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# Thermal Biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes 4 asiaticus*

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1 SHORT NOTE

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3 **Thermal Biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes***  
4 ***asiaticus***

5

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14

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23 **Abstract**

24 *Wyochernes asiaticus* (Arachnida: Pseudoscorpiones: Chernetidae) is a pseudoscorpion  
25 distributed across Beringia, the areas of Yukon, Alaska and Siberia that remained unglaciated  
26 at the last glacial maximum. Along with low temperatures, its streamside habitat suggests  
27 that submergence during flood events is an important physiological challenge for this species.  
28 We collected *W. asiaticus* in midsummer from 66.8°N Yukon Territory, Canada, and  
29 measured thermal and immersion tolerance. *Wyochernes asiaticus* is freeze avoidant, with a  
30 mean supercooling point of -6.9 °C. It remains active at low temperatures (mean critical  
31 thermal minimum,  $CT_{min}$ , is -3.6 °C), and has a critical thermal maximum ( $CT_{max}$ ) of 37.8 °C,  
32 which is lower than other arachnids, and consistent with its restriction to high latitudes. Fifty  
33 percent of *W. asiaticus* individuals survived immersion in oxygen-depleted water for 17 days,  
34 suggesting that this species has high tolerance to immersion during flooding events. To our  
35 knowledge, these are the first data on the environmental physiology of any pseudoscorpion,  
36 and a new addition to our understanding of the biology of polar microarthropods.

37

38

39 **Keywords:** Pseudoscorpion, microarthropod, cold tolerance, critical thermal limits,

40 immersion

## 41 **Introduction**

42 At high latitudes, microarthropods (small-bodied arthropods, including Collembola, and  
43 mites and other arachnids) can dominate soil and tundra ecosystems (Bale et al. 1997; Block  
44 1994; Convey and Stevens 2007; Hodkinson and Coulson 2004; Hodkinson et al. 1996;  
45 Hodkinson et al. 1998). In the Antarctic, and to a lesser extent, the Arctic, the environmental  
46 physiology of mites and springtails has received considerable attention (e.g. Cannon and  
47 Block 1988; Coulson et al. 1995; Sømme 1981). Polar springtails and mites are almost  
48 universally freeze-avoidant, and are killed by the formation of internal ice. They avoid  
49 freezing by depressing the supercooling point (SCP, the temperature at which their bodies  
50 freeze) by some combination of polyol and proteinaceous cryoprotectants, or (more rarely)  
51 via cryoprotective dehydration (Cannon and Block 1988; Coulson et al. 1995; Holmstrup and  
52 Sømme 1998; Sinclair et al. 2006; Worland et al. 1998). The activity ranges of arthropods  
53 are usually delimited by the critical thermal maximum ( $CT_{max}$ , the high temperature at which  
54 coordinated movement is lost and spasms begin) and critical thermal minimum ( $CT_{min}$ , the  
55 low temperature at which ability to move is lost; Sinclair et al. 2015). Polar and sub-polar  
56 mites and springtails usually show some evidence of cold-adaptation, with relatively low  
57  $CT_{max}$  and  $CT_{min}$  (Addo-Bediako et al. 2000; Sinclair et al. 2006; Slabber et al. 2007).

58

59 In addition to low temperatures, polar organisms must withstand other environmental  
60 stressors (Convey 2011; Sømme 1995). Because of their small size and dependence on soil  
61 structure, soil disturbance and flooding can also cause significant physiological stress in any  
62 season, whether it is from ice-cover-induced hypoxia (Coulson et al. 2000), or long-term  
63 immersion in water (Hertzberg and Leinaas 1998). This is particularly the case in riparian  
64 zones, where seasonal snowmelt can cause significant flooding. Some animals such as

65 Collembola, appear to survive inundation by being hydrophobic and rafting on the surface of  
66 water (Coulson et al. 2002; Hawes et al. 2008). Alternately, microarthropods may survive  
67 inundation either through anaerobiosis (Sømme and Conradi-Larsen 1977), or perhaps via  
68 adaptations that allow oxygen to be stored (Burmester 2004) or extracted from the  
69 surrounding water (Seymour and Matthews 2013).

