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ECTOPARASITIC WATER MITE LARVAE OF THE GENUS *ARRENURUS* ON THE DAMSELFLY *COENAGRION PUELLA* (LINNAEUS) (ZYGOPTERA: COENAGRIONIDAE)

R.A. BAKER[1], P.J. MILL[1] and A. ZAWAL[2]
[1] School of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom
pabrab@leeds.ac.uk / p.j.mill@leeds.ac.uk
[2] Department of Invertebrate Zoology and Limnology, University of Szczecin,
Waska 13, PO-71-415 Szczecin, Poland
zawal@univ.szczecin.pl

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Parasitic larval mites of the genus *Arrenurus* have been found on *C. puella*. *A. bicuspidator, A. cuspidator* and *A. maculator* make up over 80% of the total mites identified. The other spp. found were *A. bruzelii, A. claviger* and members of the *A. affinis* complex. Mites are found mainly between the second and third pairs of legs and behind the third pair. *A. bicuspidator* and *A. cuspidator* share these sites with numbers spread roughly equally on both sites. *A. maculator* is found almost exclusively behind the third pair of legs and on the first abdominal segment of the host. Smaller numbers are found on the abdominal segments where *A. claviger* is the dominant species. The larval mites show a preference for ♀ hosts. Size differences between the *Arrenurus* spp. are considered.

INTRODUCTION

The biology of mites on odonates has been studied by several workers including CASSAGNE-MEJEAN (1966), LANCIANI (1970), MITCHELL (1959, 1967, 1968), ROLFF (2000) and ZAWAL (2004a, 2004b). STECHMANN (1977) provided descriptions and a key for the identification of some of the European larval species of *Arrenurus*. The subject has also been reviewed by CORBET (1999), DAVIDS (1997), SMITH (1988) and SMITH & OLIVER (1976, 1986). Until recently, however, little attention has been paid to the distribution and site selection of the various species of larval *Arrenurus* on their zygopteran hosts in Europe. ZAWAL (2006a, 2006b, 2006c, 2006d, 2007) recently described a number of species from this genus, based on their larval morphology and this has provided sufficient information to identify the species accurately. BAKER et al. (2007) published some preliminary observations on host preferences and selection sites of larval *Arrenurus* species on several odonate host species. The present paper examines one of these hosts in more detail, namely *C. puella*, and its associated parasitic mites.

MATERIAL AND METHODS

The damselflies were collected in 2006 during the peak period of adult activity in areas around Szczecin, in northwest Poland. Damselfly identification and the sex of individuals were verified later. Material was pooled from several sites in the area. Additional information from earlier collections (BAKER et al., 2007) has been used in the analysis of choice of male and female hosts. Damselflies with attached mites were preserved in 70% ethyl alcohol. Mites were counted and their host selection site noted, then removed and mounted individually on slides in Hoyers medium, each slide representing one host specimen. Counting larvae while attached to their hosts gives only a rough number and an accurate count of mites present can only be obtained by removing and mounting each one. Inevitably some mites were lost in the transfer from host to slide, other larvae became detached in the host fixative and their selection site is therefore unknown and others were damaged, making identification difficult or impossible.

RESULTS

THE PARASITES FOUND

596 larvae have been identified, using ZAWAL's (2006a, 2006b, 2006c, 2006d, 2007) descriptions and all of these have been measured. Five *Arrenurus* species have been recorded from *C. puella*: *A. bicuspidator, A. bruzelii, A. claviger, A. cuspidator* and *A. maculator*, along with members of the *A. affinis/neumani/vietsi*-complex. Three species, *A. bicuspidator* (14.3%), *A. cuspidator* (35.9%), and *A. maculator* (32.5%) make up over 80% of the total number identified. Percentage figures for the other species are: *A. claviger* 9.4%, *A. affinis*-complex 6%, *A. bruzelii* 1.8%. In addition there are some larvae that have been identified to *Arrenurus* sp. only.

PREVALENCE AND INTENSITY OF INFESTATION

306 *C. puella* were examined for parasites and sexed. Of these, 27.5% (84) were infected and 222 uninfected and the total number of mites found was 738 (Fig. 1). The mite load on infected individuals varied from 1-62, with a mean of 8.8. Only 22 of the infected hosts had mites on their abdomen. Also the number of mites from this part of the body, compared with the total number found, was comparatively low (14%).

