Proc. Arthropod. Embryol. Soc. Jpn. 50, 11–13 (2016)

ISSN 1341–1527

[SHORT COMMUNICATION]

Is Abdominal Tergal Chaetotaxy Reliable for Species Diagnosis of Japanese Soil-Dwelling *Mundochthonius* Pseudoscorpions (Pseudoscorpiones: Chthoniidae)?

Hajime OHIRA[1], Shingo KANEKO[2] and Tadaaki TSUTSUMI[2]

[1] Graduate School of Symbiotic Systems Science and Technology, Fukushima University, 1 Kanayagawa, Fukushima, Fukushima 960–1296, Japan
[2] Department of Environmental System Management, Faculty of Symbiotic Systems Science, Cluster of Science and Technology, Fukushima University, 1 Kanayagawa, Fukushima, Fukushima 960–1296, Japan
E-mail: ohira.hajime@gmail.com (HO)

Pseudoscorpions are small arachnid animals living in various habitats, *i.e.*, in leaf litter, under stones or barks, in crevices of rocks along seashore, in caves, and even in desert. Studies on the embryonic and postembryonic developments and reproductive biology, including reproductive behavior, of pseudoscorpions have used specimens collected directly from the field, because it is difficult to establish cultures (*e.g.*, Morikawa, 1960, 1962; Sakayori, 1989, 2002b, 2003, 2014b; Kato and Tsutsumi, 2004). Japanese soil-dwelling pseudoscorpions are mainly classified based on the chaetotaxy of carapace and abdominal tergites, and pedipalpal femur morphology (Morikawa, 1960; Sato and Sakayori, 2015). However, these features are variable within species (Tsutusmi, 2012; Sakayori, 2014a) and chaetotaxy is known to change with growth (Sakayori, 2014b).

In the previous studies dealing with the developmental and reproductive biologies on pseudoscorpions, *Muncochthonius* species have also been used as materials in Japan (Kato and Tsutsumi, 2004). In the identification of *Mundochthonius* species and subspecies (Chamberlin, 1929; Morikawa, 1954; Sakayori, 2002a, 2009), including *M. japonicus japonicus* Chamberlin, *M. j. scolytidis* Morikawa, *M. kiyoshii* Sakayori, and *M. itohi* Sakayori, abdominal tergal chaetotaxy (ATC, Fig. 1) in the first, second, and third abdominal segments are used as the most important characters for classification (Sakayori, 2010), and three types of ATC are currently distinguished: "4–4–4" in *M. j. japonicus*, "4–4–6" in *M. j. scolytidis*, and "4–6–6" in *M. kiyoshii* and *M. itohi* (Sakayori, 2010). The three numerals that are used to describe ATC represent the number of tergal setae that occur in the first to third abdominal segments and are basically even numbers, because tergal setae are symmetrical and not on the median line. However, ATCs with an odd number are frequently observed and recognized as an irregularity and/or variation within a species. For example, in *M. itohi*, several irregular types of ATC such as "4–5–6" and "4–6–7" are found in addition to their regular type of ATC, "4–6–6" (Sakayori, 2009; Tsutsumi, 2012). Moreover, Tsutsumi (2012) reported that one of the total 374 samples examined in *M. itohi* had an irregular "4–4–6" ATC, which is the ATC typical to *M. j. scolytidis*. It may be possible that the ATC of the regular type in one species might be found as an irregular type in other species. Therefore, it should be examined whether the ATC is reliable for the identification of *Muncochthonius* species. In the present study, we test the reliability of ATC in the identification of *Mundochthonius* species, using molecular phylogenetic analysis that was used for critical taxonomical