70

71 Although mites and springtails are the only microarthropods in Antarctica, pseudoscorpions  
72 (Arachnida: Pseudoscorpiones) are present in the sub-Arctic and the Arctic (Buddle 2015;  
73 Koponen 1994; Koponen and Sharkey 1988; Muchmore 1990). Pseudoscorpions are small  
74 predators, and some species in alpine Europe and Manitoba, Canada are active under the  
75 snow during winter (Aitchison 1979; Vanin and Turchetto 2007). Although there is evidence  
76 that extreme high temperatures may decrease reproductive success of tropical  
77 pseudoscorpions (Zeh et al. 2012), to our knowledge there have been no investigations of the  
78 environmental physiology of any pseudoscorpions, including those of Northern latitudes.

79

80 *Wyochernes asiaticus* Redikorzev 1922 (Arachnida: Pseudoscorpiones: Chernetidae) is a  
81 large (female body length 2-2.5 mm) Holarctic pseudoscorpion whose distribution in  
82 Northern Yukon, Alaska, and Eastern Siberia suggests it is a Beringian relict (Buddle 2015).  
83 In the Yukon Territory of Canada, *W. asiaticus* lives under rocks on seasonally-flooded  
84 stream beds north of 64.28°N. Because all life stages were present in all collections, Buddle  
85 (2015) inferred that this species has a multi-year life cycle; although this remains to be  
86 confirmed with winter collections, it seems likely that adults and juveniles both overwinter.  
87 Here, we measured the critical thermal limits and supercooling points of adult and sub-adult  
88 *W. asiaticus* shortly after mid-summer collections. We also measured immersion tolerance to

89 explore the capacity of this species to withstand submergence during seasonal flooding  
90 events. To our knowledge, this represents the first ecophysiological study on a  
91 pseudoscorpion, and an extension of our understanding of the ecophysiology of polar  
92 microarthropods beyond mites and springtails.

93

#### 94 **Methods**

95 We collected c. 200 *W. asiaticus* by hand from beneath stones on the gravel banks of Sheep  
96 Creek, Yukon Territory, Canada (66.8°N, 136.3°W, 562 m elevation). The pseudoscorpions  
97 were separated into individual perforated 1.5 mL microcentrifuge tubes and kept together in a  
98 plastic bag with humidity maintained via wet cotton wool in a perforated 15 mL plastic  
99 centrifuge tube in an insulated container. We returned them to Western University, and held  
100 them at a constant 12 °C under 24 h light (consistent with summer conditions during the  
101 collection period). A maximum of nine days elapsed between collection and use in  
102 experiments. During this period, females which had been carrying egg sacs dropped them,  
103 but only five of 200 animals died during transport, and no controls died during the  
104 experiments.

105

#### 106 *Thermal Biology*

107 We measured critical thermal minima ( $CT_{min}$ ) and maxima ( $CT_{max}$ ) using an approach similar  
108 to that described by Sinclair et al. (2006). Briefly, we placed individual pseudoscorpions into  
109 depressions (1.9 mm diameter, 2 mm depth) milled into an aluminium block cooled by 50%  
110 ethylene glycol circulated from a VWR 1157P recirculating chiller (VWR, Mississauga, ON,  
111 Canada), and covered with a glass microscope slide to prevent escape. We observed them  
112 using a dissecting microscope during cooling or heating. For  $CT_{min}$ , we cooled the

113 pseudoscorpions from 12 °C at 0.25 °C min<sup>-1</sup>, periodically poked them with a fine paintbrush;  
114 we defined the CT<sub>min</sub> as the temperature where an individual's legs curled, and it no longer  
115 moved in response to stimulation from the paintbrush. For CT<sub>max</sub>, we heated the  
116 pseudoscorpions at 0.25 °C min<sup>-1</sup> from 12 °C; we defined the CT<sub>max</sub> as the temperature  
117 where they jerked briefly and no longer responded to stimulus from the paintbrush. We  
118 report mean ± SEM for CT<sub>min</sub> and CT<sub>max</sub>.

119

120 To measure the supercooling point (SCP), we chased an individual into the narrow end of a  
121 10 µL pipette tip, and used cotton wool to hold it in contact with a 36 AWG Type-T  
122 thermocouple (copper-constantan, Omega, Laval, QC, Canada) interfaced to a computer via a  
123 TC-08 thermocouple interface (Pico Technology, Cambridge, UK). We recorded the  
124 temperature every 0.5 s using Picolog software (v 5.24.2 Picotech). We placed the pipette  
125 tips containing pseudoscorpions in holes milled in an aluminium block cooled by 50%  
126 methanol circulating from a Lauda Proline RP855 circulator (Lauda, Würzburg, Germany).  
127 We cooled them at 0.1 °C min<sup>-1</sup> from 12 °C, and recorded the SCP as the lowest temperature  
128 reached before the exotherm indicating ice formation (Lee 2010).

