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Identity of a Japanese spider species recorded as "*Pasilobus bufoninus*" (Araneae: Araneidae), with a description of the male considering the sequence of mtDNA

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Abstract — It was found that a Japanese species of *Pasilobus* (Araneidae) that has been recorded under the name of *P. bufoninus* (Simon 1867) is conspecific with *P. hupingensis* Yin, Bao & Kim 2001 described from China by the comparison of Japanese specimens with the type specimens of the two species. Redescription of the species is presented on the basis of specimens collected from Japan. Males of the species is described for the first time. Partial sequences of mitochondrial DNA (COI gene) were used to confirm the species identify of the males, because of the extreme sexual dimorphism in *Pasilobus*.

Key words — Taxonomy, DNA, COI, *Pasilobus hupingensis*, *Pasilobus bufoninus*, Okinawa

The *Pasilobus* spider that has a curious shaped abdomen and constructs a triangular web is very rare in Japan and has been recorded less than ten times (Yaginuma & Shinkai 1975, Shinkai 1998, Tanikawa 2005) since its first record by Kayashima (1959). Previous authors identified the Japanese *Pasilobus* spiders as a single species, *P. bufoninus* (Simon 1867). We examined the type specimens of *Pasilobus* spiders in order to confirm the identification by previous authors.

Another point to note is that males of *Pasilobus* have not yet been known despite 12 species being described in the genus. We performed DNA analysis for identification of the male specimen because 1) it was impossible to identify males by general appearace in cyrtarachnine spiders due to their extreme male dwarfism accompanied by extreme morphological changes; 2) it also seems to be impossible to find *Pasilobus* mating in the field due to their rarity.

Voucher specimens used for the taxonomic study are deposited in the collections of the Department of Zoology, National Science Museum, Tokyo (NSMT), and University Museum, University of the Ryukyus, Okinawa (RUMF). An acronym MNHN is used for Museum National d'Histo ire Naturelle, Paris. The DNA sequences have been submitted to National Center for Biotechnology Information (NCBI: http: //ncbi.nim.nih.gov/).

Reidentification of female

First of all, we compared four females of Japanese *Pasilobus* available to us with the holotype of *Pasilobus bufoninus* Simon 1867 [♀ from Gilolo, MNHN 32 (using images drawn by H. W. Levi)], and 1♀ from Java, MNHN 23172. The epigyna of Japanese specimens did not agree with those of these specimens (Figs. 1–2, 10–11 vs 3–4, 14), and hence we concluded that the Japanese specimens are not *Pasilobus bufoninus*.

Then we examined the holotype of *Pasilobus hupingensis* Yin, Bao & Kim 2001, using the digital images taken by Dr. Yin. Because the epigynum of the female holotype of *P. hupingensis* exactly agreed with those of Japanese specimens of *Pasilobus*, we identified the Japanese specimens as *P. hupingensis*.

DNA analysis for identification of the male

Species analysed. The species used for the analysis and their sampling data are shown in Table 1. Among them, males have not yet been described for three species: *Paraplectana sakaguchii*, *P. tsushimensis* and *Pasilobus hupingensis*. *Gasteracantha kuhli* C. L. Koch 1837 (Gasteracanthinae) was used as an out-group.

DNA extraction, polymerase chain reaction and

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Table 1 Collection sites and Genbank accession numbers (A. No.) of cyrtarachnin spiders used for DNA analyses in this study and Gasteracantha kuhli (Gasteracanthinae) as an out-group.

