1 Short communication

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3 Mating duration and prolonged post-copulatory associations in *Arrenurus* water mites 4 (Actinotrichida: Parasitengonina: Hydrachnidia)

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13

14 Abstract

15 Conflicts of interest between the sexes over control of mating can be reflected in various aspects of
16 morphology and behaviour, including structure of genitalia and copulation duration. In *Arrenurus* water
17 mites (Hydrachnidia: Arrenuridae) there are two main patterns of sperm transfer that differ in degree of
18 potential male control of sperm uptake by females. In some species, males are equipped with an
19 intromittent organ (the ‘petiole’) that is used to forcefully insert sperm into the female reproductive
20 tract. In others, males lack an intromittent organ and females appear to push sperm into their
21 reproductive opening themselves. Theory suggests that the amount of time spent in courtship after
22 sperm transfer should differ between males with and without an intromittent structure. We predicted
23 that male *Arrenurus* able to push sperm into the female’s reproductive tract (petiolate males) should
24 spend less time courting females after transferring sperm than apetiolate males, which may have to
25 ‘convince’ females to take up their sperm. Here, we examined durations of mating for 10 species of

26 *Arrenurus* with males that differ in genital morphology: six species with males equipped with a well
 27 developed petiole (= ‘petiolate’ species) and four species with males that either completely lack a
 28 petiole or have a minute peg-like petiole that does not appear to function as an intromittent organ (=
 29 ‘apetiolate’ species). We tested whether males of petiolate species spend less time in the stage of
 30 courtship that takes place after sperm transfer (= ‘post-transfer courtship’) than apetiolate males. In
 31 contrast to our prediction, we found that species with well developed petioles spent significantly more
 32 time in post-transfer behaviours than species lacking petioles. The possible function of protracted post-
 33 transfer courtship in the genus *Arrenurus* is discussed.

34

35 *Keywords:* sexual selection; sexual conflict; cryptic female choice; genitalia; reproductive morphology

36

37 **1. Introduction**

38 Sexual selection theory was initially proposed to explain the existence of sexual dimorphism in species
 39 in which there is little ecological differentiation between the sexes (Darwin 1859). The originally
 40 described scenarios relate to situations in which males compete for access to females through combat
 41 or through courtship displays in which females choose the winner (Darwin 1871). However, there are
 42 situations where males show morphological, physiological and behavioural adaptations that appear to
 43 bypass female choice. Sexual conflict theory argues that in some species, male genitalia and pre-
 44 copulatory behaviour evolved to force females to mate (= coercion); if this reduces female fitness, there
 45 is then selection for female resistance to male coercive morphology and behaviour (Arnqvist & Rowe
 46 2005). Evolutionary conflicts of interest can apply to which sex controls mating rate, copulation
 47 duration, female remating propensity and ultimately fertilization of eggs (Edvardsson & Canal 2006).

48 Similarly, although females may benefit from mating with additional ‘higher quality’ males
 49 after a first mating, it is in the first male’s interest to prevent females from remating (Arnqvist & Rowe
 50 2005; Firman et al. 2017). There are various adaptations to reduce polyandry which may take the form

51 of using mating plugs by males, transferring seminal toxins or postcopulatory mate-guarding (Arnqvist
52 & Rowe 2005; Firman et al. 2017). It has been experimentally shown for some species that prolonging
53 the postcopulatory stage of mating increases probability of fertilization of eggs by sperm of a particular
54 male, since more sperm and accessory ejaculate substances is transferred over time (Eberhardt 1985;
55 Arnqvist & Rowe 2005). In response to male adaptations to prolong mating duration, females evolved
56 counter-adaptations such as struggling in order to dislodge males (Edvardsson & Canal 2006; Bergsten
57 & Miller 2007).

58 Sperm-transfer behaviour in water mites of the genus *Arrenurus* Dugès, 1834 (Actinotrichida:
59 Parasitengonina: Hydrachnididae) ranges from low to high complexity among around 1000 described
60 species (Smit 2020; Więcek et al. 2021). In males, the hindbody (the cauda) may be simple and only
61 slightly differ from female's hindbody, whereas in other species the males have elongated or complexly
62 sculptured cauda (Fig. 1A, B). The most complex cauda are equipped with pygal lobes, protrusions and
63 an elaborate median intromittent organ (the petiole) (Fig. 1B). The male's cauda and more anterior
64 region of the hindbody has pairs of glandularia that produce an adhesive secretion during mating that is
65 used to glue the female to the male's hindbody (Jin et al. 1997). Moreover, males of some species have
66 a spur-like extension on the fourth leg that is used to clasp the female's legs and held her during the
67 early stages of pairing (Jin et al. 1997; Proctor & Wilkinson 2001).

