1 **Defining host range: host-parasite compatibility during the non-infective phase of**
2 **the parasite also matters**

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14 SUMMARY

15 Host range and parasite specificity determine key epidemiological, ecological and
16 evolutionary aspects of host–parasite interactions. Parasites are usually classified as
17 generalists or specialists based on the number of hosts they feed on. Yet, the
18 requirements of the various stages of a parasite may influence the suitability of a given
19 host species. Here we investigate the generalist nature of three common ectoparasites
20 (the dipteran *Carnus hemapterus* and two species of louse flies, *Pseudolynchia*
21 *canariensis* and *Ornithophila metallica*), exploiting two avian host species (the
22 European roller *Coracias garrulus* and the Rock pigeon *Columba livia*), that frequently
23 occupy the same breeding sites. We explore the prevalence and abundance of both the
24 infective and the puparial stages of the ectoparasites in both host species. Strong
25 preferences of *Pseudolynchia canariensis* for pigeons and of *Carnus hemapterus* for
26 rollers were found. Moderate prevalence of *Ornithophila metallica* was found in rollers
27 but this louse fly avoided pigeons. In some cases, the infestation patterns observed for
28 imagoes and puparia were consistent whereas in other cases host preferences inferred
29 from imagoes differed from the ones suggested by puparia. We propose that the adult
30 stages of these ectoparasites are more specialist than reported and that the requirements
31 of non-infective stages can restrict the effective host range of some parasites.

32 Key words: *Carnus hemapterus*, louse fly, *Pseudolynchia canariensis*, *Ornithophila*
33 *metallica*, host range, generalist.

34 Key Findings:

- 35 - Host preferences of three allegedly generalist diptera ectoparasites are described.
- 36 - The infective stages of *Pseudolynchia canariensis*, *Ornithophila metallica* and
37 *Carnus hemapterus* show clear host preferences.

- 38 - For some parasites, host preferences inferred from imagoes differed from the
- 39 ones suggested by puparia.
- 40 - The requirements of non-infective stages of some parasites can restrict the
- 41 effective host range.

42 INTRODUCTION

43 Host range is a key element of parasites' ecology and evolution (Appelgren *et al.* 2016).
44 According to host range, parasites (and other organisms such as herbivores or
45 parasitoids) are usually classified as generalists or specialists (see, for instance, Barrett
46 and Heil 2012; McCoy *et al.* 2013; Loxdale and Harvey 2016), even though such
47 categories are vague and are currently under review (Jorge *et al.* 2014; Loxdale and
48 Harvey 2016). Parasites success depends on both host profitability (in terms of
49 resources acquisition) and the microenvironment provided by the host, which together
50 define host-parasite compatibility and can differ between hosts (Lemoine *et al.* 2011).
51 Therefore, the breadth of environments/hosts in which a parasite species can succeed is
52 ultimately determined by the full pattern of its vital rates in each environment/host,
53 including all the life stages (egg, larva, pupa, and imago) (Caswell 1983).

54 Animals are expected to select resources according to their impact on fitness
55 (Brodeur *et al.* 1998; Krasnov *et al.* 2004). However, an imperfect concordance between
56 host selection and insect fitness has been frequently reported for phytophagous and
57 parasitic insects (Thompson 1988; Courtney and Kibota 1990; Horner and Abrahamson
58 1992; Caron *et al.* 2010). This disagreement can arise from a variety of determinants.
59 For instance, among parasites, host availability plays a key role, which depends on host
60 densities but also on parasites' ability for finding a host (Kortet *et al.* 2010; McCoy *et*
61 *al.* 2013). Increasing the range of hosts (e.g. by ecological fitting, Agosta *et al.* 2010;

62 Araujo *et al.* 2015) could increase the chances of survival but the new hosts could be
63 suboptimal since the real host range will be determined by the fitness the parasite gets in
64 each of the hosts (Ward *et al.* 1998). Another reason for an imperfect concordance
65 between host selection and parasites' fitness is the inability of the latter to assess host
66 suitability (reviewed by Fox and Lalonde 1993), that can occur, among other reasons,
67 by the fact that different life cycle stages (e.g. larval, puparial or imaginal stages in
68 insects) have different levels of specialization (Loxdale and Harvey 2016). Our
69 knowledge on the requirements of the non-infective phases of many parasites has
70 increased substantially. Yet, more research is needed since integration of the ecology of
71 all life stages of parasites is necessary for a better understanding of the epidemiology of
72 parasitic diseases (e.g. Pietrock and Marcogliese 2003; O'Connor *et al.* 2006).