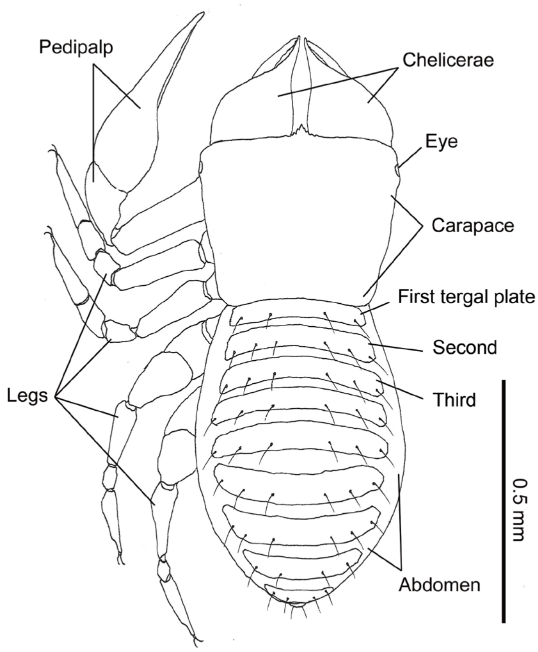

Fig. 1 *Mundochthonius itohi* Sakayori, dorsal view. Chaetotaxy is shown only on abdominal tergal plates. Right pedipalp and legs are omitted.

revisions of pseudoscorpions (*e.g.*, Murienne *et al.* 2008; Harrison *et al.* 2014).

Mundochthonius specimens were collected in Japan from Kanayama (37° 43′ 49.7″ N, 140° 3′ 13.6″ E) and Sobara (37° 41′ 22.1″N, 140° 4′ 0.5″E) in Kitashiobara, Fukushima Prefecture and from Chinone, which is the type locality of *M. itohi* (36° 35′ 27.5″N, 140° 34′ 27.6″E), in Hitachiota, Ibaraki Prefecture. Specimens were extracted from leaf litter and directly placed into absolute ethanol using Tullgren funnels. Extracted specimens were transferred to and preserved in absolute ethanol. One pedipalp was removed using a thin tungsten needle under a stereomicroscope and transferred to a PCR tube for DNA extraction; the body was then stored in a 0.2-ml tube with absolute ethanol and then processed into a microscopic slide preparation using a conventional procedure (cf. Okajima, 2006). Specimen sex was determined, and setae in the first, second, and third abdominal tergal plates were counted. All specimens examined were deposited in our laboratory at Fukushima University.

Total genomic DNA was extracted from one pedipalp using an improved method of DNA extraction from a single pollen grain (Suyama, 2011). Partial sequences of 18S ribosomal RNA gene were amplified using the following primer sets: 1F-5R, 3F-18Sbi, and 18Sa2.0–9R (Giribet *et al.*, 1996; Whiting *et al.*, 1997). All nucleotide sequences were automatically aligned using MAFFT version 7 (Katoh and Standley, 2013). The sequence gaps were treated as missing data using trimAI (Capella-Gutiérrez *et al.*, 2009). Two species of *Chthonius*, which belongs to the same tribe as *Mundochthonius*, Chthoniini, were used as outgroups for our phylogenetic analysis; their sequences were obtained from GenBank (accession numbers JN018288 and JN018289). Phylogenetic reconstruction was performed using the neighbor-joining method (Saitou and Nei, 1987), and nodal support of the phylogenetic tree was measured with 1000 bootstrap replicates (Felsenstein, 1985) and implemented in MEGA version 6 (Tamura *et al.*, 2013).

The specimens examined, 18 *Mundochthonius* individuals, showed several variations in ATC, including odd numbers of setae ("4–6–7", "4–5–6", and "5–4–6"), and the variations are not related to sex (Table 1). Two of these types of ATC, "4–6–7" and "4–5–6", had already been reported (Sakayori, 2009; Tsutsumi, 2012), but "5–4–6" was a new irregular type. The neighbor-joining dendrogram based on the partial sequences of nuclear DNA 18S ribosomal RNA gene (1244 bp) revealed three distinct clades with high bootstrap values (Clades 1–3, Fig. 2). The present analysis revealed that these three clades cannot be clearly distinguished by the ATC types. The ATC types "4–6–6" and "4–4–6" predominated in the clades 2 and 3 respectively, but different ATC types "4–5–6" and "5–4–6" were included as minorities in the clades 2 and 3. The clade 1 included three different types "4–4–6", "4–6–6",

Table 1 Specimens examined in this study

Individual	Locality	Sex*	ATC**	DDBJ accession no.
M1	Kanayama	F	4–6–7	LC082336
M2	Kanayama	F	4–6–6	LC082337
M3	Kanayama	M	4–6–6	LC082339
M4	Kanayama	M	4–6–6	LC082338
M5	Kanayama	M	4–4–6	LC082343
M6	Sobara	F	4–6–6	LC082341
M7	Sobara	M	4–6–6	LC082342
M8	Sobata	M	4–4–6	LC082340
M9	Chinone	M	4–6–6	LC082344
M10	Chinone	M	4–5–6	LC082345
M11	Chinone	F	4–5–6	LC082346
M12	Chinone	F	4–4–6	LC082350
M13	Chinone	F	5–4–6	LC082351
M14	Chinone	F	4–6–6	LC082349
M15	Chinone	M	4–4–6	LC082352
M16	Chinone	M	4–6–6	LC082347
M17	Chinone	F	4–4–6	LC082353
M18	Chinone	F	4–6–6	LC082348

*F: female, M: male.

