Further notes on the spiders of the genus *Dolomedes* (Araneae: Pisauridae) from Japan

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Abstract — *Dolomedes senilis* Simon 1880 is newly recorded from Japan using the specimens collected from Hokkaido. The species is easily separated from other Japanese congeners by its unique appearance. One new species *Dolomedes pegasus* is also described using specimens collected from Tohoku district, northern part of Honshu, Japan. *Dolomedes pegasus* resembles *D. sulfureus* and *D. angustivirgatus*, but can be distinguished from them by shorter legs, barrel-shaped epigynum, shorter male palpal tibia and smaller tibial apophysis. The mt-COI partial sequence data of these species as well as *D. zatsun* are added to the previous data set and their phylogenetic placements among Japanese species are inferred. As a result, *D. senilis* and *D. pegasus* share the same clade with *D. sulfureus*, and *D. zatsun* shares the same clade with *D. raptor*.

Key words — new records, new species, *Dolomedes senilis*, *Dolomedes pegasus*, COI, phylogeny

Introduction

In the previous study, 11 species of the genus *Dolomedes* were found to occur in Japan (Tanikawa 2003, Tanikawa & Miyashita 2008). Here I report two more species of the genus that are newly found in northern Japan, Hokkaido, Aomori and Yamagata Prefectures. Through morphology, I identified the specimens collected from Hokkaido (Figs. 1–2) as *Dolomedes senilis* Simon 1880, and recognized other specimens, collected from Aomori and Yamagata Prefectures (Figs. 3–6), as a new species. Their phylogenetic placement among Japanese species was inferred using partial sequence data of mitochondria cytochrome oxidase subunit I (COI).

The type specimens designated in this paper are deposited in the collection of the Department of Zoology, National Museum of Nature and Science, Tokyo (NSMT). Nucleotide sequence data analyzed in this paper are available in the DDBJ/EMBL/GenBank databases.

Taxonomy

Family Pisauridae

Genus *Dolomedes*

Dolomedes senilis Simon 1880

[Japanese name: Kamui-hashiri-gumo]

(Figs. 1–2, 7–15)

Dolomedes senilis Simon 1880, p. 101. [holotype from Beijing, China, lost]; Song & Zheng 1982, p. 156, figs. 3–4; Hu 1984, p. 258, figs. 271. 1–3; Song 1987, p. 205, fig. 165; Zhang et al. 2004, p. 377, figs. 44–49; Feng 1990, p. 159, figs. 134.

1–4.

Dolomedes senilis: Song et al. 1999, p. 347, figs. 202Q, 203J; Song et al. 2001, p. 266, fig. 166.

Dolomedes strandi Bonnet 1929, p. 268, figs. 1–3 [syntypes from Russia, depository not clear]; Marusik 1988, p. 1471, figs. 1. 6–7.

Specimens examined. 3♀5♂1juv., Uenae, Tomakomai-shi, Hokkaido, Japan, 13-VII-2010 (females: NSMT-Ar 9813–9815, males: NSMT-Ar 9817–9821, juv.: NSMT-Ar 9816), these specimens were collected by A. Tanikawa as juveniles and became adults after rearing other than 1 juvenile specimen.

Description. Coloration and markings. Female (Fig. 1, NSMT-Ar 9813). Carapace blackish brown with a longitudinal white midline and a pair of lateral white lines. Dorsum of abdomen blackish brown with several pairs of small white spots, indistinct in alcohol, laterally with reticulate white marking. Male (Fig. 2, NSMT-Ar 9821). Carapace blackish brown with a longitudinal white midline and a wide white border. Abdomen as in female.

Measurements. Based on 1♀1♂ from Hokkaido. (NSMT-Ar 9813, 9821), measurements in parentheses indicate the range among specimens examined. Body ♀19.38 (18.25–19.63), ♂16.50 (15.25–16.50) long. Carapace ♀8.80 (8.80–9.70), ♂8.25 (7.15–8.25) long; ♀7.40 (7.40–8.10), ♂7.08 (6.31–7.08) wide. Length of legs [female/male; tarsus + metatarsus + tibia + patella + femur = total]: I, 3.08 + 4.81 + 5.23 + 3.77 + 6.62 = 23.51/4.08 + 5.77 + 5.92 + 3.69 + 7.54 = 27.00; II, 2.92 + 5.00 + 5.23 + 3.62

Figs. 1–6. Habitus. 1–2. *Dolomedes senilis* Simon 1880. 1. Female (NSMT-Ar 9813); 2. Male (NSMT-Ar 9821). 3–6. *Dolomedes pegasus* new species. 3. Female (holotype, NSMT-Ar 9822); 4. Male (paratype, NSMT-Ar 9828); 5. Juvenile; 6. Male (paratype, NSMT-Ar 9830). Scales=10 mm.

