václav *et al.* 2008) but also on the habitat used by its hosts (Guiguen *et al.* 1983). Similarly to Fargallo *et al.* (2001) and Calero-Torralbo *et al.* (2013) we found that nest boxes were more infected than natural cavities. Microclimatic differences among nest types (see Amat-Valero *et al.* 2014) could influence parasites' choice and/or survival. Alternatively, differences in the cleaning efficiency of both nest types by adult birds can result in different amounts of detritus (and diapausing pupae) left in the cavities.

Coexistence of *H. anthracina* and *C. hemapterus* was therefore restricted to nest boxes. We found co-occurrence of both species in 50% of the nest boxes, a value somewhat higher than the 35% found by Valera *et al.* (2006b). Being non-parasites, *Hemeromyia* species are probably less dependent on the innate characteristics of the bird species, but they can still depend on host-related attributes such as the type of nest material used by the bird. Nest boxes in our study area were prepared for rollers and, thus, had a sandy layer. Nonetheless, some of them also had some vegetal matters (twigs, leaves) introduced by other birds (e.g. Spotless starling *S. unicolor*) in the nest before the rollers took ownership of it. Valera *et al.* (2006b) found that *C. hemapterus* avoided nests lined with vegetable matter but the combination of sand and vegetal material seemingly resulted in an acceptable habitat for *C. hemapterus* and for *H. anthracina*. Moreover, the high variability in the abundance of *C. hemapterus* observed in the nest boxes sampled could result from differences in the amount of vegetal matter in them. Thus, the relative abundance of each fly species may depend on host nesting behaviour and on the bird species occupying the nest box. This is important because slight interspecific differences in habitat selection criteria (even within the same general habitat type) by carnid species, may result in some spatial segregation and reduced interspecific competition.

The reasons why a sandy substrate or a vegetal one within a cavity could be unsuitable for each insect species and the particular stage(s) of the insects sensitive to the type of substratum remain to be investigated. Future studies should also quantify the abundance of *C. hemapterus* and *Hemeromyia* species for each type of substratum.

Acknowledgements. We are grateful to Esmeralda Urea for assistance with the SEM. Fran Moyano (Universidad de Almería) supported this research and gave useful comments. Jesús Porras, Javier Prieta and José Carlos Lopez kindly helped to locate nest boxes in Extremadura. An anonymous referee improved earlier versions of this paper. Junta de Andalucía and Junta de Extremadura kindly provided permits to sample birds' nests.

Financial support. F.V. and E.M. received financial support from the Spanish Ministry of Economy and Competitiveness (grant no. CGL2014-55969-P) and the European Regional Development Fund. J.V. was funded by the Spanish Ministry of Economy, Industry and Competitiveness by means of a predoctoral grant (grant no. BES-2015-075951).

References

- Amat-Valero M, Václav R, Martínez T and Valera F (2012) Mixed life-history strategies in a local population of the ectoparasitic fly *Carnus hemapterus*. *Parasitology* **139**, 1045–1053.
- Amat-Valero M, Calero-Torralbo MA and Valera F (2013) Temperature during the free living phase of an ectoparasite influences the emergence pattern of the infective phase. *Parasitology* **140**, 1357–1367.
- Amat-Valero M, Calero-Torralbo MA, Václav R and Valera F (2014) Cavity types and microclimate: implications for ecological, evolutionary and conservation studies. *International Journal of Biometeorology* **54**, 1983–1994.

