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A newly-described host-symbiont interaction: first record of *Dinocheirus panzeri* (Pseudoscorpiones: Chernetidae) associated with *Cyanistes caeruleus* (Paridae) nests

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Running title: New host for *Dinocheirus panzeri*

Pseudoscorpions are arachnids that prey upon other invertebrates. Currently, 3,385 species have been described worldwide (Harvey 2013) including 27 species in 18 genera in the UK (Legg & Farr-Cox 2016; Legg 2017).

Pseudoscorpion ecology is generally poorly understood, especially outside the tropics. This is partly due to their small size (globally specimens are typically <4mm in length; all but three of the UK species <3mm) and partly because their favoured habitats – leaf litter, decaying wood, tree bark, compost piles – are frequently overlooked in ecological surveying (Christophoryová *et al.* 2011; Mahnert *et al.* 2011).

Pseudoscorpions have been found previously in avian nesting environments. The earliest reports are those of Butterfield (1908) and Kew (1911) (UK) and Nordberg (1936) (Finland). More recently, an exhaustive global review of pseudoscorpions in bird nests was conducted by Turienzo, Di Iorio & Mahnert (2010). In total, 85 pseudoscorpion species (63 genera; 14 families) were found to be associated with bird nests. The greatest species diversity was in Procellariidae and Turdidae nests, with Turdidae nests also being notable for high pseudoscorpion prevalence. Despite this global picture, there are only 13 recorded incidences of pseudoscorpions in known wild bird nests in the UK, which are mainly based on one individual in a single nest (see Table 1 and references therein); there were two additional records by Jones (1975) where the host species was unknown.

We report the discovery of a female *Dinocheirus panzeri* (Chernetidae) (C.L. Koch 1837) in a *Cyanistes caeruleus* (Linnaeus 1758) nest built in a wooden nestbox at Nagshead Nature Reserve, Gloucestershire, UK (2°34'0" W, 51°47'0" N). The nest was collected within 24 hours post-fledging under English Nature licence 20060590 as part of a larger project and double sealed to prevent cross-contamination. It was immediately

frozen to kill and preserve any arthropods. Following freezing, nest material was manually searched using a 1mm diameter sieve to separate feather dust from vegetative material, which was then thoroughly teased apart using forceps under 10x magnification.

The pseudoscorpion specimen was a female with a well-developed egg mass on the opisthosoma containing at least 15 eggs (Fig. 1; Fig 2). Identification was undertaken using a Nikon SMZ800 compound microscope with images projected onto a computer using Nikon NIS Elements version D3.2. Initial identification of *Dinocheirus panzeri* was based on cephalothorax and pedipalp colouration, together with the number, colouration and division of the opisthosomal tergites (Legg & Farr-Cox 2016; Legg 2017). Identification was confirmed by the presence of: (1) accessory teeth on both movable and fixed fingers of the pedipalps (n = 4 and 5, respectively) (Nassirkhani, Sharaf & Azimi 2015; Legg 2017; Fig1c) and (2) long subdistal tricobothrium-like tactile setae on pedal tarsus IV (Christophoryová, Šťáhlavský & Fedor 2011; Nassirkhani, Sharaf & Azimi 2015; Legg 2017). The individual was fairly large at 2.9mm in length (2.1-2.6mm is typical (Legg & Farr-Cox 2016) but lengths of 2.9mm have been recorded for females previously (Nassirkhani, Sharaf & Azimi 2015)). Given that positive identification was possible using features present with the only specimen intact, and the need to retain a specimen in an archive as per journal policy, we did not dissect the individual to examine the spermatheca. The specimen has been placed in permanent storage in the University of Gloucestershire Archives (accession number GB1960 U/04/17/1; Francis Close Hall, Swindon Road, Cheltenham, GL50 4AZ; archives@glos.ac.uk; +441242714851).

This is the seventh record of *Dinocheirus panzeri* being associated with wild birds in the UK and the fourth definite avian species with which it has been associated (Table 1; Butterfield 1908; George 1961; Jones 1975). More importantly, this is: (1) the first record of any pseudoscorpion of species occurring in *C. caeruleus* nests in the UK (Turienzo, Di Iorio & Mahnert 2010); and (2) the first ever record of *Dinocheirus panzeri* being associated with *C. caeruleus* anywhere despite the species co-occurring in 27 countries throughout Europe (Harvey 2013; Birdlife International 2017). The species has not yet been assessed by IUCN in terms of rarity but only 23 UK records were collated by Jones (1980) between 1960 and 1980. The National Biodiversity Network atlas only has 8 UK records, which all pre-date 1980.

Theoretically, bird nests constitute ideal habitat for pseudoscorpions. They comprise dead or decaying vegetation and those built inside cavities also have a warm and humid microclimate (Jones 1978). Moreover, nests are likely to be food rich as they support numerous ectoparasitic and free-living arthropods (Phillips & Dindal 1990; Goodenough & Hart 2012), many of which are small enough to be pseudoscorpion prey (Woodroffe 1953). In the particular nest where this specimen was found, there were Acari and Phthiraptera, as well as larval Siphonaptera, Coleoptera, and Lepidoptera. Bird nests thus have the potential to be a high-quality habitat for pseudoscorpions, some of which may be nest-specialists (Mahnert *et al.* 2011).