+ 6.92 = 23.69/3.69 + 5.31 + 5.69 + 3.69 + 7.23 = 25.61; III, 2.77 + 5.08 + 5.08 + 3.31 + 6.54 = 22.78/3.31 + 5.23 + 5.15 + 3.15 + 6.77 = 23.61; IV, 3.38 + 6.92 + 6.69 + 3.77 + 7.69 = 28.45/4.31 + 7.00 + 6.54 + 3.38 + 7.77 = 29.00. Abdomen ♀10.00 (8.30–10.00), ♂7.92 (7.50–8.50) long; ♀7.25 (6.50–7.75), ♂5.25 (5.00–6.50) wide.

Female and male. Carapace longer than wide [length/width ♀1.19 (1.18–1.21), ♂1.16 (1.13–1.18)]. Median

ocular area almost as long as wide [length/width ♀0.99 (0.93–0.99), ♂1.00 (0.93–1.01)]; wider behind than front [anterior width/posterior width ♀0.66 (0.63–0.68), ♂0.64 (0.64–0.65)]. Fang furrow of chelicera with 3 (3–4 in male) promarginal and 4 (3–4 in male) retromarginal teeth. Labium wider than long [length/width ♀0.86 (0.81–0.91), ♂0.84 (0.80–0.92)]. Sternum almost as long as wide [length/width ♀0.99 (0.94–1.00), ♂1.06 (0.95–1.06)]. Length of leg I/length of carapace ♀2.67 (2.45–2.67), ♂3.27

(2.93–3.27). Male palp (Figs. 13–15): shapes and arrangement of sclerites as for the *fimbriatus*-group style (Carico 1973); lateral projection of fulcrum (Fig. 13, arrow) diagnostic; tibial apophysis V-shaped (Fig. 15). Abdomen longer than wide [length/width ♀1.38 (1.27–1.39), ♂1.51 (1.31–1.51)]. Female genitalia (Figs. 7–12): shape of epigynum *fimbriatus*-group style (Carico 1973),

spermatheca well sclerotized, long and winding (Figs. 10–12).

DNA barcode. COI sequence data are available in the DDBJ/EMBL/GenBank databases. Accession numbers are: AB687493 (NSMT-Ar 9813, 9814, same sequence), AB687494 (NSMT-Ar 9816) and AB687495 (NSMT-Ar 9821).

Figs. 7–15. *Dolomedes senilis* Simon 1880. 7–9. Epigynum, ventral view. 10–12. Internal genitalia, dorsal view. 13–15. Male left palp. 13. Ventral view, arrow shows lateral projection of fulcrum; 14. Lateral view; 15. Tibia, lateral view. (7, 10, NSMT-Ar 9813; 8, 11, NSMT-Ar 9814; 9, 12, NSMT-Ar 9815; 13–15, NSMT-Ar 9821.) Scales=1 mm.

Remarks. *Dolomedes senilis* can be easily distinguished from the other Japanese congeners by general appearance (Figs. 1–2). Lateral projection of fulcrum of male palp (Fig. 13, arrow) is the most diagnostic feature. No other species of East Asian *Dolomedes* spiders has such a projection.

Distribution. Japan (Hokkaido), China, Russia.

***Dolomedes pegasus* new species**

[Japanese name: Michinoku-hashiri-gumo]

(Figs. 3–6, 16–26)

Type series. Holotype: ♀, Tashirotai, Aomori-shi, Aomori Pref., Japan, 16-VII-2009 (NSMT-Ar 9822). Paratypes: 7♂, the same data as the holotype (NSMT-Ar 9826–9832, all of the specimens were collected by A. Tanikawa as juveniles and became adults after rearing).

Other specimens examined. 1♀, the same locality as for the holotype, 30-VIII-1997, A. Fukushima leg. (NSMT-Ar 9823). 2♀, Oguni-machi, Nishi-okitama-gun, Yamagata Pref., 17-VII-2010, A. Tanikawa and A. Andoh leg. (NSMT-Ar 9824, 9825)

Description. Coloration and markings. Female (Fig. 3, holotype, NSMT-Ar 9822). Carapace and abdomen dark brown, with a pair of longitudinal lateral white bands. Dorsum of abdomen with several pairs of small white spots, inconspicuous in alcohol. Male (Fig. 4, one of paratypes, NSMT-Ar 9828). Almost the same as in female. Color variation. Some of the specimens examined uniformly dark brown without a lateral white band (Figs. 5–6).