73 Here we examine host choice by three allegedly generalist avian, nest-based
74 ectoparasites, the dipteran *Carnus hemapterus* and two species of louse flies (Family
75 Hippoboscidae), exploiting two avian host species, the European roller *Coracias*
76 *garrulus* and the Rock pigeon *Columba livia*. *Carnus hemapterus* is a widespread bird
77 parasite in the Holarctic and Nearctic (Grimaldi 1997; Brake 2011). Hippoboscid flies
78 (Hippoboscidae) are worldwide distributed, obligatory parasites attacking a wide variety
79 of bird species (Boyd 1951; Maa 1969). Whereas the imagoes of both species feed on
80 birds, the non-parasitic stages of their life cycle dwell in birds' nests. The European
81 roller and the Rock pigeon are secondary hole-nesting birds whose nesting
82 environments are ecologically similar but that, otherwise, differ in several key life-
83 history traits (migration, breeding phenology, clutch size, composition of the nest
84 material), that may impose divergent selective pressures on parasites.

85 We hypothesise that host selection by the infective phase of the parasites is
86 correlated with the suitability of the host and its environment for the development of the

whole life cycle of the parasite. We predict that all stages of the parasites should perform better on the host where imagoes (the choosing stage) reach the higher prevalence and abundance. If so, such estimates of parasitization will be good indicators of host-parasite compatibility and can be used for defining host range. Alternatively, prevalence and abundance of the imago in a given host will not correlate with prevalence and abundance of other stages of the parasite in the same host, so that imago's selection will not be a good indicator of host suitability and parasites' host range. To test this hypothesis we evaluated during two years the parasitization of *Carnus hemapterus*, *Pseudolynchia canariensis* and *Ornithophila metallica* on two different avian hosts and estimated puparial abundance in the nests as a surrogate of host-parasite compatibility during the non-infective stage.

MATERIALS AND METHODS

Study species and study area

The study area (around 50 km²) is located in the Desert of Tabernas (Almería, SE Spain, 37°05'N, 2°21'W). The landscape mostly consists of open shrubland with olive and almond groves interspersed among numerous dry riverbeds with steep sandstone banks – ramblas –. The climate is temperate, semiarid Mediterranean with strong water deficit during the long, hot summer months. The mean annual rainfall is ca. 230 mm, with high inter-annual and intra-annual variability (Lázaro *et al.* 2001). The average temperature is 18°C, with mild inter-annual oscillations of 3–4 °C and significant intra-annual fluctuations (Lázaro *et al.* 2004).

Carnus hemapterus (hereafter *Carnus*) is a 2-mm long, nidicolous ectoparasitic fly that colonizes nestling birds of several dozens of species (Grimaldi 1997; Brake 2011). Its life cycle comprises an adult phase, three larval stages and a puparial phase

(Guiguen *et al.* 1983). The puparia are found in the detritus of the nests of the host species. The imagoes (the infective stage) emerge from the puparia after winter diapause and throughout the spring when nestling hosts are available (Valera *et al.* 2003; Calero-Torralbo *et al.* 2013). After their emergence, adults are initially winged, but lose their wings as soon as they locate a suitable host (Roulin, 1998). Once emerged, adult *Carnus* cannot survive a long time without feeding and its dispersal period is seemingly short (around 2–3 days; Calero-Torralbo 2011; Veiga *et al.* unpubl. data). Mating occurs on the host and eggs are laid in the nest. Larvae are saprophagous and perform two moults (Papp 1998). After the third larval stage, the pupa enters into diapause. In most cases, imagoes emerge the next breeding season. However, prolonged diapause has been recorded for this parasite, so that some pupae remain longer in diapause and adult flies emerge after two or more wintering seasons, therefore enabling *Carnus* to persist in the nest for several years (Valera *et al.* 2006). This haematophagous parasite (Kirkpatrick and Colvin 1989; Dawson and Bortolotti 1997) can have detrimental effects on nestling health (Whitworth 1976; Cannings 1986; Soler *et al.* 1999, but see Kirkpatrick and Colvin 1989; Dawson and Bortolotti 1997; Liker *et al.* 2001).

