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First records of parasitizing water mite larvae (Hydrachnidia) on damselflies (Odonata: Zygoptera) from southwestern Poland

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Abstract. We describe the relationships between the water mites *Arrenurus* s. str. and their damselfly (Zygoptera) hosts from small water bodies in the Silesian Lowland (SW Poland). We found 1088 larvae of five water mite species on 75 adult odonates belonging to six species. The prevalence of infestation was very high (92.3%) due to the cumulative effect of water temperature. This is the first record of parasitism of *Arrenurus cuspidator* on *Enallagma cyathigerum*, *A. maculator* on *Coenagrion hastulatum*, and *A. tetracyphus* on *Lestes sponsa* and *Enallagma cyathigerum*.

Key words: *Arrenurus,* damselflies, Zygoptera, parasite, host

The larvae of most water mite species (Hydrachnidia) parasitize aquatic insects, and in this manner water mites disperse and colonize new water bodies (Bohonak 1999, Bilton et al. 2001, Zawal 2003a, 2003b, Bohonak et al. 2004, Martin 2008). Water mites can be divided into two groups depending on the insects they infest. The first group consists of parasites of insects predominantly inhabiting in water and leaving this environment only periodically, i.e. aquatic beetles and true bugs (Cichocka 1995, Zawal 2002, 2003c, Fairn et al. 2008, Incekara & Erman 2008, Zawal et al. 2013, Abe et al. 2015). The other group parasitizes insects whose larvae live in the water while the imagines permanently reside outside of the water (Böttger 1976, Davids 2004, Zawal 2004a, 2004b, 2006a, 2006b, Esteva et al. 2007, Buczyńska et al. 2015, Stryjecki et al. 2015). These two groups of water mites infest their hosts in different ways. In the first case the infestation occurs in the water. In the second case the lifespan of the larva has two phases: an aquatic pre-parasitic (phoretic) and a terrestrial/aerial parasitic phase. The transition between these phases requires the mite larva to move from the exoskeleton of the final pre-imaginal instar to the integument of the enclosing adult.

The phoretic phase concerns odonates parasitized by the larvae of *Arrenurus* s. str. (sensu stricte) (Zawal 2004a, 2004b, 2006a, 2006b, Baker et al. 2006, 2007, 2008, Zawal & Jaskuła 2008, Radhakrishnan et al. 2010, Kulijer et al. 2012, 2013, Kanisto et al. 2015, Młynarek et al. 2015). Due to the lack of an identification key for *Arrenurus* larvae, knowledge on the host-parasite relationship is still fragmentary. The use of identification key (Zawal 2008) has made it possible to expand this knowledge.

The objective of this study was to characterize the parasitism of water mite larvae on damselflies in small water bodies of SW Poland.

Adult odonates for the study were captured in small water bodies surrounded by forest and meadows in the Silesian Lowland (SW Poland). Infested specimens were only recorded in July and August during previous field studies including months from April to October in 2012 year. Samples were taken at monthly intervals by entomological net.. For this reason the reference point for the analyses of parasitism was material collected in these months, consisting of 78 imagines (15♀♀ and 63♂♂) of six damselfly species (Table 1).

Water mite larvae were removed with tweezers, preserved in 95% ethanol, and slides were mounted in Hoyer's medium. The body length of the water mite larvae was measured from the frontal part of the gnathosoma to the end of the idiosoma by AxioVision 4.8 software using AxioScope.A1 Zeiss microscope. A total of 1088 water mite larvae of the subgenus *Arrenurus* s. str. were collected. The larvae were identified using the key by Zawal (2008).

Two indices were used in the statistical analyses: 1) prevalence of infestation (percentage of infested hosts) and 2) intensity of infestation (number of parasites on a given host).

Statistical significance (alpha value) was determined by the Kruskal-Wallis test and U Mann-Whitney test. The calculations were performed with Statistica 12.5 software.

Five water mite species of the subgenus *Arrenurus* s. str. were recorded at the stage of parasitic larva (Table 2).

Infested damselfly specimens were only re-

Table 1. Infestation of damselfly host species by water mite larvae from SW Poland.

	Coenagrion puella	*Coengrion pulchellum*	*Coenagrion hastulatum*	*Enallagma cyahigerum*	*Pyrrhosoma nymphula*	*Lestes sponsa*
average	16.8	11.4	26	5.3	14.7	9.3
standard deviation	17.90	7.30		4.13	19.40	7.37
range	1-80	3-18	26	1-14	2-37	1-15
total	923	57	26	42	44	28

Table 2. Number of particular water mite parasites recorded on particular damselfly host species in SW Poland.