68 Among *Arrenurus* there are two main modes of sperm transfer that appear to differ in the
69 amount of control the female has over moving sperm into her genital tract. In one mode, males
70 forcefully push sperm into the female's genital tract with the use of well developed intromittent organ
71 (e.g., *Arrenurus valdiviensis* K.O. Viets, Böttger 1965). In the second mode, males lack an intromittent
72 organ. Males of these species deposit spermatophores on the substrate and lower the female on top of
73 the sperm packets on the spermatophores. Females may have their genital flaps open at this point, or
74 they may be closed, and they later use their legs to move the accumulated sperm from their external
75 genital flaps into their genital openings, as in *Arrenurus globator* (O.F. Müller) (Lundblad 1929;

76 Böttger 1965) and *A. manubriator* Marshall (Proctor 2003). Sexual conflict is likely more intense in
 77 species with males possessing well developed intromittent organs that appear to bypass female choice
 78 of sperm uptake, while female choice should be the stronger force of selection in species with males
 79 not equipped with such an organ (Proctor & Smith 1994; Proctor & Wilkinson 2001).

80 Both male and female *Arrenurus* are known to mate repeatedly in their life span and therefore
 81 potential conflicts over mating rates and courtship duration may take place (Proctor & Wilkinson 2001;
 82 Proctor 2003). The duration of mating in different *Arrenurus* species seems to be largely controlled by
 83 males through various methods: the male gluing the female to his hindbody, clasping and maneuvering
 84 the female before and during coupling with the use of a spur-like extension on the fourth leg, and (in
 85 some species) using an intromittent organ to insert sperm into the female. Separation of male and
 86 female at the end of the mating sequence also sometimes seems to be controlled by the male pushing
 87 the female in a way that breaks the binding of the glue (Proctor & Wilkinson 2001). Here, we observed
 88 full mating sequences and examined mating durations of *Arrenurus* species differing in presence of an
 89 intromittent structure (the petiole) in the male. We predicted that apetiolate males should spend more
 90 time on post-deposition courtship in order to ‘encourage’ females to move sperm higher into their
 91 genital tracts. In contrast, we predicted that petiolate males equipped with a well developed intromittent
 92 organ that forcefully pushes sperm into the female genital tract should spend less time on courtship
 93 following sperm transfer.

94

95 **2. Materials and methods**

96 **2.1. Mite collection and maintenance**

97 *Arrenurus* mites were collected using a net with a mesh size of 250 µm in four locations in Poland:
 98 ponds at Adam Mickiewicz University campus in Poznań (52°27'59.3"N 16°55'57.2"E), Bagnisko lake
 99 (53°29'55.7"N 16°28'42.3"E), Łagowo lake (52°19'33.6"N 15°17'16.8"E) and an unnamed pond near
 100 Zielona Góra (51°55'03.8"N 15°28'50.6"E). Data for species originating from Canada were obtained

101 from Proctor and Wilkinson (2001). Live specimens were sorted under a stereoscope microscope in the
102 laboratory and determined to species using a key in Gerecke et al. (2016).

103 Deutonymphs collected in the field were maintained in its own wells with 2 cm in diameter and
104 1 cm deep until they transformed into an adult. Adult mites sampled from ponds were separated by
105 species and sex, and maintained together in the laboratory in tissue culture plates with diameter 5 cm
106 and 3 cm deep at ambient temperatures (~20-25 °C). Both adults and deutonymphs were fed with
107 ostracods, cladocerans and copepods from laboratory colonies established based on zoological material
108 collected in local water bodies.

109

110 **2.2. Mating observations**

111 Female and male mites (whenever possible, virgin specimens) were held individually for 24 h before
112 observation in microaquaria that were half filled with tap water. The well-plates had diameter of 2 cm
113 and depth of 1 cm with the scratched bottom to create a stable substrate for the mites during mating.