Hippoboscid flies are hematophagous ectoparasites. More than 200 species are recognised, most of them parasitise birds belonging to 18 orders (Maa 1969; Lloyd 2002; Lehane 2005). Imagoes spend most of their time on the body of the bird, where they feed on blood several times a day (Coatney 1931). Hippoboscids attack more juvenile than adult birds and imagoes die usually within two or three days when removed from the host (Boyd 1951). Larval development occurs almost entirely within the female. The pupa is formed almost immediately after laying, which occurs in the nest of birds. The insects apparently overwinter as puparia in the hosts' nests (Boyd

136 1951). With the exception of the larval and puparial phase, its dependence on the birds'
137 nest is lower than in the case of *Carnus* since adult flies do not lose the wings and are
138 capable of flying between hosts (Harbison *et al.* 2009; Harbison and Clayton 2011).
139 Hippoboscids cause direct and indirect threats to the health and fitness of their hosts
140 (Waite *et al.* 2012). In our study area, we have identified two species of hippoboscid
141 parasites on birds (*Pseudolynchia canariensis* and *Ornithophila metallica*).
142 *Pseudolynchia canariensis* (hereafter *Pseudolynchia*) parasitizes mainly pigeons, but it
143 has a wider host range than closely related species and has been described attacking
144 several dozens of bird species (Maa 1966, 1969). Adults copulate on the host. Eggs
145 hatch in utero in the female fly, and then three stages of larvae feed from “milk” glands
146 in the female fly (Harwood and James 1979). The larvae pupate and female flies deposit
147 puparia in the substrate in or around pigeon nests (Bishopp 1929). Pupal development is
148 sensitive to temperature and can span 36—55 days (Klei and Degiusti 1975; Mandal
149 1989). The female produces on average eight pupae during its lifetime, which is on
150 average 24 days under laboratory conditions (range: 6—70 days) (Klei and Degiusti
151 1975). *Ornithophila metallica* (hereafter *Ornithophila*) is a poorly-known species. It has
152 been described parasitizing a variety of bird species, including several species of the
153 families Columbidae and Coraciidae (Maa 1969).

154 The European roller (hereafter roller) is a secondary cavity nesting bird. It is a
155 trans-Saharan migrant that arrives to south Spain during April. In our study area, the
156 nest is a slight depression at the sandy bottom of cavities in cliffs or in the nest boxes.
157 They lay a single clutch of two to seven eggs. Nestlings are naked at hatching but, by
158 the age of 13 days, their body is almost completely covered with closed feather sheaths
159 (Cramp 1998). Juveniles fly from the nest about 20—22 days after hatching (Václav *et*
160 *al.* 2008). Rollers do not expel their faeces from the nest-cavity (Sosnowski and

161 Chmielewski 1996), where detritus can accumulate after several breeding seasons, even
162 though nest sanitation behaviour is common.

163 Rock pigeons (hereafter pigeons) also use natural cavities and human
164 constructions to breed but not nest boxes. This resident species breeds at any time of the
165 year, but peak times in our study area are spring and summer. The nest is a light
166 platform of straw and sticks, laid under cover. Pigeons lay two eggs and incubation lasts
167 around 18 days (Johnston and Janiga 1995). The newly hatched nestlings have pale
168 yellow down and a flesh-coloured bill. For the first few days, the nestlings are fed
169 exclusively on "crop milk". The fledging period is about 30 days (Johnston and Janiga
170 1995). Droppings accumulate in the nest cavity that usually is filled becoming
171 unsuitable for breeding after several nesting events.