	Coenagrion puella	*Coengrion pulchellum*	*Coenagrion hastulatum*	*Enallagma cyahigerum*	*Pyrrhosoma nymphula*	*Lestes sponsa*
A. bicuspidator	3		2			
A. cuspidator	603	33	4	3	37	
A. maculator	306	7	2	2		
A. bruzelii	1	2	15			
A. tetracyphus				34		34
total	913	42	23	39	37	34

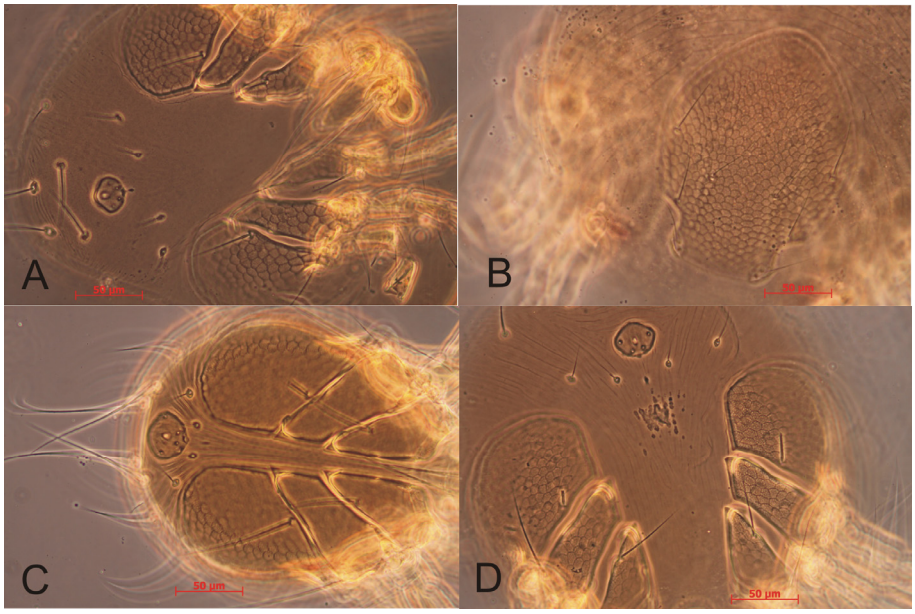

Figure 1. Water mite larvae: ventral side A–*Arrenurus cuspidator*, C – *A. bruzelii*, D – *A. tetracyphus*; B – dorsal plate *A. maculator*.

corded in July and August. A total of 78 adult odonates (15♀♀ and 63♂♂) belonging to 6 species were collected (Table 1). Water mite larvae were recorded on 75 specimens among all the odonates collected (Table 1). The Kruskal-Wallis test did not revealed statistically significant differences between the degree of infestation of individual species (H[5, n=75]=4.830365, p=0.4369).

Prevalence of infestation was 92.3% for all odonates combined, 100% for *Lestes sponsa*, *Coenagrion pulchellum*, *C. hastulatum*, *Enallagma cyathigerum* and *Pyrrhosoma nymphula*, and 91.3% for *C. puella*. The highest mean numbers of parasites occurring on a single host of a given species occurred on *C. hastulatum*, followed by *C. puella*, *P. nymphula*, *C. pulchellum*, *L. sponsa* and *E. cyathigerum* (Table 1). As there was only one individual each of *C. hastulatum*, *C. pulchellum* and *L. sponsa* and three each of *Enallagma cyathigerum* and *Pyrrhosoma nymphulla*, the data for these species are

Table 3. Infestation rate of damselfly host sexes.

	Coenarion puella		*Coenagrion pulchellum*	*Coenagrion hastulatum*	*Enallagma cyathigerum*		*Pyrrhosoma nymphula*	*Lestes sponsa*
	female	male	male	male	female	male	female	male
range	2-39	1-80	3-18	26	4-14	1-7	2-37	1-15
average	13.5	17.3	11.4	26	8.3	3.4	14.7	9.3

Table 4. Body size of parasites recorded on damselfly host.

	Arrenurus cuspidator	*Arrenurus maculator*	*Arrenurus bicuspidator*	*Arrenurus bruzelii*	*Arrenurus tetracyphus*
range	172-813	183-755	234-687	243-593	224-690
average	500.2	434.4	417.2	289.3	487.5
standard deviation	117.39	140.30	212.38	82.95	105.99

largely uncertain.