Species	Sex	Collecting sites	Date	GenBank accession number
Cyrtarachne bufo (Bösenberg & Strand 1906)	1juv.	Masuho-chô, Yamanashi	23-VII-2004	DQ518421
Cyrtarachne inaequalis Thorell 1895	1juv.	Masuho-chô, Yamanashi	23-VII-2004	DQ518415
Cyrtarachne nagasakiensis Strand 1918	1♀	Masuho-chô, Yamanashi	23-VII-2004	DQ518414
Ordgarius hobsoni (O. P.-Cambridge 1877)	1♀	Uchiko-chô, Ehime	16-VIII-2004	DQ518417
Ordgarius sexspinosus (Thorell 1894)	1♀	Masuho-chô, Yamanashi	23-VII-2004	DQ518418
Paraplectana sakaguchii Uyemura 1938	1♀	Goto-shi, Nagasaki	unknown	DQ518420
Paraplectana tsushimensis Yamaguchi 1960	1♀	Okinawajima Is., Okinawa	24-V-2004	DQ518422
Paraplectana tsushimensis Yamaguchi 1960	1♀	Amami-ôshima Is., Kagoshima	20-VI-2003	DQ518423
Pasilobus hupingensis Yin, Bao & Kim 2001	1♀	Tancha, Okinawajima Is.	II-2004	DQ518424
Unknown cyrtarachnin male	1♂	Ureshinoinoue-chô, Mie	7-III-2002	DQ518425
Poecilopachys australasia (Griffith & Pidgeon 1833)	1♀	Baiariver, Papua New Guinea	30-VII-2003	DQ518419
Gasteracantha kuhli C. L. Koch 1837	1♀	Uchiko-chô, Ehime	16-VIII-2004	DQ518416

1) All the sites except for Baiariver are in Japan

sequencing. Specimens were preserved in 75% ethanol and genomic DNA was extracted from muscle of cephalothorax or legs using Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN, USA). The mitochondrial cytochrome oxidase 1 (COI) partial sequence were amplified using the primer combination LCO-J-1490: 5'-GGT CAA CAA ATC ATA AAG ATA TAT TGG-3' with HCO-N-2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAA TCA-3' (Folmer et al. 1994). The reactants were initially denatured for 3 min at 95ºC, proceeded with 30 cycles of 60 sec at 95ºC, 60 sec at 50ºC, 60 sec at 72ºC and then the final extension at 72ºC for 10 min. PCR products were assayed by electrophoresis on 1.2% agarose minigel visualized under UV light after ethidium bromide staining. The target DNA fragments were isolated and purified by the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). The purified PCR products were sequenced using the BigDye terminator cycle sequencing kit and analysed on ABI 3100 or 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA).

Data analyses. Chromatograms and contiguous alignments were edited using Seqman version 4.00 (DNAstar Inc., Madison, WI). All sequences were aligned via Clustal W program (Thompson et al. 1994) in BioEdit version 4.7.8 (Hall 1999) and corrected manually in GeneDoc (Nicholas et al. 1997). The phylogenetic trees were constructed using MEGA version 2.0 (Kumar et al. 2001) by distance matrix method (neighbor-joining, NJ) (Saito & Nei 1987).

Results and discussion. The cladgram obtained is shown in Fig. 5. The male in question formed a clade with a female of *Pasilobus hupingensis*, with the difference in DNA sequences between the male and the *Pasilobus* female being approximately 5%.

Four genera of subfamily cyrtarachninae have been known to the Japanese fauna, that is, *Cyrtarachne*, *Ordgarius*, *Paraplectana*, and *Pasilobus*. Of these, males are unknown for two species of *Paraplectana* and a species of *Pasilobus*. The male in question differs from those of *Cyrtarachne* and *Ordgarius* by having larger sigillae and a wider abdomen, and there is no projection in the carapace of this male unlike *Ordgarius*. The large sigillae on the dorsum of abdomen are also seen in females of *Paraplectana*, but sigilla of *Paraplectana* is round and inconspicuous relative to that of *Pasilobus* female. Furthermore, as noted above, analysis of mitochondria COI gene showed that the male in question formed a clade with a female of *Pasilobus hupingensis*. Therefore, it is most likely that the male belongs to the genus *Pasilobus*. Because all the female specimens examined in this study were identified as *Pasilobus hupingensis*, and no other congeners have been recorded from Japan, we conclude that the male in question is the male of *Pasilobus hupingensis*.