**Abdominal tergal chaetotaxy (ATC) represents the number of setae in the first, second, and third abdominal tergal plates.

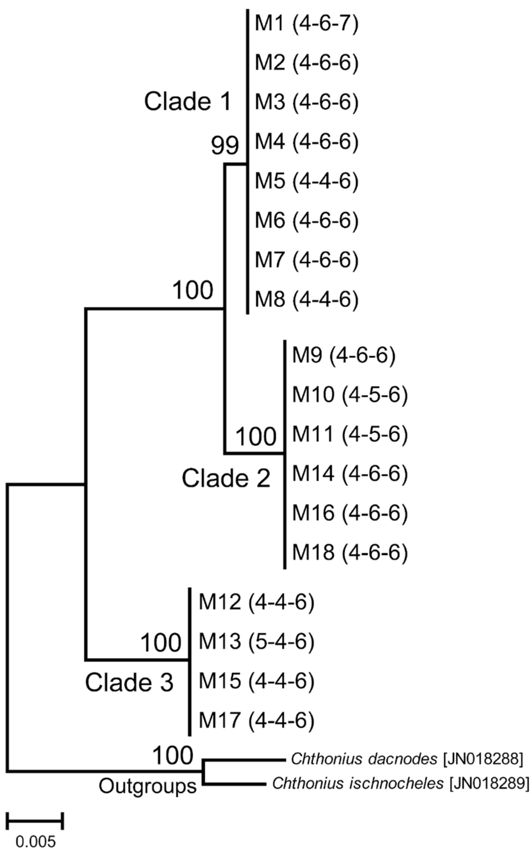

Fig. 2 Neighbor-joining dendrogram of Japanese soil-dwelling *Mundochthonius* inferred from the partial sequences (1244 bp) of 18S ribosomal RNA gene. Operational taxonomic units (OTUs) were indicated by the individual number of *Mundochthonius* shown in Table 1 (M1-M18) with the ATC in parenthesis. *Chthonius dacnodes* [GenBank accession number JN018288] and *C. ischnocheles* [JN018289]) were used as outgroups. Bootstrap support values are shown above the branches.

and "4–6–7" as well. The present study revealed that the ATC cannot clearly define the monophyletic clades, and this implies that the ATC should not be a reliable taxonomic diagnosis for *Mundochthonius*.

Sakayori (2014a) pointed out that the pedipalpal femur morphology (length:width ratio of the pedipalp femur) is not a useful character in *Allochthonius* species identification. The pedipalpal femur morphology has been employed also in the classification of *Mundochthonius* as another useful diagnosis (Sato and Sakayori, 2015). Its reliability has to be also tested for this genus, and simultaneously more reliable and practical diagnoses in *Mundochthonius* identification should be surveyed.

Acknowledgments: The present study was supported by the Research Project for Regeneration of Harmonies between Human Activity and Nature in Bandai-Asahi National Park in Fukushima University.

References

Capella-Gutiérrez, S., J. M. Silla-Martínez and T. Gabaldón (2009) trimAI: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, **25**, 1972–1973.

Chamberlin, J. C. (1929) On some false scorpions of the suborder Heterosphyronida (Arachnida-Chelonethida). *The Canadian Entomologist*, **61**, 152–155.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.

Giribet, G., S. Carranza, J. Baguñà, M. Riutort and C. Ribera (1996) First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Molecular Biology and Evolution*, **13**, 76–84.

Harrison, S. E., M. T. Guzik, M. S. Harvey and A. D. Austin (2014) Molecular phylogenetic analysis of Western Australian troglobitic chthoniid pseudoscorpions (Pseudoscorpiones: Chthoniidae) points to multiple independent subterranean clades. *Invertebrate Systematics*, **28**, 386–400.

