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Original Article

DNA barcoding suggested the existence of cryptic species and high biodiversity of South Korean pseudoscorpions (Arachnida, Pseudoscorpiones)

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A B S T R A C T

Pseudoscorpions are one of the common arthropod in soil mesofauna but are infrequently studied in East Asia. The fauna in South Korea is not adequate enough, and practical faunistic survey of pseudoscorpions have not been conducted in last 20 years. In this article, the current pseudoscorpion fauna in South Korea is reviewed, and the results of the survey in Chungcheongnam-do Province, the DNA barcoding (mitochondrial DNA cytochrome *c* oxidase I sequencing) and molecular phylogenetic analysis are shown. A total 64 specimens, including four species, *Allochthonius* (*Allochthonius*) *buanensis*, *Bisetocreagris japonica*, *Bisetocreagris turkestanica* (first record from South Korea), and *Microbisium pygmaeum*, were collected. The result of molecular phylogenetic analysis based on the cytochrome *c* oxidase I sequences (427 bp) implied the existence of a possible cryptic species within *A.* (*A.*) *buanensis*. Faunistic survey and DNA barcoding of South Korean species can greatly contribute to the understanding of East Asian pseudoscorpion systematics.

Introduction

Soil biodiversity provides sustainable ecosystem services (Lavelle 1996; Brussaard 1997; Lavelle et al 2006; Wolters 2001). However, much of the taxonomic diversity of soil organisms has not been sufficiently researched. Thus, soil communities may contain a large number of undescribed species (Dance 2008; Decaëns 2010; Rougerie et al 2009). Taxonomic studies should be promoted to accurately identify and describe the biodiversity for the conservation of soil ecosystems (Decaëns 2010; Decaëns et al 2008).

Pseudoscorpions are one of the common arthropod taxa in the soil mesofauna, but they have been largely ignored in East Asia. Over 3,500 pseudoscorpion species are currently described worldwide (Harvey 2013a,b). Few surveys of pseudoscorpions have been conducted in most countries apart from the United States, European countries, and some African countries (Harvey 2007). On the other hand, faunistic surveys could result in the discovery of new species if they are conducted in regions where they have not yet been done. In fact, recent surveys have continuously reported many new species, especially in China (e.g. Gao et al 2016, 2017a,b; Gao and Zhang 2011, 2012, 2013, 2016; Guo and Zhang 2016a,b, 2017; Harvey 2013b; Hu and Zhang 2011, 2012a,b,c,d,e, 2013; Li et al 2017; Mahnert and Li 2016; Yang and Zhang 2013; Zhang and Zhang 2014a,b,c). DNA barcoding and molecular phylogenetic analysis have suggested the existence of multiple cryptic and undescribed species, sometimes represented as a short-range endemisms (Harvey 2002), in soil-dwelling and subterranean pseudoscorpions (Cosgrove et al 2016; Harms 2018; Harrison et al 2014; Harvey et al 2016; Neethling and Haddad 2016; Ohira et al 2018).

The combined approach of faunistic surveys with DNA barcoding and molecular phylogenetic analysis may be useful for describing the taxonomic diversity of South Korean pseudoscorpions. A total 21 species of pseudoscorpions, including 16 soil-dwelling species, have been recorded from South Korea (Bang 1984; Choi and Hwang 2009; Hong 1992, 1996; Hong and Kim 1993, 1996; Hong et al 1996, 1997; Kim and Hong 1994; Lee 1981, 1982; Lee and Seo 1995; Morikawa 1970; Table 1). The number of species recorded in South Korea is lower than that of neighboring countries: 68 species from Japan, 33 species from Russia, and 98 species from China (Gao and Zhang 2011, 2012, 2013, 2016; Gao et al 2016, 2017a, 2017b; Guo and Zhang 2016a, 2016b, 2017; Harvey 2013b; Hu and Zhang 2011, 2012a,b,c,d,e, 2013; Li et al 2017;

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Mahnert and Li 2016; Yang and Zhang 2013; Zhang and Zhang 2014a,b,c). The species richness of South Korean pseudoscorpions might be underestimated because of a deficient investigation. Practical faunistic surveys of South Korean pseudoscorpions have not been conducted in the last 20 years, except for a record of maritime species from Dokdo Island (Choi and Hwang 2009). Although many new species of pseudoscorpions have been described using DNA barcoding and molecular phylogenetic analysis in current study (e.g. Harvey et al 2016; Neethling and Haddad 2016), no attempts were made for South Korean pseudoscorpions. However, it is possible to discover unrecorded and/or undescribed (or cryptic) species in the area. Faunistic survey coupled with DNA barcoding and molecular phylogenetic analysis will elucidate the understanding of the taxonomic diversity of the species, on the current situation of pseudoscorpion study in South Korea.

To show the necessity of DNA barcoding and molecular phylogenetic analysis for description of taxonomic diversity of South Korean pseudoscorpions, we performed preliminary faunistic survey of soil-dwelling pseudoscorpions in the Korean peninsula for the first time in 20 years since Hong et al (1997). We then collected *Allochthonius*, *Bisetocreagris*, and *Microbisium* species, which included a newly recorded species in South Korea. Here, we provide the mitochondrial DNA sequences of South Korean pseudoscorpions for the first time and suggest the possibility of existence of cryptic species in South Korean pseudoscorpions.