The apparent suitability of avian nests for pseudoscorpions is at odds with their low prevalence (Turienzo, Di Iorio & Mahnert 2010) and it is thus appropriate to consider whether pseudoscorpions are under-recorded in nest environments (Christophoryová *et al.* 2011) or whether they are genuinely rare. Despite bird nests supporting a miniature ecosystem of predatory, scavenging and detritivore species, they are not routinely examined for invertebrates. The potential for species to be under-recorded is thus considerable, as evidenced by Goodenough & Hart (2012) finding numerous individuals of the “rare” beetle *Gnathoncus buyssoni* (Auzat 1917) in nests of *Parus major* (Linnaeus 1758). That said, detailed analysis of another ~40 *C. caeruleus* and *P. major* nests from the same site (Goodenough, Elliot & Hart 2011; Goodenough & Hart 2012) did not reveal any other pseudoscorpions. Thus although under-recording is likely to be partly responsible for the seemingly low incidence of pseudoscorpions, the phenomenon does not seem particularly common. This concurs with work in the Neotropics by Turienzo (2012) where 353 insect species were found in 634 bird nests compared to just 7 pseudoscorpion species. Low prevalence might be partly explained by the fact that nests are temporary in nature: although the mating strategy of pseudoscorpions allows females to exploit temporary habitats (Legg & Farr-Cox 2016), low dispersal ability might be an important biological barrier.

It is unclear how pseudoscorpions arrive in bird nests. Possibilities include pseudoscorpions actively seeking out nests, being accidentally translocated in nesting material, arriving via phoresy on birds (Jones 1978), or simply being present by chance. As pseudoscorpions have previously been found attached to legs of flies (Jones 1978; Carl 1994), it is also possible that pseudoscorpions arrive on insects such as Protocalliphora, which lay their eggs in bird nests. Multiple Protocalliphora larvae/pupae occurred in the same nest as our specimen. As the majority of UK bird-pseudoscorpion associations (Table 1) involve

multi-brooded birds (*Hirundo rustica*, *Columba livia*, *Turdus merula*) or those with comparatively long incubation and chick-rearing periods (*Ardea cinerea*, *Corvus monedula*), the host nests are less ephemeral than the norm. This could increase the chances of phorasy or passive translocation and/or the reward of actively seeking out such environments.

In conclusion, we present the first evidence of pseudoscorpions using *C. caeruleus* nests in the UK and the first evidence of an interaction between *C. caeruleus* and *Dinocheirus panzeri* anywhere in the world. This is likely to be under-recorded and thus true prevalence of this newly-described host-symbiont interaction cannot be determined without further investigation. We recommend this as an area for future.

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Table 1: Previous records of pseudoscorpions found in wild birds' nests in the UK. All records come from individual nests unless indicated by a double symbol. Based on data in Turienzo, Di Iorio & Mahnert (2010) and Jones (1975).

	<i>Ardea cinerea</i> Linnaeus 1758	<i>Delichon urbica</i> (Linnaeus 1758)	<i>Hirundo rustica</i> Linnaeus 1758	<i>Corvus monedula</i> Linnaeus 1758	<i>Columba livia</i> Gmelin 1789	<i>Turdus merula</i> Linnaeus 1758	<i>Sturnus vulgaris</i> Linnaeus 1758	<i>Passer domesticus</i> (Linnaeus 1758)	<i>Parus major</i> Linnaeus 1758	Strigidae	Columbidae
<i>Cheiridium museorum</i> (Leach 1817)		+	+	+	+			+			
<i>Chelifer cancroides</i> (Linnaeus 1758)					++						
<i>Chthonius ischnocheles</i> (Hermann 1804)		+				+					
<i>Chernes cimicoides</i> (Fabricius 1793)	+										
<i>Pselaphochernes scorpioides</i> (Hermann 1804)				+							
<i>Dinocheirus panzeri</i> (C.L. Koch 1837)				+			+		++	+	+



Figure 1.

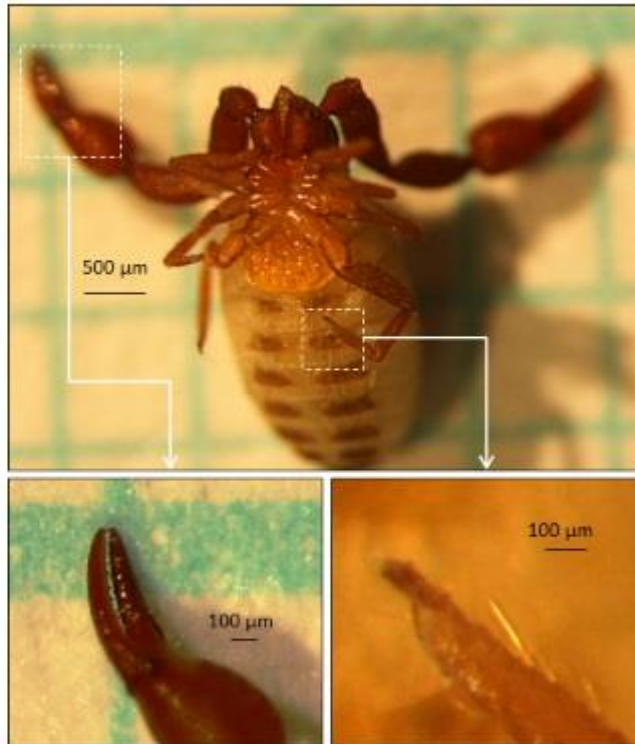


Figure 2.

Figure Legends

Figure 1: Dorsal view of a female *Dinocheirus panzeri* (composite focus-stacked image using multiple photographs in different focal planes).

Figure 2: Ventral view of a female *Dinocheirus panzeri* showing a well-developed egg mass. The two diagnostic identifying features are shown on inserts: the (left) pedipalp with accessory teeth on both movable and fixed fingers and the (right) pedal tarsus IV showing long tactile setae.

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