Measurements. Based on holotype ♀ and paratype 1♂ (NSMT-Ar 9822, 9828), measurements in parentheses indicate the range among specimens examined. Body ♀22.25 (17.13–24.13), ♂13.63 (12.00–19.00) long. Carapace ♀10.75 (8.60–11.50), ♂7.15 (6.60–10.00) long; ♀9.13 (7.10–9.25), ♂6.00 (5.53–8.25) wide. Length of legs [female/male; tarsus + metatarsus + tibia + patella + femur = total]: I, 3.50 + 5.75 + 6.75 + 4.50 + 7.92 = 28.42/4.29 + 6.25 + 6.75 + 3.33 + 7.42 = 28.04; II, 3.50 + 5.58 + 6.50 + 4.50 + 8.08 = 28.16/3.83 + 5.50 + 6.08 + 3.29 + 7.33 = 26.03; III, 3.08 + 5.25 + 5.92 + 3.92 + 7.58 = 25.75/3.08 + 4.92 + 5.00 + 2.75 + 6.42 = 22.17; IV, 4.17 + 7.67 + 7.92 + 4.25 + 9.00 = 33.01/4.08 + 6.92 + 6.67 + 3.00 + 7.50 = 28.17. Abdomen ♀11.50 (8.70–13.38), ♂6.85 (6.40–9.38) long; ♀7.75 (6.20–9.38), ♂3.62 (3.00–5.63) wide.

Female and male. Carapace longer than wide [length/width ♀1.18 (1.18–1.24), ♂1.19 (1.17–1.21)]. Median ocular area almost as long as wide [length/width ♀1.01 (0.91–1.01), ♂0.87 (0.87–1.03)]; wider behind than front [anterior width/posterior width ♀0.65 (0.65–0.70), ♂0.64 (0.62–0.66)]. Fang furrow of chelicera with 3 (2–3) promarginal and 4 retromarginal teeth. Labium slightly wider than long [length/width ♀0.92 (0.92–0.94), ♂0.90 (0.90–0.97)]. Sternum almost as long as wide [length/width ♀1.00 (0.97–1.03), ♂1.00 (1.00–1.07)]. Length of leg I/length of carapace ♀2.64 (2.64–3.10), ♂3.92 (3.34–3.92). Male palp

(Figs. 22–26): shapes and arrangement of sclerites as for the *fimbriatus*-group style (Carico 1973), tibial apophysis small (Figs. 24–26). Abdomen longer than wide [length/width ♀1.48 (1.40–1.49), ♂1.89 (1.52–2.13)]. Female genitalia (Figs. 16–21): shape of epigynum *fimbriatus*-group style (Carico 1973), spermatheca well sclerotized other than most outer part, long and winding (Figs. 19–21).

DNA barcode. COI sequence data are available in the DDBJ/EMBL/GenBank databases. Accession numbers are: AB687496 (paratypes, NSMT-Ar 9826, 9827, same sequence), AB687497 (NSMT-Ar 9824, 9825, same sequence).

Remarks. *Dolomedes pegasus* resembles *D. angustivirgatus* and *D. sulfureus* in general appearance, but can be separated from them by the relative length of the first leg and the male palpal tibia as well as the shape of female epigynum and tibial apophysis of male palp. The first leg and male palpal tibia of *pegasus* are shorter than those of *angustivirgatus* and *sulfureus*. The length of the first leg divided by the width of carapace: *pegasus* ♀2.64–3.10, ♂3.34–3.92; *angustivirgatus* ♀3.49–4.40, ♂4.49–5.27; *sulfureus* ♀4.30–5.09, ♂5.57–6.63. The length of male palpal tibia divided by width: *pegasus* 1.53–1.83, *angustivirgatus* 2.06–2.41, *sulfureus* 2.80–3.14. Epigynum of *pegasus* is barrel-shaped (Figs. 16–18) but fig-shaped in *sulfureus* and *angustivirgatus* (Tanikawa & Miyashita 2008, figs. 34–35, 37–38). Tibial apophysis of male palp of *pegasus* is smaller and has a sharp point or none (Figs. 24–26), but those of *sulfureus* and *angustivirgatus* are larger and V-shaped (Tanikawa & Miyashita 2008, figs. 24–30), having two sharp points.

Distribution. Japan (Northern part of Honshu).