DISTRIBUTION OF THE MOST COMMON *ARRENURUS* SPECIES ON THE HOST

The preferred area of attachment, for the majority of the *Arrenurus* parasites identified, is the posterior half of the thorax. *A. bicuspidator* (n = 86) and *A. cuspidator* (n = 214) appear to select similar sites for attachment, occurring almost invariably between legs two and three or behind the third pair of legs. In *A. cuspidator*, 95% occurred between the second and third pair of legs and behind the third pair, with numbers spread approximately equally between these two sites. *A. maculator* on the other hand is found almost exclusively behind the third pair of legs with small numbers on the first abdominal segment. Out of a total of 197 *A. maculator* identified, 92% occurred behind legs three on the thorax (Tab. I). When *A. cuspidator* and *A. maculator* occur alone on their host, the pattern appears essentially the same, although numbers are too low to draw any meaningful conclusions. *A. cuspidator, A. bicuspidator* and *A. maculator* appear therefore to prefer the posterior half of the thorax as their main area of attachment.

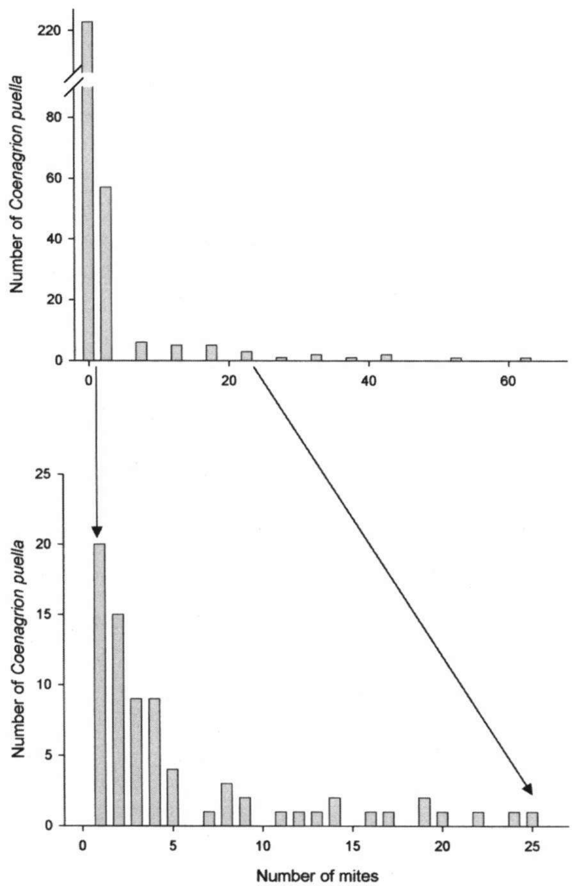

Fig. 1. Numbers of mite (all species) on all *Coenagrion puella* individuals

The situation is quite different for *A. claviger* (n = 56) where 89% were found on the abdomen, mainly on segments 3 to 6, and sometimes in the inter-segmental region between these segments. They occur most commonly on segment 4. Thus *A claviger* appears to be an abdominal parasite in terms of its selection site on the host (Tab. I).

Table I
Location of four species of *Arrenurus* on adult *Coenagrion puella*

Arrenurus	Thorax			T/A	Abdomen									Total
	1/2	2/3	3		1	2	3	4	5	6	7	8	9	
bicuspidator	4	49	30		1			2						86
cuspidator	7	98	106	3										214
maculator		2	182		13									197
claviger		4	1	1	1	1	10	24	6	7	1			56

CHOICE BASED ON SEX OF HOST

Using parasitized material from earlier collections (BAKER et al., 2007), not previously analysed for sex preference, together with the present collections, it was found that female *C. puella* carried a significantly heavier average parasite load than did male *C. puella* (Mann-Whitney test T = 2393, n (male) = 54, n (female) = 58; P<0.001) (Tab. II, Fig. 2).