172 The distribution of roller and pigeon nests along the study area is patchy and
173 breeding patches can be defined according to distinct geomorphological units (Václav *et*
174 *al.* 2011): (1) ramblas, with nest boxes for rollers and with natural cavities occupied by
175 rollers, pigeons and other cavity-nesting bird species; (2) individual bridges with
176 numerous, densely spaced cavities (ca. 2—3 m apart), suitable for rollers, pigeons and
177 other bird species, and (3) spatial aggregations of suitable nesting places— mostly trees
178 with nest boxes but also small sandstone banks with natural cavities and isolated
179 country houses with cavities. Rollers, pigeons and other cavity nesting species (mostly
180 Common kestrel *Falco tinnunculus*, Little owl *Athene noctua*, Eurasian jackdaw *Corvus*
181 *monedula*) co-occur more frequently along ramblas and bridges. Moreover, cavities in
182 sandy cliffs and in bridges or abandoned country houses are frequently used
183 successively (both within the season and among seasons) by these bird species.

184 *Ectoparasite estimation in birds*

185 Fieldwork was carried out in 2016 and 2017. Clean nest boxes provided with unsoiled
186 sand were installed at the beginning of the 2016 and 2017 breeding season for roller
187 reproduction. Cavities in sandy cliffs and in human constructions were examined in
188 search of breeding pigeons. Occupied nest boxes and cavities were monitored along the
189 breeding seasons.

190 The prevalence and abundance of *Carnus* imagoes in 251 nestling rollers (32
191 nests in 2016 and 38 in 2017) and 35 nestling pigeons (9 nests in 2016 and 10 nests in
192 2017) was determined by examining chicks at the mid-nestling stage (i.e. when they are
193 covered by closed feather sheaths), when the peak of parasite infestation occurs (Václav
194 *et al.* 2008). Roller and pigeon broods were carefully taken from the nest and placed in a
195 cotton bag. Subsequently, each nestling was taken and the number of carnid flies on the
196 body surface of each chick was counted twice and then we averaged both counts. This
197 visual census method has been found to be reliable (Roulin 1998; Václav *et al.* 2008).

198 The prevalence and abundance of hippoboscids flies in 251 nestling rollers (32
199 nests in 2016 and 38 in 2017) and 42 nestling pigeons (10 nests in 2016 and 13 nests in
200 2017) was determined in each nest coinciding with the estimation of carnid flies
201 following the same method (i.e. search of flies on body surface and between sheaths).
202 Nonetheless, since the hippoboscids flies are much more mobile than *Carnus*, quickly
203 leaving the bird when manipulated, underestimation of the actual parasite load is
204 possible. Therefore, we took advantage of successive monitoring of the nests for other
205 purposes and checked the abundance of louse flies on nestlings several times. We used
206 the maximum number of flies observed in each nest for the calculation of prevalence
207 and abundance of these parasites. Imagoes collected from both bird species as well as
208 flies emerging from collected nest detritus (see below) were used for identification
209 purposes.

210 *Sampling nest detritus*

211 During October—November 2016 and July 2017 nest boxes and cavities occupied by
212 the study bird species during the previous breeding seasons were sampled (2016: roller:
213 32 nest boxes, pigeon: 26 cavities; 2017: roller: 38 nest boxes, pigeon: 10 cavities).

214 Nest material from rollers' nests (consisting essentially of sand, excrements and
215 insect remnants) was collected by hand. Pigeons' nests, which consisted on sticks used
216 to make the nest, and a compact mass of excrements that usually included organic
217 remains like vegetable matter, shells and remains of dead nestlings, were completely
218 removed.

219 The collected material was stored in plastic bags that were transferred to the
220 Estación Experimental de Zonas Áridas where it was kept in a dark room with open
221 windows to resemble natural conditions (i.e. ambient temperature moderated by partial
222 enclosure and semi-darkness). The samples collected in 2016 were stored 2—7 months
223 until processing whereas the ones collected in 2017 were stored about two months.