129

130 To determine the cold tolerance strategy, we placed ten pseudoscorpions in pipette tips in  
131 contact with thermocouples in a cooled aluminium block, as described above. We cooled  
132 them from 12 °C at 0.1 °C min<sup>-1</sup> until five of the ten pseudoscorpions had frozen. At this  
133 point, we removed all of the individuals rapidly to room temperature and removed the cotton  
134 wool and thermocouple; survivors resumed movement after a few seconds. If all of the  
135 individuals died regardless of whether they had frozen, we would define that as chill

136 susceptibility, if only individuals that froze died, we would define that as freeze avoidance,  
137 while if individuals that froze survived, we would define that as freeze tolerance.

138

139 *Immersion tolerance*

140 To explore the ability of *W. asiaticus* to survive long periods immersed, we first submerged  
141 n= 10 individuals in 0.7 mL microcentrifuge tubes filled to overflowing with distilled,  
142 deionised water and sealed with Parafilm (Bemis Flexible packaging, Neenah, WI, USA).  
143 These tubes were kept in an incubator at 4 °C, 24 h light, and the pseudoscorpions were  
144 observed under a dissecting microscope for movement after one week. As a control, an equal  
145 number of individuals were placed in dry, perforated vials in the same incubator, and  
146 observed at the same interval as the immersed animals. We weighed each animal before and  
147 after the experiment (blotted dry on tissue paper for the immersed individuals) on a Mettler  
148 MX-5 microbalance (Mettler-Toledo, Columbus, OH, USA).

149

150 In the first immersion experiment, we observed a silvery film of air on the ventral abdomen  
151 that could be consistent with a plastron or other physical gill (Seymour and Matthews 2013),  
152 we repeated the immersion experiment, but this time with water that had been depleted of  
153 oxygen by bubbling dry N<sub>2</sub> gas through it for 2 h prior to use in the experiment (Tamburri et  
154 al. 2002). This decreased the oxygen saturation of the water from 70.0 % to 27.4 % (YSI 600  
155 Q-S dissolved oxygen meter, Yellow Springs, OH, USA). The tubes were again sealed with  
156 parafilm and held at 4 °C under 14 h daylight. A control again consisted of pseudoscorpions  
157 in similar-sized microcentrifuge tubes but that were perforated and dry, giving them full  
158 access to air. The pseudoscorpions were checked for survival after 1 week, and every 1-3



159 days thereafter until 50 % of the immersed animals had died (no sign of movement following  
160 agitation of the tube).

161

## 162 **Results & Discussion**

163 Only pseudoscorpions that froze died, suggesting that they are freeze-avoidant, in keeping  
164 with other polar microarthropods (Cannon and Block 1988). The mean SCP was  $-6.9 \pm 0.7$   
165  $^{\circ}\text{C}$  (mean  $\pm$  SE; range:  $-5.6$  to  $-10.7$ ,  $n=7$ ), which is relatively high for a small ( $0.62 \pm 0.02$   
166 mg,  $n=80$ ) microarthropod, suggesting the presence of ice nucleating agents. Antarctic  
167 springtails and mites generally have SCPs below  $-20^{\circ}\text{C}$  (Cannon and Block 1988), although  
168 springtails that are feeding can have SCPs similar to those we report for *W. asiaticus* (e.g.  
169 Sinclair et al. 2003; Worland et al. 2000), and feeding can also increase SCP in spiders  
170 (Tanaka 1994; Tanaka and Watanabe 1996). This SCP is likely too high for survival of  
171 Yukon's winter conditions, even under snow cover; in Fairbanks, Alaska, temperatures  
172 beneath snow can reach at least  $-13^{\circ}\text{C}$  (Barnes et al. 1996). Thus, we would expect  
173 substantial seasonal plasticity in cold tolerance, as has been observed in other  
174 microarthropods (e.g. van der Woude 1987). Alternately, it is possible that the moist under-  
175 rock habitat of the pseudoscorpions might be conducive to cryoprotective dehydration, as has  
176 been observed for the arctic springtail *Megaphorura arctica* (Holmstrup and Sømme 1998;  
177 Worland et al. 1998), which has a similar SCP to *W. asiaticus*. However, pseudoscorpions  
178 exposed to air for one week as controls in our immersion experiments lost relatively little  
179 mass (see below), suggesting that they may not be permeable enough to use this strategy  
180 (Holmstrup et al. 2002).