χ^2 tests indicate that this female bias occurs in *A. cuspidator* (P<0.01; n = 127 mites on ♀ *C. puella* and 87 mites on ♂ *C. puella*), *A. bicuspidator* (P<0.001; n = 75 & 10), and *A. claviger* (P<0.001; n = 55 & 1). There was a slightly greater predominance of *A. maculator* on females (101) compared to males (93) but this was not significant. Inclusion of *A. cuspidator*, *A. bicuspidator* and *A. maculator* data from previous collections (268) (there were no further individuals of *A. claviger*), bringing the total number of mites in the data set from 549 to 817, made no material difference to the first two species. *A. cuspidator* (P<0.01; n = 186 mites on ♀ *C. puella* & 135 mites on ♂ *C. puella*) and *A. bicuspidator* (P<0.001; n = 91 & 10) but now indicated a strong female preference by *A. maculator* (P<0.001; n = 230 & 109).

SEASONAL DIFFERENCES

Seasonal differences have also been examined by grouping collection dates under four headings, end of May, mid June, early July and mid July. Although only limited information is available at present, it does appear as though the number

Table II
Location of larval arrenurids on male and female *Coenagrion puella*

C. puella	Thorax			T/A	Abdomen									Total
	1/2	2/3	3		1	2	3	4	5	6	7	8	9	
Females	4	141	225	4	8	0	17	29	10	9	1			448
Males	9	57	144	3	4	0	0	0	0	0	0			217
Total	13	198	369	7	12	0	17	29	10	9	1			665

of species of *Arrenurus* increase as the year progresses. In late May only *A. cuspidator* and *A. maculator* were found. By mid June five of the six species were present in the collections, only *A. bicuspidator* being absent. By early July all of the species were recorded.

SIZE DIFFERENCES OF THE PARASITES

The median mite sizes fall into two clearly defined groups (Tab. III).

The size data for the *A. affinis* complex and for *A. bruzelii* are excluded from further analysis since the former contains more than one species and the sample size of the latter is very small. The size range of each of the remaining four species is very wide and there is considerable overlap between them (Fig. 3).

The data are not normally distributed and hence Dunn's Pairwise Multiple Comparison Method was used in the analysis. It revealed a significant difference in the size distributions at $P<0.05$ for all pairwise comparisons except *A. cuspidator* and *A. maculator* (Tab. IV). The similarity between these last two species is clear (Fig. 3) with 73% of *A. cuspidator* and 85% of *A. maculator* between 200µm and 300µm in length. Although 61% of *A.claviger* are also within this range, 27% are within the range 300-400µm compared with only 7% and 6% of *A. cuspidator* and *A. maculator* respectively. *A. bicuspidator* stands out with 41% in the range 450-500µm and a further 15% and 12% in the 400-450µm and 500-550µm ranges respectively.

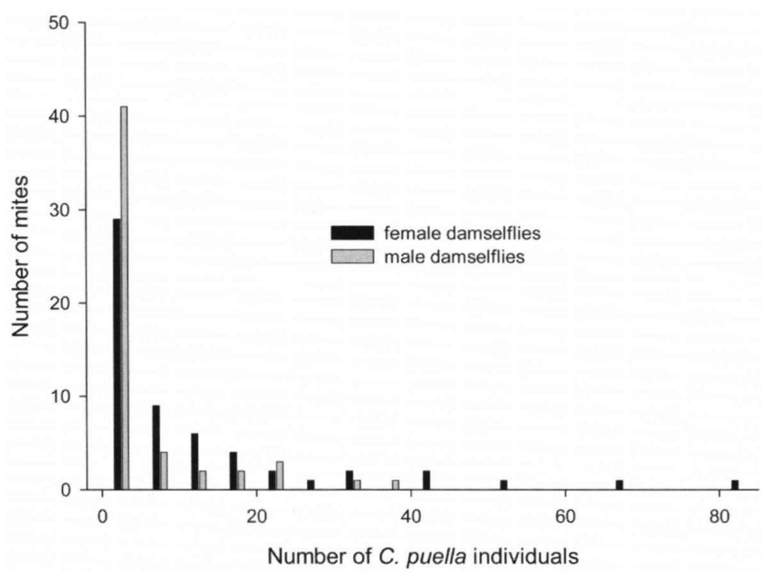

Fig. 2. Number of mites (all species) on infected *Coenagrion puella* females and males

Table III
Sizes of the 596 *Arrenurus* mites measured. All measurements are in micrometres

Arrenurus	n	Median	Mean	Minimum	Maximum
maculator	194	246	252.9	184	504
cuspidator	214	249	274.2	170	542
claviger	56	253	300.9	228	536
Affinis-complex	36	413	414.3	228	644
bicuspidator	85	456	425.3	226	528
bruzelii	11	512	488.5	324	614

Interestingly, inclusion of *A. bruzelii* in the Pairwise Comparison indicates that it is not significantly different in size from *A. bicuspidator*, but this should be viewed with caution given the small sample size of the former species.