224 *Nest material treatment*

225 *Searching for *Carnus hemapterus* puparia*

226 A sample of nest material of each roller and pigeon nests was sieved using a column of
227 4 mm, 1 mm and 0.5 mm sieves. Material ≤ 1 mm was collected and two subsamples of
228 5 g per nest were selected. During January—March 2017 (for samples from 2016) and
229 July—August 2017 (for samples from 2017) such subsamples were visually examined
230 with a binocular loupe Nikon SMZ645 in search of *Carnus* puparia, that were identified
231 following Papp (1998) and Valera *et al.* (2018) . We distinguished between apparently

232 viable puparia (intact, closed puparia) and open puparia. Intact puparia were stored in
233 transparent tubes.

234 For the calculation of the prevalence and abundance of *Carnus* puparia in
235 pigeons and rollers nests, only viable puparia were considered. Counts of each
236 subsample of 5 g per nest were averaged.

237 *Searching for Hippoboscidae pupae*

238 A second sample of nest material of each roller (except for 2017, for which only 14
239 nests were examined) and pigeon nests was sieved using a column of 4 and 1 mm
240 sieves. Material collected in the second sieve was retained. In 2016 100 gr of such
241 material was selected for rollers and pigeons, even though for this second species we
242 could not get such amount in all nests (range: 42—100 gr). In 2017 the amount of nest
243 material scanned in search of Hippoboscidae pupae ranged 16—42 gr for rollers and
244 42—310 gr for pigeons. During March—May 2017 (for samples from 2016) and
245 August 2017 (for samples from 2017) the selected material was extended in a tray and
246 puparia were sought visually. We distinguished between apparently viable puparia
247 (intact, closed puparia) and open puparia. Only intact puparia (that were stored in
248 transparent tubes) were used to calculate prevalence and abundance and values were
249 extrapolated to 5 gr in both years.

250 We found just one type of puparium that was identified as *Pseudolynchya*
251 *canariensis* after the emergence of the corresponding imagoes from the puparia
252 individually stored in plastic tubes (following Hutson 1984).

253 In both years, nest detritus from rollers and pigeons not examined in search of
254 pupae was also stored during the autumn-winter and scanned subsequently until next

spring in search of emerged flies. In this way, we got some hippoboscids from pigeon detritus that were identified.

Statistical analyses

Prevalence (proportion of infected nests among all the nests examined) and mean intensity of imagoes and puparia of each parasite species (mean number of individuals found in the infected nests) and their respective 95% confidence intervals were calculated. Fisher's exact tests and bootstrap 2-sample t-tests were used for comparing prevalences and mean intensities, respectively; 2,000 replications were used for estimation of confidence intervals and bootstrap t-tests. Unless otherwise noted, average values and standard errors are given and the tests performed are 2-tailed. Statistical significance was set at $p < 0.05$.

Statistical tests were done with the program Quantitative Parasitology 3.0 (Reiczigel and Rózsa 2005) and Statistica Academic 13 (Dell Inc. 2016).

Ethical note

Trapping and handling of birds undertaken in this study was approved by the Dirección General de Gestión del Medio Natural, Consejería de Medio Ambiente, Junta de Andalucía.

RESULTS

Occurrence of infective and non-infective phases of the parasites in two host species

Prevalence of imagoes and puparia of each parasite in each host species did not differ between years for any of the parasite species (Fisher tests, $P > 0.20$ in all cases), so that data from both years were pooled for each parasite. Similarly, no interannual differences

277 in mean intensity of imagoes and puparia per infected nest were found except for
278 *Pseudolynchia* puparia in pigeon (see below). Thus, data for both years are shown
279 separately only for this case.

280 *Carnus hemapterus*

281 The prevalence and intensity of imagoes of *Carnus* in rollers' nests is high.
282 Correspondingly, the prevalence and intensity of puparia is also high (Table 1).