Description

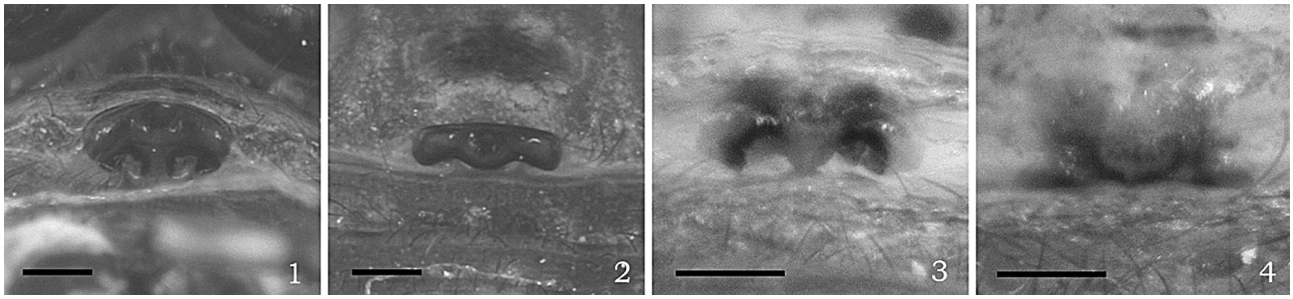
Genus *Pasilobus* Simon 1895
Pasilobus hupingensis Yin, Bao & Kim 2001
[Japanese name: Wakudo-tsukiji-gumo]
(Figs. 1–2, 6–13)

Pasilobus hupingensis Yin, Bao & Kim 2001, p. 174, figs. 1–4 [female holotype from Mt. Hupin, China, preserved in Hunan Normal University, digital images examined].

Pasilobus bufoninus: Kayashima 1959, p. 11; Yaginuma 1960, p. 64, pl. 27, fig. 157; Yaginuma 1986, p. 113, pl. 29, fig. 5; Tanikawa 2005, p.103. [nec *Pasilobus bufoninus* (Simon 1867), misidentification]

Specimens examined. AICHI PREF.: Kita-shitara-gun, Shitara-chô, Kawamuki, 1♂, 6-X-2004, Y. Suzuki leg. (NSMT-Ar 5923, left palp only). Toyota-shi, Kyogamine, 1♂, 13-IV-2003, T. Sugiyama leg. (NSMT-Ar 5924). MIE PREF.: Matsusaka-shi, Ureshino-inoue-chô, 1♂, 7-III-2002, K. Kumada leg. MIYAZAKI PREF.: Higashiusuki-gun, Kadogawa-chô, Kawachi, 1♀, 9-X-1959, M. Tsukiji leg. (NSMT-Ar 5925). OKINAWA PREF. Okinawajima Is.: Kunigami-son, Yona, 1♀, 10-VI-2004, A. Tanikawa leg. (NSMT-Ar 5926); Kin-chô, Kisenbaru, 1♀, 29-IV-2003, N. Murayama leg. (RUMF-ZA-00600). On-na-son, Tancha, 1♀, 14-VI-2004, M. Sugimoto leg..

Description. Coloration and markings. Female (Figs. 6–



Figs. 1–4 Epigyna of two species of *Pasilobus* — 1, 3, ventral view; 2, 4, ventral view. 1–2, *Pasilobus hupingensis* Yin, Bao & Kim 2001 (NSMT-Ar 5926); 3–4, *Pasilobus bufoninus* Simon 1867 (specimen from Java, MNHN, no 23172). (Scales: 0.3 mm)