114 We took videos of the entire mating sequences of seven species of *Arrenurus*: five with petiolate males
115 (*A. bicuspidator*, *A. bruzelii*, *A. claviger*, *A. cuspidator*, *A. tricuspidator*) and two with apetiolate males
116 (*A. globator* and *A. stecki*). Details of mating behaviour of these species are in Więcek (2016). Video
117 recording began when a male was introduced to a container with a conspecific female. Recording was
118 terminated upon physical separation of individuals after mating. In order to obtain a larger sample size
119 to test our predictions, the results were pooled with the data obtained by Proctor and Wilkinson (2001)
120 for three North American species: petiolate *A. nr. reflexus* and apetiolate *A. manubriator* and *A.*
121 *rufopyriformis*. The observations of mating behaviour were made with Zeiss Stemi 2000-C
122 stereomicroscope and videotyped with the use of attached to the stereomicroscope DIC illumination
123 and digital camera Olympus DP71 with CellD 2.8 software (Olympus Soft Imaging Solutions GmbH).

124 We divided mating sequences into the following categories, listed in temporal order: pre-pairing
125 stage, behaviours from initial pairing to the end of spermatophores deposition and post-deposition

behaviour. In all categories except pre-pairing, the female was attached to the male. For statistical analysis we calculated the following: pre-pairing duration = total time from introduction of a male into a female's container to the female being glued onto the male; total duration of mating = total time that male and female were attached (i.e. glued together), including time spent in pre-deposition behaviour, spermatophore deposition and sperm transfer (deposition of spermatophores was assumed based on male 'dipping' movements), and post-deposition behaviour; post-deposition behaviour = time male and female were still attached to each other after sperm transfer. Unlike in Lundblad (1929) and Więcek (2016), we treated the male behaviours of lateral waving and alternate bending of left and right legs as part of post-deposition behaviour.

SEM photographs of couples in copula were made in the Department of Earth and Atmospheric Sciences, University of Alberta. After dehydration through an alcohol-HMDS (hexamethyldisilazane) series, mites were mounted on stubs using double-sided tape, sputter coated with gold, and examined using a JEOL 630 I field emission scanning electron microscope (SEM) in the Department of Earth and Atmospheric Sciences, University of Alberta. SEM photographs were edited in Photoshop 6.0.

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141 **2.3. Statistical analyses**

Data were tested for significant deviations from normal distribution with Shapiro-Wilk test. No significant deviations from normal distribution for comparing all groups in analyses were found, and therefore parametric two sample *t*-tests (two tailed) were applied. The sample sizes for statistical analyses were based on numbers of species of petiolate and apetiolate *Arrenurus* (n=6 and 4, respectively). The data on difference in percentage of time spent on post-spermatophore-transfer behaviours were changed to proportions and arcsine-transformed (Sokal & Rohlf 1995). Moreover, mean values and confidence intervals (95%) for the estimate of the mean were calculated based on the standard errors.

The duration of pre-pairing stage of mating can be affected by female age and whether they were previously exposed to males (H. Proctor, pers. obs.). Because mating status and age of mites were not standardized, pre-pairing duration was excluded from statistical tests. All analyses were run with the statistical software PAST 4.03 (Hammer et al. 2001).

3. Results

In this study 31 pairings of 7 *Arrenurus* species were observed (*A. bicuspidator*, *A. bruzelii*, *A. claviger*, *A. cuspidator*, *A. tricuspidator*, *A. globator*, *A. stecki*), which resulted in about 160 hours of mating trials (Table 1). Moreover, observations of *A. manubriator*, *A. nr. reflexus* and *A. rufopyriformis* were included in the statistical analyses (Proctor & Wilkinson 2001). Total duration of mating duration ranged from 29.00 ± 5.00 minutes in apetiolate *A. stecki* to 630.00 ± 79.00 minutes in petiolate *A. bicuspidator* (Table 1, SEM photographs of mites in copula are in Fig. 2). Total duration of mating in petiolate species was significantly longer (mean = 455.7 ± 55.16 min, n=6) than in apetiolate species (mean = 85.45 ± 25.32 min, n=4) (*t*-test: 6.10, two-tailed $P < 0.001$) (Fig. 3A). There was no significant difference in duration of behaviours from initial pairing to the end of spermatophores deposition between petiolate and apetiolate species (*t*-test: 0.81, two-tailed $P = 0.44$). Petiolate species spent a significantly higher percentage of total mating time on post-deposition behaviour (mean = $93.69 \pm 1.06\%$) than apetiolate species (mean = $55.27 \pm 12.47\%$) (*t*-test: 3.61, two-tailed $P = 0.033$) (Fig. 3B).