283 Pigeons seem to be less attractive than rollers for *Carnus*, given that both the
284 prevalence and intensity of imagoes per infected nest is significantly lower (Prevalence,
285 Fisher test: $P < 0.001$, Intensity: bootstrap 2-sample t-test: $t = 6.8$, $P < 0.001$, $n = 19$,
286 70). Importantly, the prevalence of carnid puparia in pigeon nests is more than nine
287 times smaller than the prevalence of *Carnus* imagoes in nestling pigeons and a single
288 puparium was found in samples of thirty-six nests (Table 1).

289 *Ornithophilla metallica*

290 The prevalence of imagoes in rollers is ca. 17% and we found a mean intensity of one
291 fly per infected nest. In contrast, we did not find a single puparium in samples from
292 forty-six roller nests (Table 1).

293 Pigeons were not infected by *Ornithophilla metallica*: neither imagoes nor
294 puparia were found in nestling pigeons and nests (Table 1).

295 *Pseudolynchia canariensis*

296 No imago or puparium were found in nestling rollers or nests (Table 1). In contrast, the
297 prevalence of imagoes on nestling pigeons was high as it was the intensity of imagoes
298 (mean: 2.65 flies per nest, range: 1–6). We also found that at least 36% of the nests

299 harboured *Pseudolynchia* puparia. The intensity of puparia in pigeon nests varied
300 significantly between years (bootstrap t-test: $t = 2.6$, $P = 0.04$, $n = 10, 3$) (Table 1).

301 DISCUSSION

302 Here we provide information about the parasitisation of three allegedly generalist
303 ectoparasitic flies on two secondary hole-nesting bird species whose nesting
304 environments are ecologically similar. Whereas these parasitic flies are widely
305 distributed we were unable to find detailed information about their parasitic load on our
306 study species or on other bird species (see below). Data on puparia in the nests are even
307 scantier so that comparisons are done only when information was found. Thus, our data
308 contribute to a better knowledge of the epidemiology of these common parasites.
309 Moreover, we compare the parasitisation pattern of these ectoparasitic flies considering
310 both the prevalence and abundance of the infective, imaginal stage and the puparial
311 stage on both bird species. In some cases (e.g. for *Carnus* in rollers, for *Pseudolynchia*
312 in pigeons and rollers and for *Ornithophila* in pigeons) the pattern observed for imagoes
313 and puparia were consistent whereas in other cases (e.g. *Carnus* in pigeons and
314 *Ornithophila* in rollers) host preferences inferred from imagoes differed from the ones
315 suggested by puparia.

316 All three parasite species have been frequently quoted as generalist ones. *Carnus*
317 has been reported parasitizing 64 host species (including the roller and the pigeon) from
318 24 avian families from raptors to passerines (Grimaldi 1997; Brake 2011 and references
319 therein). Similarly, although *Pseudolynchia canariensis* shows preference for
320 Columbiformes, it has been described attacking many other bird species, including the
321 genus *Coracias* (Maa 1966, 1969). Klei and Degiusti (1975) and references therein
322 report lack of host specificity in laboratory colonies. *Ornithophila metallica* was

classified by Maa (1969) in the group of louse flies with very wide host range, citing this parasite species in 134 bird genera, including the genus *Coracias* and two Columbidae. In our study area rollers and pigeons commonly breed interspersed, frequently at short distances from each other and even using successively the same cavities. So, detection of each parasite in both bird species would be expected. Yet, our results suggest strong host preferences and rejections. Considering the parasitic stage we found that: i) *Carnus* prefers rollers over pigeons. The high prevalence and parasitic load of imagoes in nestling rollers found in this study agrees with previous information (Václav *et al.* 2008, see also Soltész *et al.* 2018 for other species). We were unable to compare our results on pigeons since, to our knowledge, there is no published information; ii) adult *Pseudolynchia* flies were frequently found on nestling pigeons but never on nestling rollers. Pigeons are known to be a preferred host of this louse fly, and the load of adult flies per nest in our study area is within the range reported for the species (Maa 1966; Adang *et al.* 2009, but see Amaral *et al.* 2013 for a higher load). Concerning rollers, we could find only a record of a *Coracias* sp. parasitized by *P. canariensis* (Maa 1966); iii) adult *Ornithophila* flies were never recorded in pigeons but they were found parasitizing nestling rollers in ca. 20% of nests. Again, comparisons of our results are limited by the scant data available. These results therefore suggest that the low host specificity reported for these flies cannot be generalized.