- Bequaert J (1942) *Carnus hemapterus* Nitzsch, an ectoparasitic fly of birds, new to America (Diptera). *Bulletin of Brooklyn Entomological Society* **37**, 140–149.
- Brake I (2011) World catalog of the family Carnidae (Diptera, Schizophora). *Myia* **12**, 113–169.
- Calero-Torralbo MA, Václav R and Valera F (2013) Intra-specific variability in life-cycles synchronization between an ectoparasitic fly and its avian host. *Oikos* **122**, 274–284.
- Capelle KJ and Whitworth TL (1973) The distribution and avian hosts of *Carnus hemapterus* (Diptera: Milichiidae) in North America. *Journal of Medical Entomology* **10**, 525–526.
- Carles-Tolrá M (1992) New species of Carnidae and Lauxaniidae (Diptera) from Spain. *Entomologist's Monthly Magazine* **128**, 63–67.
- Carles-Tolrá M. (2002) Catálogo de los Diptera de España, Portugal y Andorra (Insecta). In Sociedad Entomológica Aragonesa (SEA) (ed.). *Monografías de la Sociedad Entomológica Aragonesa*, vol. **8**. Zaragoza: Sociedad Entomológica Aragonesa, 323pp.
- Dawson RD and Bortolotti GR (1997) Ecology of parasitism of nestling American Kestrels by *Carnus hemapterus* (Diptera, Carnidae). *Canadian Journal of Zoology* **75**, 2021–2026.
- Dearn JM (1977) Variable life history characteristics along an altitudinal gradient in three species of Australian grasshopper. *Oecologia* **28**, 67–85.
- Dell Inc. (2016) Dell Statistica (data analysis software system), version 13. Software.dell.com.
- Dingle H (ed.) (1978) *Evolution of Insect Migration and Diapause*. New York/Heidelberg/Berlin: Springer, 284pp.
- Fargallo JA, Blanco G, Potti J and Viñuela J (2001) Nest box provisioning in a rural population of Eurasian kestrels: breeding performance, nest predation and parasitism. *Bird Study* **48**, 236–244.
- Grimaldi D (1997) The bird flies, Genus *Carnus*: species revision, generic relationships and a fossil *Meoneura* in amber (Diptera: Carnidae). *American Museum Novitates*. N° 3190, American Museum of Natural History, New York.
- Guiguen C, Launay H and Beaucornu JC (1983) Ectoparasites des oiseaux en Bretagne. I. Répartition et écologie d'un diptère hematophage nouveau pour la France: *Carnus hemapterus* Nitzsch. *Revue Française d'Entomologie* **5**, 54–62.
- Hairston NG Jr, Ellner S and Kearns CM (1996). Overlapping generations: the storage effect and the maintenance of biotic diversity. In Rhodes OE Jr, Chesser RK and Smith MH (eds). *Population Dynamics in Ecological Space and Time*. Chicago: University of Chicago, pp. 109–145.
- Leishnam PT, LaDeau SL and Juliano SA (2014) Spatial and temporal habitat segregation of mosquitoes in Urban Florida. *PLoS ONE* **9**, e91655.
- Martin-Vivaldi M, Ruiz-Rodríguez M, Méndez M and Soler JJ (2006) Relative importance of factors affecting nestling immune response differs between junior and senior nestlings within broods of hoopoes *Upupa epops*. *Journal of Avian Biology* **37**, 467–476.
- Papp L (1984). Family Carnidae. In Soos A and Papp L (eds). *Catalogue of Palearctic Diptera*, vol. **10**. Budapest: Akademiai Kiadó, pp. 118–124.
- Papp L (1998). Family Carnidae. In Papp L and Darvas B (eds). *Manual of Palearctic Diptera*. vol. **3**. Budapest: Science Herald, pp. 211–217.
- Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2017) Nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131. 2017. Available at <https://CRAN.R-project.org/package=nlme>.
- R Core Team. (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2017. Available at <https://www.R-project.org/>.
- Reiczigel J and Rózsa L (2005) *Quantitative Parasitology 3.0*. Budapest. Distributed by the authors.
- Roulin A (1998) Cycle de reproduction et abondance du diptère parasite *Carnus hemapterus* dans les nichées de chouettes effraies *Tyto alba*. *Alauda* **66**, 265–272.
- Roulin A (1999) Fécondité de la mouche *Carnus hemapterus*, ectoparasite des jeunes chouettes effraies *Tyto alba*. *Alauda* **67**, 205–212.
- Sabrosky CW (1987) Carnidae. In McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR and Wood DM (eds). *Manual of Nearctic Diptera*, vol. **II**. Agriculture Canada Research Branch Monograph vol. 28. Ottawa: Canada Communication Group Pub, pp. 909–912.
- Soler JJ, Møller AP, Soler M and Martínez JG (1999) Interactions between a brood parasite and its host in relation to parasitism and immune defence. *Evolutionary Ecology Research* **1**, 189–210.
- Soler JJ, De Neve L, Martín-Gálvez D, Molina-Morales M, Pérez-Contreras T and Ruiz-Rodríguez M (2014) Do climatic conditions affect host and

- parasite phenotypes differentially? A case study of magpies and great spotted cuckoos. *Oecologia* **174**, 327–338.
- Stuke JH** (2016) Carnidae (Diptera) in the Canadian National Collection of insects (Ottawa), with the description of five new species. *Zootaxa* **4084**, 540–556.
- Tauber CA and Tauber MJ** (1981) Insect seasonal cycles: genetics and evolution. *Annual Review of Ecological Systems* **12**, 281–308.
- Tauber CA and Tauber MJ** (1982) Evolution of seasonal adaptations and life history traits in *Chrysopa*: response to diverse selective pressures. In Dingle H and Hegmann JP (eds). *Evolution and Genetics of Life Histories*. New York: Springer Verlag, pp. 51–72.
- Temeyer KB** (2009) Nutritional limitation on growth and development of horn fly (Diptera: Muscidae) larvae. *Southwestern Entomologist* **34**, 263–272.
- Tilman D.** (1982). *Resource Competition and Community Structure*. Princeton, New Jersey, USA: Princeton University Press.
- Tsuda Y and Takagi M** (2001) Survival and development of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) larvae under a seasonally changing environment in Nagasaki, Japan. *Environmental Entomology* **30**, 855–860.
- Václav R, Calero-Torralbo MA and Valera F** (2008) Ectoparasite load is linked to ontogeny and cell-mediated immunity in an avian host system with pronounced hatching asynchrony. *Biological Journal of the Linnean Society* **94**, 463–473.
- Valera F and Zidková L** (2012) Reproductive anatomy and fecundity estimation of the haematophagous ectoparasite *Carnus hemapterus*. *Parasitology Research* **110**, 1733–1739.
- Valera F, Hoi H, Darolová A and Kristofik J** (2004) Size vs health as a cue for host choice: a test of the Tasty Chick Hypothesis. *Parasitology* **129**, 59–68.
- Valera F, Casas-Crivillé A and Calero-Torralbo MA** (2006a) Prolonged diapause in the ectoparasite *Carnus hemapterus*: how frequent is it in parasites? *Parasitology* **133**, 179–188.
- Valera F, Martín-Vivaldi M and Carles-Tolrá M** (2006b) Life-history variation in three coexisting species of Carnid flies (Diptera: Carnidae), *Carnus hemapterus*, *Hemeromyia anthracina* and *Hemeromyia longirostris*. *European Journal of Entomology* **103**, 347–353.
- Williams H and Richardson AMM** (1983) Life history responses to larval food shortages in four species of necrophagous flies (Diptera: Calliphoridae). *Australian Journal of Ecology* **8**, 257–263.