181

182 The  $CT_{min}$  of *W. asiaticus* was  $-3.6 \pm 0.5$  °C (range: -0.7 to -4.8, n= 9), and the  $CT_{max}$  was  
183  $37.8 \pm 1.1$  °C (range: 33.3 to 43.6 °C, n=10). Pseudoscorpions have been reported active  
184 beneath the snow in Southern Manitoba (Aitchison 1979), and although the  $CT_{min}$  we  
185 observed is consistent with low temperature activity (at least extending the active season), we  
186 expect that these animals will spend much of the winter inactive, assuming that under-snow  
187 temperatures are similar to those reported by Barnes et al. (1996). Both the  $CT_{min}$  and  $CT_{max}$   
188 are broadly similar to those reported for oribatid mites from the maritime Antarctic (Everatt  
189 et al. 2013). Thus, the  $CT_{min}$  of *W. asiaticus* is consistent with that of other polar  
190 microarthropods, and would likely extend activity during the otherwise short growing season  
191 well into the spring and autumn. We do note that the  $CT_{max}$  of *W. asiaticus* we report here is  
192 lower than the c. 45 °C recorded for wolf spiders from the same region, even though the  
193  $CT_{min}$  for *W. asiaticus* is broadly similar to that of these spiders (S.E. Anthony, unpublished  
194 observations). This may indicate that spiders and *W. asiaticus* experience different selection  
195 pressures on  $CT_{max}$ , even in nearby habitats.

196

197 The near-stream riparian habitat of *W. asiaticus* is regularly flooded in the spring, leading us  
198 to explore the capacity of this species to tolerate immersion in water. In our first experiment,  
199 we observed no mortality in control animals, and survival of 9/10 individuals held  
200 submerged. We observed a silvery film of air on the abdomen of the submerged individuals,  
201 and most individuals clung to the vial wall, trapping a larger bubble between their body and  
202 the vial; we agitated the vials to remove this large bubble at the beginning of the experiment.  
203 To test the hypothesis that the trapped air on the abdomen acts as a gill (Seymour and  
204 Matthews 2013), we repeated this experiment with deoxygenated water. After one week,  
205 mortality was the same as in oxygenated water (no mortality in control, 1/10 in submerged),  
206 but the time to 50 % mortality was seventeen days for both treatment and control, suggesting

207 that factors other than immersion were responsible for mortality. Historical river flow data  
208 from Eagle Creek (2.5°S of our collections) suggest that flood events in this part of Yukon  
209 Territory generally last 2-7 days, with occasional high discharges persisting for 10 days  
210 (Environment Canada: [www.wateroffice.ec.gc.ca](http://www.wateroffice.ec.gc.ca), station 09FB002).

211

212 During the first immersion experiment, we also observed changes in mass (assumed to be due  
213 to change in water content); while the air-exposed controls lost  $4.6 \pm 0.6$  % (range: 1.3 to 8.2  
214 %) of their body water over this time, the surviving immersed individuals gained  $6.2 \pm 1.3$  %  
215 (range: 1.8 to 13 %) of their body water. The individual that died gained 14.9 % mass. It is  
216 possible that the pseudoscorpions were slightly dehydrated at the start of the experiment (they  
217 did not have access to liquid water), and that the mass gain we observed was a function of  
218 rehydration by drinking. However, the submerged individuals did appear engorged (S.E.  
219 Anthony pers. Observations), suggesting that this may instead be a case of ‘overhydration’  
220 (cf. Lopez-Martinez et al. 2009), which might imply that long periods of immersion  
221 eventually lead to osmotic stress. Under this scenario, we hypothesise that mortality of the  
222 control and immersed individuals in our second immersion experiment could be from  
223 different causes: desiccation in the air-exposed controls, but overhydration in the submerged  
224 individuals. Given the significant variation in water availability between summer and winter,  
225 and during flooding, water balance of this species merits future attention.

226

227 In conclusion, *W. asiaticus* appears to be relatively cold-adapted, with a low  $CT_{min}$  and  
228  $CT_{max}$ , but we predict it will show significant seasonal plasticity in cold hardiness. It can  
229 easily withstand immersion for one week, and does not appear to be reliant on oxygen from  
230 the water for this survival. These are the first direct measures of environmental physiology

231 for any pseudoscorpion, and an important taxonomic extension of our understanding of the  
232 physiology of Arctic microarthropods. Given the relative accessibility of this species, it may  
233 be a useful model for understanding pseudoscorpion physiology in general.

234

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