174 Table 1. Mean time ($\pm$ SE) spent on particular stages of mating in different species of the genus
 175 *Arrenurus*. The post-deposition behaviour is part of pairing behaviour and is expressed as percentage of
 176 the total time spent on mating (also given in min). The total duration of mating does not include pre-
 177 pairing stage.

species	intromittent organ	pre-pairing duration (min)		total duration of mating (min)		post-deposition behaviour (min)		post-deposition behaviour (%)	
<i>A. sp. nr. reflexus</i> [†]	n = 8	9.85 $\pm$ 4.97		325.67 $\pm$ 19.65		307.10 $\pm$ N.A.		94.3 $\pm$ 0.45	
<i>A. cuspidator</i>	n = 3	43.33 $\pm$ 28.87		598.00 $\pm$ 79.37		553.33 $\pm$ 74.37		92.47 $\pm$ 1.65	
<i>A. bicuspidator</i>	n = 2	35.00 $\pm$ 5.00		630.00 $\pm$ 79.00		610.5 $\pm$ 76.5		96.9 $\pm$ 0.0	
<i>A. tricuspidator</i>	n = 4	petiolate	25.68 $\pm$ 5.09	439.0 $\pm$ 67.00*	455.7 $\pm$ 55.16	423 $\pm$ 70.0*	428.66 $\pm$ 54.24	96.15 $\pm$ 1.25*	93.69 $\pm$ 1.06
<i>A. bruzelii</i>	n = 9	29.6 $\pm$ 12.37		303.55 $\pm$ 20.42		272.78 $\pm$ 17.15		89.95 $\pm$ 1.23	
<i>A. claviger</i>	n = 4	18.00 $\pm$ 4.24		438.00 $\pm$ 10.92		405.25 $\pm$ 19.72		92.37 $\pm$ 2.42	
<i>A. globator</i>	n = 7	84.00 $\pm$ 16.00		123.57 $\pm$ 20.62		87.43 $\pm$ 15.50		70.35 $\pm$ 1.50	
<i>A. stecki</i>	n = 2	apetiolate	32.00 $\pm$ 22.00	39.91 $\pm$ 23.51	29.00 $\pm$ 5.00	85.45 $\pm$ 25.32	16 $\pm$ 7.00	43.79 $\pm$ 15.61	52.55 $\pm$ 15.05 55.27 $\pm$ 12.47
<i>A. manubriator</i> [†]	n = 7	N.A.		132.6 $\pm$ 19.97		28.11 $\pm$ N.A.		21.2 $\pm$ 3.91	
<i>A. rufopyriformis</i> [†]	n = 9	3.73 $\pm$ 1.05		56.63 $\pm$ 4.98		43.61 $\pm$ N.A.		77.00 $\pm$ 2.5	

[†] data from Proctor & Wilkinson (2001)

* calculated from two of the four pairings

178

179 4. Discussion

180 We expected that *Arrenurus* males with an intromittent structure (the petiole) would spend less time in
 181 courtship behaviours after sperm transfer than apetiolate males, which have less coercive control over
 182 whether sperm enters the female's genital opening. Contrary to our prediction, males of petiolate
 183 species spent more time in post-deposition behaviours than males of apetiolate species, both absolutely
 184 and relatively (as proportion of total time spent mating). Although both petiolate and apetiolate species
 185 exhibited prolonged post-deposition associations, in petiolate species post-deposition behaviour made

up > 90% of total mating time in contrast to ~50% in apetiolate species. However, there was a considerable variation in time spent on post-transfer behaviours among apetiolate species.

Why might males of petiolate species show such long periods of attachment to females after introducing sperm into her genital tract? Protracted courtship or simply male attendance following sperm transfer is known to occur in different animal groups and is thought to reduce the probability of the female to be inseminated by a subsequent male (mate guarding, Arnqvist & Rowe 2005). Also, in this stage of mating males can manipulate female's physiology by stimulating oviposition or decreasing production of sex pheromones attracting males (Arnqvist & Rowe 2005; Mazzi et al. 2009). Radwan and Siva-Jothy (1996) demonstrated that males of the terrestrial mite *Rhizoglyphus robini* (Acaridae) increase fertilisation of eggs by their sperm by prolonging attachment to a female. We hypothesize that this could be an explanation of the function of periods of motionlessness and courtship behaviour after sperm transfer in the examined *Arrenurus* spp.