Kato, Y and T. Tsutsumi (2004) Life cycle and breeding period in a soil pseudoscorpion, *Mundochthonius japonicus* Chamberlin (Arachnida: Pseudoscorpiones) in Iino town, Fukushima Prefecture, inferred from their oogenesis and seasonal fluctuation. *Proceedings of Arthropodan Embryological Society of Japan*, **39**, 55–58. (in Japanese).

Katoh, K. and D. M. Standley (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.

Morikawa, K. (1954) Two new species of Chthoniinea from Japan. *Japanese Journal of Zoology*, **11**, 329–331.

Morikawa, K. (1960) Systematic studies of Japanese pseudoscorpion. *Memoirs of Ehime University (2B)*, **4**, 85–172.

Morikawa, K. (1962) Ecological and some biological notes on Japanese pseudoscorpions. *Memoirs of Ehime University (2B)*, **4**, 417–435.

Murienne, J., M. S. Harvey and G. Giribet (2008) First molecular phylogeny of the major clades of Pseudoscorpiones (Arthropoda: Chelicerata). *Molecular Phylogenetics and Evolution*, **49**, 170–184.

Okajima, S. (2006) *The Insects of Japan, Vol.2, The Suborder Tubulifera*. Touka Syobo, Fukuoka.

Saitou, N. and M. Nei (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sakayori, H. (1989) Postembryonic development of a neotenic pseudoscorpion, *Microbisium pygmaeum* (Ellingsen, 1907). *Acta Arachnologica*, **38**, 55–62.

Sakayori, H. (2002a) Two new species of the family Chthoniidae from Kyushu, in Western Japan (Arachnida: Pseudoscorpionida). *Edaphologia*, **69**, 1–7.

Sakayori, H. (2002b) Postembryonic development of a Japanese soil-dwelling pseudoscorpion, *Tyrannochthonius japonicus* (Pseudoscorpion, Chthoniidae). *Bulletin of Ibaraki Nature Museum*, **5**, 57–67.

Sakayori, H. (2003) External morphology of nymphal stages of *Allochthonius tamurai* Sakayori, 1999 (Pseudoscorpion, Chthoniidae). *Bulletin of Ibaraki Nature Museum*, **6**, 23–31.

Sakayori, H. (2009) A new species of the genus *Mundochthonius* from Ibaraki Prefecture, Central Japan (Arachnida: Pseudoscorpionida: Chthoniidae). *Bulletin of Ibaraki Nature Museum*, **12**, 1–4.

Sakayori, H. (2010) The current situation in the classification of the genus *Mundochthonius* pseudoscorpions in Japan. *In* Ibaraki Nature Museum (ed.), *Report of Comprehensive Surveys of Plants, Animals and Geology in Ibaraki Prefecture by the Ibaraki Nature Museum -Trends of Insects and Other Invertebrates in 2009-*, pp. 51–52. Ibaraki Nature Museum, Bando. (in Japanese).

Sakayori, H. (2014a) Redescription of *Allochthonius (Allochthonius) opticus* collected from Okayama city, West Honshu, Japan (Pseudoscorpionida, Chthoniidae). *Bulletin of Ibaraki Nature Museum*, **17**, 1–6. (in Japanese).

Sakayori, H. (2014b) Postembryonic development of *Allochthonius (Allochthonius) shintoisticus* Chamberlin, 1929 (Pseudoscorpionida: Chthoniidae). *Bulletin of Ibaraki Nature Museum*, **17**, 7–17. (in Japanese).

Sato, H. and H. Sakayori (2015) Pseudoscorpiones. *In* Aoki, J. (ed.), *Pictorial Keys to Soil Animals of Japan, 2nd Ed.*, pp.105–118. Tokai Press, Hadano. (in Japanese).

Suyama, Y. (2011) Procedure for single-pollen genotyping. *In* Y. Isagi and Y. Suyama (eds.), *Single-Pollen Genotyping*, pp.7–15. Springer, Tokyo.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.

Tsutsumi, T. (2012) Impact to soil animal fauna in Soma, Fukushima Prefecture by the Tsunami caused by the Great East Japan Earthquake -The survey on the pseudoscorpions and thrips after The Great East Japan Earthquake-. *The Report of Project Research at Fukusihima University: Nature and Its Humanization*, **9**, 1–12. (in Japanese).

Whiting, M. F., J. C. Carpenter, Q. D. Wheeler and W. C. Wheeler (1997) The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, **46**, 1–68.