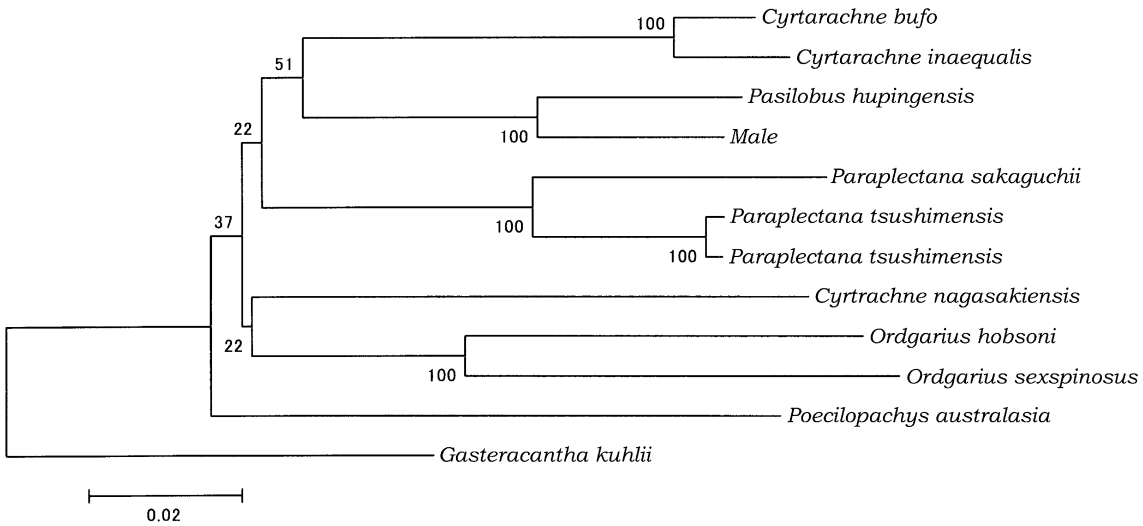


Fig. 5 A phylogenetic tree obtained by neighbor-joining method for the species examined. Scores at each node are bootstrap values. Bar shows 2% sequence divergence.

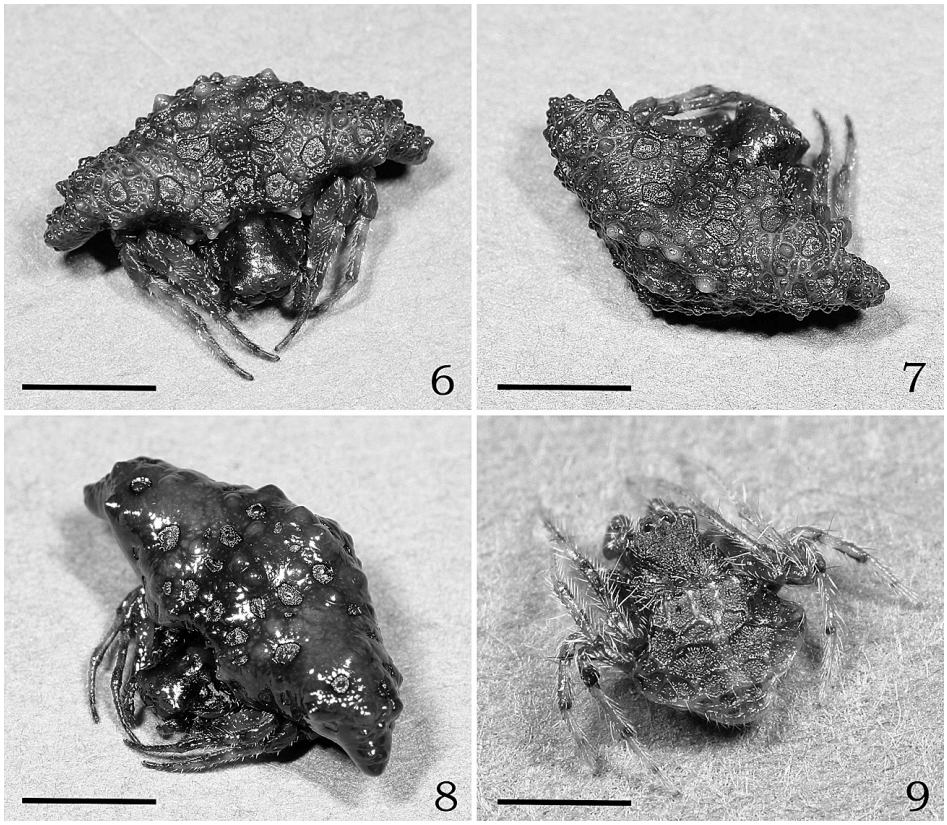
8): whole body dark reddish brown, carapace darker than abdomen. Male (Fig. 9): whole body blackish brown, carapace darker.