It is well known that in various invertebrate taxa the risk of sperm competition can be selective force in males and can be expressed by using mating plugs and mate guarding that are supposed to prevent future inseminations (Dougherty et al. 2016; Firman et al. 2017). Male courtship and postmating behaviours may also encourage preferential use of sperm of a particular male and thus can be under selection by cryptic female choice (Firman et al. 2017). It was shown that *Arrenurus* spp. can mate repeatedly (e.g. *A. manubriator*; Proctor 2003), which potentially leaves space for both selective pressures associated with sperm competition between males and physiological processes in females affecting preferential use of sperm of a particular male. Males of apetiolate and petiolate species may differ in the amount of sperm competition they face, if, in addition to pushing sperm into the female's genital opening, the petiole also acts to remove or displace sperm deposited by previous males as does, e.g., the genitalia of male damselflies (Waage 1979). If so, the greater duration of both pre- and post-transfer behaviour in petiolate species may reflect the lengths to which such males must go to increase the likelihood that females will use their sperm and not that of a previous or subsequent mate. To test

211 this idea, the paternity of egg clutches could be assessed in matings in which the second male is
212 allowed to complete the entire mating sequence or is interrupted at various times after sperm transfer.

213 Finally, although optimal copulation duration can be a subject of conflict between the sexes,
214 which may be the case in the examined *Arrenurus*, it has been observed that females can benefit from
215 prolonged copulations, for instance, because they receive larger ejaculates (e.g. bruchid beetles,
216 Edvardsson & Canal 2006). Therefore, further research should be done to determine if prolonged post-
217 copulatory associations in *Arrenurus* mites influence female's lifetime fecundity as well as male's
218 paternity.

219

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226

227 **Conflict of interest**

228 The authors have no conflict of interest to declare.

229

230 **References**

- 231 Arnqvist G, Rowe L. 2005. *Sexual Conflict*. Princeton: Princeton University Press.
232 doi:10.1515/9781400850600
- 233 Bergsten J, Miller KB. 2007. Phylogeny of diving beetles reveals a coevolutionary arms race between
234 the sexes. PLoS One. 2:e522. doi:10.1371/journal.pone.0000522

235 Böttger K. 1965. Zur Ökologie und Fortpflanzungsbiologie von *Arrenurus valdiviensis* K. O. Viets
236 1964 (Hydrachnellae, Acari). [Ecology and reproductive biology of *Arrenurus valdiviensis* K. O.
237 Viets 1964 (Hydrachnellae, Acari)]. Z. Morphol. Oekol. Tiere. 55:115–141.

238 Darwin C. 1859. *On the Origin of Species by Means of Natural Selection*. 1st edition. J. Murray,
239 London.

240 Darwin C. 1871. *The Descent of Man and Selection in Relation to Sex*. 1st edition. J. Murray, London.

241 Dougherty LR., Simmons LW, Shuker DM. 2016. Postcopulatory sexual selection when a female
242 mates once. Anim Behav. 116:13–16. doi: 10.1016/j.anbehav.2016.03.003

243 Eberhardt WG. 1985. *Sexual Selection and Animal Genitalia*. Cambridge, Mass.: Harvard University
244 Press.

245 Edvardsson M, Canal D. 2006. The effects of copulation duration in the bruchid beetle *Callosobruchus*
246 *maculatus*. Behav Ecol. 17:430–434. doi:10.1093/beheco/arj045

247 Firman RC, Gasparini C, Manier MK, Pizzari T. 2017. Postmating female control: 20 years of cryptic
248 female choice. Trends Ecol Evol. 32:368–382. doi: 10.1016/j.tree.2017.02.010

249 Gerecke R, Gledhill T, Pešić V, Smit H. 2016. Süßwasserfauna Von Mitteleuropa, Bd 7/2–3.
250 Chelicerata: Acari III. Heidelberg: Springer.