In four out of six study cases (three parasites and two hosts) the information provided by the prevalence and abundance of puparia of each parasite in each host nest agrees with the one obtained from imagoes on nestling hosts: i) parallel to imagoes, *Carnus* puparia are abundant in rollers nests (see also Valera *et al.* 2018); ii) the occurrence of *Pseudolynchia* puparia in pigeon nests is compatible with the occurrence of imagoes in nestling pigeons; iii) the absence of *Pseudolynchia* imagoes on nestling

348 rollers agrees with the nil abundance of puparia in rollers nests; iv) similarly, the
349 absence of *Ornithophila* imagoes on nestling pigeons matches with the absence of
350 puparia in the nests. In these cases, clear and consistent preference/rejection criteria can
351 be deduced.

352 In contrast, for two other systems we found that host choice by the imago did not
353 correspond with the occurrence of the puparial, non-parasitic stage in the host' nest.
354 Carnid flies showed a moderate prevalence in pigeon nests (26%) whereas the
355 occurrence (both prevalence and abundance) of puparia in the nests is very low. Pigeons
356 often nest in cavities previously occupied by other birds, most commonly rollers, that
357 usually contain diapausing carnid puparia. Therefore, parasitization of the nestling
358 pigeons by *Carnus* is very likely the result of the use of cavities infected with
359 diapausing puparia (i.e. involuntary host shifting, see Calero-Torralbo and Valera
360 2008). Since the amount of puparia in rollers nests can be very high (e.g. here we found
361 ca. 10 puparia/5 gr and more than 0,5 kg of detritus can accumulate in a roller nest
362 during a breeding season) and the mean intensity of adult flies in nestling pigeons is
363 very low, we suspect that freshly emerged flies in pigeon nests migrate in search of
364 other host species and that nestling pigeons are, in fact, rejected by *Carnus*. Similarly,
365 *Ornithophila* flies were relatively common in roller nests and the parasitic load found (1
366 fly/nest) is probably underestimated (Maa 1969 reports that the highest density per
367 infested bird was 3 flies). However, no puparium was found in any nest during two
368 breeding seasons.

369 It could be argued that our sampling effort has not been intense enough to detect
370 parasites in some cases. However, we think that our results are reliable because: i) the
371 number of sampled nests and nestlings of both species is appropriate and the results for
372 both breeding seasons are consistent in nearly all cases; ii) subsequent monitoring of the

373 nestlings of both species for other purposes did not render different results; iii) we did
374 not find *Carnus*, *Ornithophila* or *Pseudolynchia* imagoes emerging from non-
375 monitored, stored detritus of pigeon and roller nests respectively whereas we did record
376 emergence of *Pseudolynchia* from stored pigeon nest detritus.

377 The cases where host suitability deduced from the occurrence of the infective
378 and non-infective phase differs suggests that host compatibility filters occur at the later
379 stage of the parasite. Pigeon nests does not seem a suitable environment for *Carnus*
380 because, in contrast to rollers nests: i) organic material (e.g. insect remains) is scarce in
381 the nest so that food for the saprophagous larvae is probably scant, ii) the nest
382 substratum is probably adverse for *Carnus* eggs, larvae and puparia. Dung of nestling
383 pigeons acts to cement the nesting material together into a sturdy adobe-like mound that
384 has also been reported to inhibit the development of some ectoparasites (Johnston and
385 Janiga 1995). Thus, the tiny eggs and larvae of *Carnus* can easily get embedded in the
386 faeces of pigeons. In this case, adult *Carnus* flies are probably physiologically able to
387 feed on nestling pigeons but parasite fitness is negatively affected given that the nest
388 may jeopardize egg, larval and/or puparia survival. Rejection of pigeon as hosts by adult
389 carnid flies (suggested by the very low load) is consistent with the unsuitability of this
390 species for other life stages of the parasite. The misleading prevalence of adult flies on
391 pigeons should be interpreted as an indirect consequence of other ecological pressures
392 (nesting behaviour of pigeons when nest sites are limiting, Václav *et al.* 2008).