Material and methods

Collection sites and sampling

We collected pseudoscorpions from six sites in Chungcheongnam-do Province, South Korea, from September 30 to October 1, 2016 (Figure 1; Table 2). The collection sites were chosen based on convenience and accessibility primarily because of our time-limited survey. Specimens were collected by sifting leaf litter (Figure 2A–2F) and using the following procedure: (1) Leaf litters and soil are sieved and passed through a soil sieve (5 mm) on white tray; (2) Soil animals will be dropped with leafs and soil debris on the tray; (3) Look for pseudoscorpions among other soil animals, leafs, and soil debris dropped; and (4) Pick up a pseudoscorpion using brush, and then put it into screwcap tubes with absolute ethanol. All examined specimens are stored at Changwon National University of Korea and in the University Archives and Collections, Fukushima University of Japan, respectively.

Morphological observation and species identification

An appendage was removed from the specimen's body using a thin needle (tungsten needle) under a stereoscopic microscope SMZ645 (Nikon, Tokyo, Japan). Each appendage removed from the body was transferred individually to a 0.2 ml polymerase chain reaction (PCR) tube with absolute ethanol for DNA extraction. The remaining part of the body was then stored in a 1.5 ml PCR tube with absolute ethanol until it was mounted to a microscope slide.

The stage or instar of each specimen was determined by counting the trichobothrial setae on the movable finger of the pedipalp under a stereoscopic microscope. Sex of specimens was determined by confirming the morphology of the genital area (Harvey 1992; Weygoldt 1969). Some specimens were prepared for microscopic slides using conventional procedures (cf. Okajima 2006). Microscopic slides were observed under an optical microscope ECLIPSE E200 (Nikon, Tokyo, Japan), and specimens were identified based on their morphological characteristics.

Species were identified based on the references indicated in Table 1. However, because several *Bisetocreagris* specimens were

Table 1. Species list of South Korean pseudoscorpions.

Taxon	Habitat	References
Suborder Epiocheirata		
Family Pseudotyrannochthoniidae		
Allochthonius (*Allochthonius*) *buanensis* W.K. Lee, 1982[*]	Soil	Lee (1982); Lee & Seo (1995); Hong et al (1996); Hong et al (1997); this study
Allochthonius (*Allochthonius*) *coreanus* Morikawa, 1970[*]	Cave	Hong et al (1996); Hong et al (1997); Lee & Seo (1995); Morikawa (1970)
Allochthonius (*Allochthonius*) *opticus* (Ellingsen, 1907)	Soil	Hong et al (1996); Lee & Seo (1995)
Pseudotyrannochthonius dentifer (Morikawa, 1970)[*]	Cave	Hong et al (1996); Morikawa (1970)
Family Chthoniidae		
Mundochthonius bifurcatus Kim & Hong, 1994[*]	Soil	Hong (1992); Hong et al (1996); Kim & Hong (1994)
Mundochthonius minusculus Kim & Hong, 1994[*]	Soil	Hong (1992); Hong et al (1996); Hong et al (1997); Kim & Hong (1994)
Tyrannochthonius assimilis Hong & Kim 1993[*]	Soil	Hong & Kim (1993); Hong et al (1996)
Tyrannochthonius spinatus Hong, 1996[*]	Soil	Hong et al (1996)
Tyrannochthonius suppressalis Hong, 1996[*]	Soil	Hong et al (1996)
Suborder Iocheirata		
Family Cheiridiidae		
Cheiridium minor Chamberlin, 1938	House	Lee (1981)
Family Chernetidae		
Allochernes japonicus (Morikawa, 1953)	Tree	Bang (1984)
Family Garypidae		
Garypus japonicus Beier, 1952	Seashore	Choi & Hwang (2009)
Family Neobisiidae		
Bisetocreagris japonica (Ellingsen, 1907)	Soil	Hong et al (1997); this study
Bisetocreagris pygmaea (Ellingsen, 1907)	Soil	Hong et al (1997)
Bisetocreagris turkestanica (Beier, 1929)[†]	Soil	this study
Microbisium pygmaeum (Ellingsen, 1907)	Soil	Hong et al (1997); Lee (1981); this study
Microcreagris microdivergens Morikawa, 1955	Soil	Lee (1981)
Microcreagris pseudoformosa Morikawa, 1995	Soil	Bang (1984)
Parobisium longipalpum Hong, 1996[*]	Soil	Hong (1996); Hong et al (1997)
Parobisium magnum chejuense Morikawa, 1970[*]	Soil	Hong (1996); Morikawa (1970)
Parobisium magnum ohuyeanum Morikawa, 1952	Soil	Bang (1984)
Parobisium robustiellum Hong, 1996[*]	Soil	Hong (1996); Hong et al (1997)
Family Syarinidae		
Pararoncus japonicus (Ellingsen, 1907)	Soil	Hong & Kim (1996); Hong et al (1997)

* Endemic species/subspecies to South Korea.
† New record in South Korea. This species was identified using Ćurčić (1983, 1985).

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