251 Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for
252 education and data analysis. Palaeontol Electronica. 4:p. 9. Available from: [https://palaeo-](https://palaeo-electronica.org/2001_1/past/issue1_01.htm)
253 [electronica.org/2001_1/past/issue1_01.htm](https://palaeo-electronica.org/2001_1/past/issue1_01.htm)

254 Jin D, Li L, Wiles R. 1997. The structure and evolution of male cauda and petiole with a cladistic
255 analysis of Chinese species of the genus *Arrenurus* (Acari : Arrenuridae).
256 Syst Appl Acarol. 2:195–209. doi: 10.11158/saa.2.1.29

257 Lundblad O. 1929. Über den Begattungsvorgang bei einigen *Arrenurus*-Arten. [Mating behaviour of a
258 few *Arrenurus* species]. Z. Morphol. Oekol. Tiere. 15:705–722.

259 Mazzi D, Kesäniemi J, Hoikkala K, Klappert K. 2009. Sexual conflict over the duration of copulation
260 in *Drosophila montana*: why is longer better? BMC Evol Biol. 9:132. doi:10.1186/1471-2148-9-
261 132

262 Proctor H. 2003. Male mating effort and female remating in the water mite *Arrenurus manubriator*
263 (Acari: Arrenuridae). In: Smith IM, editor. *An Acarological Tribute to Dave Cook*. West
264 Bloomfield: Indira Publishing House. ISBN 0-930337-18-2 (refereed). p. 211–221.

265 Proctor HC, Smith BP. 1994. Mating behaviour of the water mite *Arrenurus manubriator* (Acari:
266 Arrenuridae). J Zool, London. 232:473–483. doi:10.1111/j.1469-7998.1994.tb01588.x

267 Proctor H, Wilkinson K. 2001. Coercion and deceit: water mites (Acari: Hydracarina) and the study of
268 intersexual conflict. In: Halliday RB, Walter DE, Proctor HC, Norton RA and Colloff MJ,
269 editors. *Acarology: Proceedings of the 10th International Congress*. CSIRO Publishing,
270 Melbourne. p. 155–169.

271 Radwan J, Siva-Jothy MT. 1996. The function of post-insemination mate association in the bulb mite,
272 *Rhizoglyphus robini*. Anim Behav. 52:651–657. doi:10.1006/anbe.1996.0209

273 Smit H. 2020. Water mites of the world, with keys to the families, subfamilies, genera and subgenera
274 (Acari: Hydrachnidia). Monografieën van de Nederlandse Entomologische Vereniging 12:1–774.

275 Sokal RR., Rohlf, JF. 1995. *Biometry: the principles and practice of statistics in biological research*.
276 W. H. Freeman and Company, New York.

277 Waage JK. 1979. Dual function of the damselfly penis: sperm removal and transfer. Science. 203:916–
278 918. doi: 10.1126/science.203.4383.916

279 Więcek M. 2016. Effects of the evolution of intromission on courtship complexity and male and female
280 morphology: water mites of the genus *Arrenurus* (Acari; Hydrachnida) from Europe and North
281 America [Ph.D. Thesis]. Poznań, Poland. Adam Mickiewicz University.

282 Więcek M, Broda Ł, Proctor H, Dabert M, Smith BP, Dabert, J. 2021. Species boundaries among
 283 extremely diverse and sexually dimorphic *Arrenurus* water mites (Acariformes: Hydrachnidiae:
 284 Arrenuridae). bioRxiv: <https://doi.org/10.1101/2021.04.04.438411>

289 | Figure 1. Scanning electron micrographs of males of apetiolate *Arrenurus fontinalis* (B) and petiolate
 290 *Arrenurus bicuspidator* (B); the petiole – p, indicated with an arrow.

291 Figure 2. Scanning electron micrographs of *Arrenurus* spp. in copula. A, B. *Arrenurus globator* in mating
292 position (lateral view): female glued to males hindbody (no petiole). C, D. *Arrenurus* sp.: female glued to males
293 dorsum; the petiole inserted in to female genital opening is shown. Abbreviations: p – petiole (intromittent
294 organ), mc – male's cauda (hindbody), g - adhesive secretion enabling gluing female to male's hindbody
295 produced by glandularia, fg – female's genital opening.

Figure 3. Mean time (and standard errors) spent on mating and post-deposition behaviours: **A**. Mean time spent on mating (dark grey) versus post-deposition stage (light grey) in petiolate and apetiolate species (in min). **B**. Mean time spent on post-deposition behaviours (expressed as percentage of the total time spent on mating). Sample sizes and SE are shown.