DISCUSSION

The present paper supplements earlier work by the present authors (BAKER et al., 2007) using additional information and seeks to analyse aspects of the biology of the mites and their relationship with *C. puella*, giving particular attention to the identification and distribution of the species of *Arrenurus* on its host, choices based on the sex of the host and size differences of these parasitic larval mites.

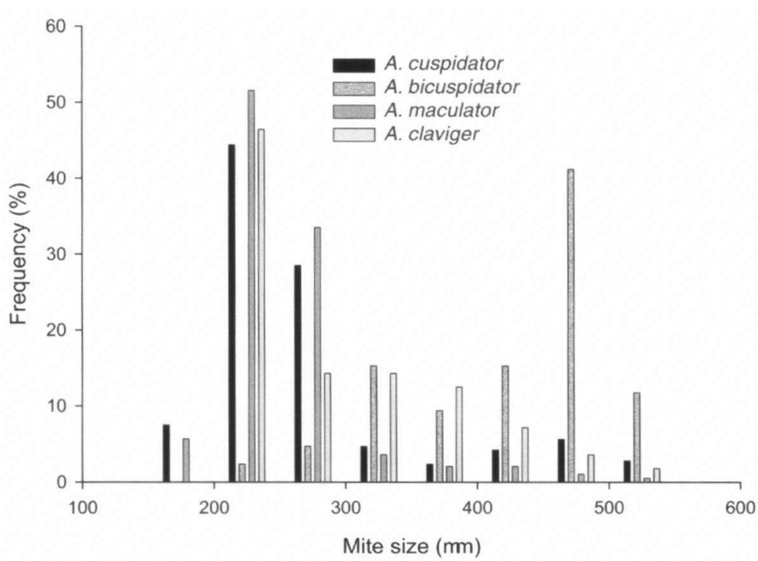

Fig. 3. Frequency size distribution of four species of mites found on *Coenagrion puella*

Table IV
Pairwise Multiple Comparisons (Dunn's Method) between the sizes of four species of arrenurid mites. Significance at P = <0.05. The multiple comparisons on ranks do not include an adjustment for ties

Arrenurus	*cuspidator*	*bicuspidator*	*maculator*	*claviger*
cuspidator	-	Yes	No	Yes
bicuspidator		-	Yes	Yes
maculator			-	Yes
claviger				-

It is clear from the present study that, in the case of *C. puella*, the majority of mites occur on the posterior half of the thorax, between the second and third pair and behind the third pair of their host. They are found in smaller numbers on the abdomen. Occasionally, but in very low numbers, mites are also found at other sites such as the head and between the first and second pair of legs. The mean intensity of the parasites in infected *C. puella* was 8.8, agreeing closely with a figure of 8 parasitic larvae per individual, in work by GRANT & SAMWAYS (2007).

The *Arrenurus* species appear to be generalist parasites, each species being found on a number of genera of host damselflies (DAVIDS, 1997; BAKER et al., 2007). DAVIDS (1997), for example, recorded the larvae of *A. bruzelii* on eight different zygopteran hosts, and recorded nine different species of *Arrenurus* from *C. puella*. However, DAVIDS (1997) review relies, to an extent, on older records which in some cases may not have provided adequate descriptions of the larvae involved. Zawal's recent descriptions of the larval species give more detailed accounts. In an earlier paper, BAKER et al. (2007) recorded four species of *Arrenurus* on *C. puella* but speculated that, with further collections, more species were likely to be found. The present study adds *A. claviger* and the *A. affinis/neumani/vietsi*-complex to that list. The most common species found are *A. bicuspidator*, *A. cuspidator* and *A. maculator,* which together make up over 80% of the total of identified mites.