Coenagrion puella and *C. hastulatum* were infested by four species of parasites, *C. pulchellum* and *E. cyathigerum* by three species, and *L. sponsa* and *P. nymphula* by one parasitic species (Table 2).

The highest number of hosts occurred in the case of *Arrenurus cuspidator* – five, followed by *A. maculator* with four, *A. bruzelii* with three, and *A. bicuspidator* and *A. tetracyphus* with two host species each (Table 2).

Differences in the infestation of each of the sexes were observed for only one host species (*Coenagrion puella*) (Table 3), but the differences was not statistically significant (U Mann-Whitney test (Z=-0.4663, p=0.64).

The body size of water mite larvae varied from 183 to 813 µm. The widest range of values occurred for *A. cuspidator*, which was also distinguished by the largest larvae and the largest mean body size (Table 4).

The damselfly species noted here are among those most often used as hosts by water mite larvae of the subgenus *Arrenurus* s. str., and nearly all the parasite species recorded here have previously been found on the same host species (Davids 1997, 2004, Baker et al. 2006, 2007, 2008, Zawal & Dyatlova 2006, 2008, Baker & Zawal 2011, Zawal & Szlauer-Łukaszewska 2012, Kulijer et al. 2012, 2013, Zawal & Buczyński 2013). Exceptions are *A. cuspidator* on *E. cyathigerum*, *A. maculator* on *C. hastulatum* and *A. tetracyphus* on *L. sponsa* and *E. cyathigerum*, recorded for the first time.

The fact that parasitism was detected only in July and August is likely linked to the effect of water temperature on the degree of infestation (Robb & Forbes 2005, Hassall et al. 2010) and confirms previously noted peaks in numbers of parasites in the late-summer months (Zawal 2006b, Zawal & Dyatlova 2006, Zawal & Buczyński 2013).

A surprising observation is the unusually high prevalence of infestation observed in this study. Prevalence of water mites on odonates is relatively high in comparison to other insects (Cichocka 1995, Zawal 2002, 2003, 2004a, 2006a, Martin & Stur 2006), but in this case it was nearly 100%. This is an exceptional situation and was probably due to the synchronization of the appearance of water mite larvae and transforming damselfly larvae in the study area, as a result of a substantial increase in water temperature in the entire basin of the small water bodies in July and August. This temperature increase likely served to synchronize the development of water mites and damselflies, which led to high prevalence as well as to parasitism exclusively in these months. The temperature is usually an element synchronizing hatching water mite larvae with emergence of damselflies, but the prevalence is slightly lower (Rolff 2000).

The intensity of infestation was consistent with data obtained in previous studies (Baker et al. 2006, 2007, 2008, Zawal & Dyatlova 2006, 2008, Baker & Zawal 2011, Zawal & Szlauer-Łukaszewska 2012, Kulijer et al. 2012, 2013, Zawal & Buczyński 2013). The number of parasites found on one host does not appear to depend on the host species but on the moment when the parasite encounters the host and how many hosts are available then.

Both the number of host species per parasite and the number of parasite species occurring on individual hosts confirm data from previous studies (Baker et al. 2006, 2007, 2008, Zawal & Dyatlova 2006, 2008, Baker & Zawal 2011, Zawal & Szlauer-Łukaszewska 2012, Kulijer et al. 2012, 2013, Zawal & Buczyński 2013), which is indicative of opportunism of the parasites. This does not

mean that there are no differences in preferences for host species or in the length of lists of host species for particular species of parasites. However, confirming such preferences requires analysis of data from numerous studies conducted in different areas. The greater number of host species noted for *Arrenurus cuspidator* and *A. maculator* likely results from their wider habitat spectrum and high abundance in most environments.

Previous data indicate differences in infestation of the two sexes in damselflies (Zawal 2004a, 2006b, Ilvonen et al. 2015). In the case of *Coenagrion puella*, parasites have shown greater preference for females (Zawal 2004a, Zawal & Szlauer-Łukaszewska 2012, Zawal & Buczyński 2014). This was not confirmed in the present study. The explanation for this may be the same (substantial synchronization of hatching of water mite larvae and damselfly transformation due to an increase in water temperature) as for the high prevalence, and is an exceptional situation. However, the host sex preferences of parasites must be verified by further research on a greater quantity of material.

The size of the water mite larvae was consistent with previous data on the size of parasitizing larvae (Baker et al. 2008, Kulijer et al. 2013). On the other hand, they were predominantly much larger than newly hatched larvae (Zawal 2006c, 2006d, 2006e), which indicates that infestation had taken place about 1-2 weeks before collection.

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