Measurements (in mm). Based on 1♀ from Okinawa (NSMT-Ar 5926) and 1♂ from Aichi (NSMT-Ar 5924), measurements in parentheses indicate the range among whole specimens used in this paper. Body ♀8.00 (8.00–9.10), ♂2.35 (2.35–2.76) long. Carapace ♀3.32 (3.20–3.40), ♂1.05 (1.05–1.22) wide. Length of legs [female/male; tarsus＋metatarsus＋tibia＋patella＋femur＝total]: I, 0.67＋1.57＋2.03＋1.40＋3.00＝8.67/0.38＋0.73＋0.84＋0.50＋1.28＝3.73; II, 0.68＋1.53＋1.97＋1.40＋2.90＝8.48/0.38＋0.69＋0.79＋0.46＋1.15＝3.47; III, 0.57＋1.00＋1.17＋0.90＋1.93＝5.57/0.35＋0.44＋0.52＋0.31＋0.79＝2.41; V, 0.53＋1.23＋1.80＋1.20＋2.83＝7.59/0.32＋0.44＋0.61＋0.37＋0.95＝2.69. Abdomen ♀6.31 (6.31–6.63), ♂1.13 (1.13–1.56) long; ♀14.38 (13.13–14.38), ♂1.44 (1.44–2.11) wide.

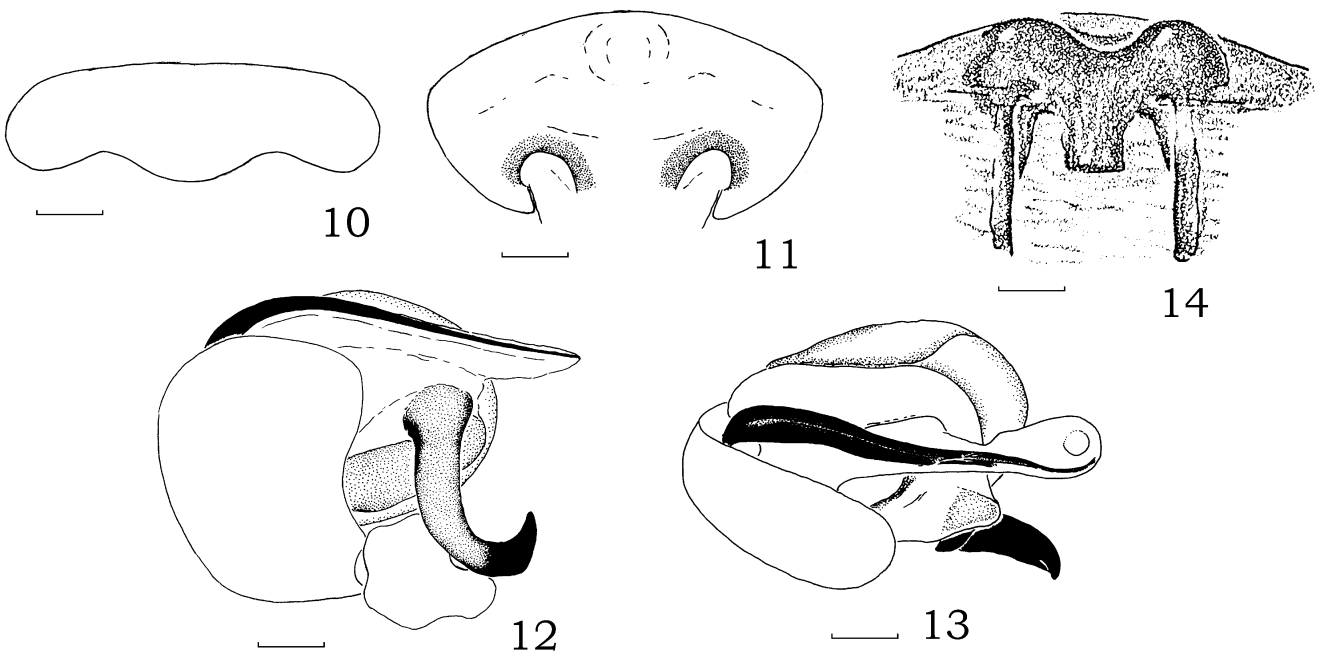
Female. Carapace almost as long as wide [length/width 1.04 (0.95–1.04)]. Median ocular area wider than long [length/width 0.84 (0.79–0.84)]; wider in front than behind [anterior width/posterior width 1.09 (1.09–1.22)]. Promargin of fang furrow of chelicera with 3 teeth, retromargin with 1 (1–2) tooth. Labium wider than long [length/width 0.69 (0.69–0.74)]. Sternum almost as long as wide [length/width 1.00 (1.00–1.02)]. Length of leg I/length of carapace 2.52 (2.52–2.53). Abdomen wider than long [length/width 0.44 (0.44–0.49)], with many protuberances and sigillae. Epigynum (Figs. 1–2, 10–11) simple, without scape