Although arrenurid mites do not have single species hosts, their choice of attachment site is clearly defined in some of the species studied. In these cases resource partitioning may occur. Thus *A. claviger* is found mainly on the middle segments of the abdomen, *A. maculator* selects the region behind the third pair of legs and *A cuspidator* is found in roughly equal numbers between the second and third pair of legs and behind the third pair. Site specificity has been recorded for other species of *Arrenurus.* MITCHELL (1968) described two species of *Arrenurus* larvae from North America attached to *Paracercion hieroglyphicum* (formerly in *Cercion*), *A. mitoensis* on the thorax and *A. agrionicola* on the abdomen. In MITCHELL's work (1968) the incidence of the thoracic parasite was low but the abdominal parasite was abundant, with 98% of the hosts infected. These results are in contrast to the present findings. BOTMAN et al. (2002) also found species specific selection sites in two species of *Arrenurus* on the damselfly *Ischnura posita*. *A. major* was attached to the thorax and abdominal segments one to three, whereas *A. americanus* was attached to abdominal segments five to eight. ROLFF (2000) working with *C. puella* and *C. hastulatum*, and ANDRES & CORDERO (1998) working on *Ceriagrion tenellum* demonstrated a clumped distribution of mites on their hosts but not in all individuals. It seems clear that each species of mite has a clearly defined attachment site as SMITH (1988) records. Although initial contact could be made at an alternative site, the mites take up a characteristic final location on their host, depending on the species, and do not behave in a random manner.

The *Arrenurus* species described here showed preferences in their attachment sites. Choice of different sites may reduce interspecific competition for feeding and growing space. MITCHELL (1968) found that the response to mite overcrowding was to move to new sites. In the present study, *A. maculator*, although typically occurring at the posterior end of the thorax, is also found on the first abdominal segment, suggesting that overcrowding on its favoured site may lead to occupation of an adjacent area. A. *claviger*, although invariably found on the abdomen, is occasionally found in small numbers further forward on the thorax.

SMITH (1988) stated that ectoparasitic mites associate with hosts already carrying mites. It had been assumed that clumping of the same species at attachment sites is the result of the synchronization of larval activity and should result in larvae of similar size being found. However, in the present study, there are considerable size differences between individual mites of the same species, which could reflect the period of time they have been attached to the host, if indeed they arrive at different times. It is thought that the larger specimens were feeding or had fed and were about to detach and the smaller specimens, recently arrived at their sites, were producing stylostomes in order to start feeding.

ZAWAL, in his morphological descriptions (2006a, 2006b, 2006c, 2006d, 2007), found unengorged larvae with the following lengths: *A. maculator* 200-210, *A. cuspidator* 199-204, *A. bruzelii* 212-228, *A.affinis* complex 224-244, *A. bicuspidator* 228-252 and *A. claviger* 234-260 µm. The largest are therefore *A. claviger*, *A. bicuspidator* and the *A. affinis*-complex. In the present study, which included both unengorged and engorged larvae and hence produced much wider size ranges, *A. maculator*, *A. cuspidator* and *A. claviger* had lower median and mean sizes than those in the *A. affinis*-complex, *A. bicuspidator* and *A. bruzelii*. Of note is that *A. claviger* was the largest of the unengorged mites recorded by ZAWAL (2006d) but did not show the largest engorged individuals in the present study.

It is known that most larval mites remain on their odonate host for up to 20 days (CORBET, 1999) but some do not reach maturity and die on the host, most likely due to overcrowding and/or the inability to feed and this may in part explain the differences we found.

CORBET (1999) in his review states that, "mites infest male and female Odonata apparently without bias". This is certainly supported by a number of studies, for example, ROLFF (2000) and McKEE et al. (2003) However, in contrast, ROBB & FORBES (2006), working with *Lestes disjunctus*, found female sex bias in parasitism by larval *A. pollictus*; LAJEUNESSE et al. (2004) found sex biases in parasitism by the mite *Limnochares americana* in field surveys and FORBES et al. (2004), in their findings involving recapture techniques, reported a situation in *A. planus* in which mature females carried more mites than mature males. Mature males also had fewer mites than newly emerged males not recaptured. In the present study on *C. puella,* the authors found significant differences in numbers, with mites apparently prefering females to males. Since the female damselfly returns to water to oviposit, males flying mainly over vegetation adjacent to water, it would be of some advantage for the mite to show a female-biased parasitism in order more easily to return to water to continue the aquatic part of their life cycle.

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