393 Concerning *Ornithophila*, we ignore the reasons why puparia are absent in roller
394 nests. We do not think that the reasons given for *Carnus* in pigeon nests also hold for
395 *Ornithophila* since louse flies lay their pupae in crevices and under layers of nest
396 material (pers. obs. on *Pseudolynchia*, see also Waite *et al.* 2012). Temperature is
397 known to play an important role in puparial development of *Pseudolynchia canariensis*

(Klei and Degiusti 1975; Mandal 1989) and it could also be the case for *Ornithophila*. Since the insulation ability of nest boxes is poor, with oscillations above 30 °C within one day occurring frequently in our study area (Amat-Valero *et al.* 2014), it could be that artificial breeding places such as nest boxes are unsuitable for development of louse flies. Interestingly, an exhaustive study of dipteran assemblages in nests boxes used by different bird species did not record hippoboscids flies (Soltész *et al.* 2018). Alternatively, predation could account for the absence of puparia in the nests. Kaunisto *et al.* (2016) found remarkable predation rates of deer ked (*Lipoptena cervi*) puparia presumably by lizards, spiders, harvestmen (Opiliones) and Formicidae-ants. This could also be the case for *Ornithophila* puparia, since ants are frequently found in roller nests. More research is necessary to highlight the requirements of *Ornithophila* and the likely filters imposed by its host species and/or their close environment.

The current debate about the terms generalist and specialist warns about several flaws such as the ambiguous definition of the term or the problem raised by the abundance of cryptic species in many taxa (Loxdale and Harvey 2016). Our study suggests that the adult stages of these allegedly generalist parasites are more specialist than reported. We also suggest that the host range can differ among different phases of a parasite and that the requirements of some stages can be particularly restrictive (see also Dapporto and Dennis 2013). Thus, it is not only that simple species records are not enough to determine whether a parasite is a true host generalist (McCoy *et al.* 2013) but also that different phases of the parasite should be considered to define an organism selective environment.

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1 Table 1. Prevalence and mean intensity (with 95% confidence intervals and number of nests sampled in square brackets) of imagoes and pupae of
 2 three ectoparasitic flies, *Carnus hemapterus*, *Ornithophila metallica* and *Pseudolynchia canariensis*, on nests of two bird species, the European
 3 roller and the Rock Pigeon (data from 2016 and 2017 pooled except for the intensity of pupae of *P. canariensis* in pigeon nests).

4

		<i>Carnus hemapterus</i>		<i>Ornithophila metallica</i>		<i>Pseudolynchia canariensis</i>	
Host		Imagoes/nest	Pupae/ 5 gr. detritus	Imagoes/nest	Pupae/ 5 gr. detritus	Imagoes/nest	Pupae/ 5 gr. detritus
Roller	Prevalence	88.6% [62/70] 0.79-0.95	64.3% [45/70] 0.52-0.75	17.1% [12/70] 0.09-0.28	0.0% [0/46]	0.0% [0/70]	0.0% [0/46]
	Mean intensity	20.87 16.45-27.19	10.51 8.02-14.0	1.0 0-0	0.0	0	0
Pigeon	Prevalence	26.3% [5/19] 0.09-0.51	2.8% [1/36] 0.0007-0.14	0.0% [0/23]	0.0% [0/36]	73.9% [17/23] 0.52-0.88	36.1% [13/36] 0.21-0.54
	Mean intensity	2.20 1.20-3.20	1.0 0-0	0.0	0.0	2.65 1.76-3.65	2016: 0.19 (0.11-0.29) 2017: 0.05 (0.03-0.07)

5