Male. Carapace longer than wide [length/width 1.12 (1.10–1.12)]. Median ocular area wider than long [length/width 0.91 (0.89–0.95)]; wider in front than behind [anterior width/posterior width 1.10 (1.05–1.10)]. Promargin of fang furrow of chelicera with 3 teeth, retromargin with 0 (0–1) tooth. Labium wider than long [length/width 0.65 (0.60–1.04)]. Sternum wider than long [length/width 0.95 (0.95–0.98)]. Length of leg I/length of carapace 3.16 (3.08–3.16), metatarsi I, II and tibiae I, II prolaterally with a row of weak spines (Fig. 9). Palp (Figs. 12–13): embolus apically wrapped by conductor; median apophysis hook shaped; palpal tibia with one bristle. Abdomen wider than long [length/width 0.79 (0.72–0.79)], with many sigillae (Fig. 9).

Remarks. *Pasilobus hupingensis* closely resembles *P. bufoninus* in general appearance, but can be distinguished



Figs. 6–9 *Pasilobus hupingensis* Yin, Bao & Kim 2001 — 6–7, female (NSMT-Ar 5926) after egg-laying; 8, same, before egg-laying; 9, male (NSMT-Ar 5924). (Scales: 6–8, 5 mm, 9, 1 mm)



Figs. 10–14 Genital organ of two species of *Pasilobus*. — 10, epigynum, ventral view; 11,14, same, posterior view; 12, male palp, ventral view; 13, same, dorsal view. 10–13, *Pasilobus hupingensis* Yin, Bao & Kim 2001 (10–11, NSMT-Ar 5926; 12–13, NSMT-Ar 5923), 14, *Pasilobus bufoninus* Simon 1867 (holotype from Molucca, MNHN, no 30, drawn by H. W. Levi). (Scales: 0.1 mm.)

from the latter by the shape of epigynum (Figs. 1–2, 10–11 vs 3–4, 14). In ventral view, the epigynum is thick in *hupingensis* (Fig. 2, 10) but thin in *bufoninus* (Fig. 4).

The male of *P. hupingensis* differs from those of *Cyrtarachne* and *Ordgarius* by having large sigillae and a wide abdomen, and there is no projection in the carapace unlike *Ordgarius*. The large sigillae on dorsum of abdomen are also seen in females of *Paraplectana*, but sigilla of *Paraplectana* is round and inconspicuous relative to that of *Pasilobus* female.

Distribution. Japan (Honshu, Kyushu, Nansei Isls.), China.

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