Etymology. Pegasus is the immortal winged horse in Greek mythology. The type locality of this species is in Tohoku district, northern part of Honshu Island, where many people died or suffered heavy damage from the strong earthquake and huge tsunami on March 11, 2011. I am praying for the recovery of Tohoku like Pegasus flying through the sky.

Phylogeny

Taxon analyzed. All of the Japanese species were analyzed in this study. The sequence data of *D. senilis* and *D. pegasus* (accession numbers and specimen numbers, see taxonomy parts), as well as *Dolomedes zatsun* Tanikawa 2003 (female collected from the type locality on 2-VII-2011 by N. Ohshiro, accession no. AB687498) were added to the data set used in the previous study (Tanikawa & Miyashita 2008). *Hygropoda higenaga* (Kishida 1936) was used as an out-group.

DNA extraction, polymerase chain reaction and sequencing. Specimens were preserved in 99.5% ethanol, and genomic DNA was extracted from muscle of legs using DNeasy Blood & Tissue kit (Qiagen, Inc.). The mitochondrial cytochrome oxidase subunit I (mt-COI) partial sequences were amplified using the primer combination

Figs. 16–26. *Dolomedes pegasus* new species. 16–18. Epigynum ventral view. 19–21. Internal genitalia, dorsal view. 22–26. Male left palp. 22. Ventral view; 23. Lateral view; 24–25. Tibial apophysis. (16, 19, holotype, NSMT-Ar 9822; 17, 20, NSMT-Ar 9824; 18, 21, NSMT-Ar 9825; 22–23, paratype, NSMT-Ar 9826; 24, NSMT-Ar 9828; 25, paratype, NSMT-Ar 9831; 26, paratype, NSMT-Ar 9827.) Scales=1 mm.

LCOI-1498: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' with HCOI-2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al. 1994). The reactants were initially denatured for 2 min at 95°C, proceeded with 40 cycles of 15 sec at 95°C, 20 sec at 47°C, 30 sec at 72°C. PCR products were purified using the

ExoSAP-IT (GE Healthcare Bio-Sciences, Co. Ltd.). The purified PCR products were sequenced using the BigDye terminator cycle sequencing kit (ver.3.1) and analyzed on ABI 3130xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Chromatograms were checked by eye using BioEdit Ver. 7.0.5.0.3 (Hall 1999).

Fig. 27. 50% majority rule consensus tree topologies obtained from Bayesian inference. Posterior probabilities are shown just before nodes. Details of sampling data of *zatsun*, *senilis* and *pegasus* are shown in the text. Numbers in parentheses correspond to the specimen numbers in Tanikawa & Miyashita 2008. Scale: 0.02 substitution/site.

Sequence alignments were done by Clustal W program (Thompson et al. 1994) in MEGA version 5.05 (Tamura et al. 2011).

Data analyses. The perl script KAKUSAN 4 (Tanabe 2011) and Tree Finder (Jobb et al. 2004) were used to determine the appropriate model of DNA evolution by BIC for Bayesian analyses. MrBays ver. 3.1.2 (Ronquist & Huelsenbeck 2003) was employed to infer the phylogeny. Four concurrent Markov Chain Monte Carlo (MCMC) chains were run for 5,000,000 generations, saving a tree every 100 generations. Topologies prior to *ln* stabilization (“burn-in”) were discarded and posterior clade probabilities were computed from the remaining trees.

Results. I obtained 610 bp of mt-COI partial sequence from the specimens used. The best-fit models of sequence evolution determined by KAKUSAN 4 were HKY+I for the first codon position, F81 homogenous for the second position and HKY+G for the third position and codon proportional rather than codon separate. Bayesian inference resulted in a phylogenetic tree shown in Fig. 27. *Dolomedes horishanus* is a sister to all other species comprising two reciprocally monophyletic clades as in the previous study (Tanikawa & Miyashita 2008). In this study, *D. zatsun* is revealed to share the first clade (A in Fig. 27) with *raptor*, *yawatai*, *japonicus*, and *orion*. Although the clade *raptor* + *yawatai* + *japonicus* is supported strongly (pp=100), other parts in the clade A seem to be unresolved because of the weak support (pp=85, 73). *Dolomedes senilis* and *pegasus* are united in the second clade (B in Fig. 27). The clade B comprises three subclades (C, D & E in Fig. 27). *Dolomedes pegasus* shares the clade D (pp=100) with

angustivirgatus, *sulfureus*, and *fontus*. The clade E is uniting *silvicola* and *saganus*, resting clade C consists of only *senilis*. The relationships among these three clades are not clear and seem to be an unresolved trichotomy due to the weak support.

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