TAXONOMIC RE-EVALUATION OF THE WOLF SPIDER Wadicosa okinawensis (Tanaka, 1985) THROUGH MORPHOLOGICAL EXAMINATION AND DNA BARCODING, WITH ITS REDESCRIPTION AND A NEW SYNONYMY (Araneae, Lycosidae, Pardosinae)

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ABSTRACT

The genus *Wadicosa* Zyuzin, 1985 comprises medium-sized, free-hunting wolf spiders characterized by an anterior-retrolateral process on the male palpal tegulum and rounded or oval foveolae in the female epigyne. Among its 19 valid species, *Wadicosa okinawensis* (Tanaka, 1985) has limited diagnostic information on its genital structures and inadequate illustrations for identification. This study addresses these gaps providing a thorough redescription of *W. okinawensis* and detailed photographs of its diagnostic characters based on specimens from its type locality and surrounding areas. A comparative morphological analysis of *W. okinawensis* and *Wadicosa daliensis* Yin, Peng & Zhang, 1997 from China and Laos reveals no significant morphological differences between the two species. Consequently, we propose *W. daliensis* as a junior synonym of *W. okinawensis*. For the first time, *W. okinawensis* is included in DNA barcoding which supports its separation from the morphologically similar *Wadicosa fidelis* (O. Pickard-Cambridge, 1872). This work enhances the taxonomy of *Wadicosa*, facilitating species identification and serving as a valuable reference for understanding the diversity of the family Lycosidae.

Keywords: The Ryukyus, junior synonym, taxonomy, type locality, molecular phylogeny.

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INTRODUCTION

Wadicosa Zyuzin, 1985 is a relatively small genus of middle-sized, free-hunter spiders of the family Lycosidae Sundevall, 1833. It was first established by Zyuzin (1985) based on species previously included in Lycosa Latreille, 1804 and Pardosa C.L. Koch, 1847; its type species, Wadicosa fidelis (O. Pickard-Cambridge, 1872), was later fixed by Kronestedt & Zyuzin (2009). The genus' key features are an anterior retrolateral process in the male palpal tegulum and oval or rounded foveolae (pockets) in the epigyne (see Kronestedt & Zyuzin, 2009).

Currently, Wadicosa comprises 19 valid species most of which are restricted to the Afrotropical and Indomalayan realms (WSC, 2024). In Asia, seven species have been recorded: Wadicosa commoventa Zyuzin, 1985 from Iran and Turkmenistan; Wadicosa ghatica Kronestedt, 2017 and Wadicosa prasantae Ahmed, Anam, Saikia, Manthen & Saikia, 2014 from India; Wadicosa quadrifer (Gravely, 1924) from India and Sri Lanka; Wadicosa daliensis Yin, Peng & Zhang, 1997 from Mainland China and Laos; and Wadicosa okinawensis (Tanaka, 1985) from the Ryukyus (southwest archipelago of Japan) and Hainan Island. One species, Wadicosa fidelis (O. Pickard-Cambridge, 1872), shows a remarkably wide distribution ranging from North Africa to the Philippines and Sumatra Island (WSC, 2024). One additional but still unnamed species is recorded in Borneo (Omelko, 2024).

Most species of *Wadicosa* are relatively well described or were re-described over time (Kronestedt & Zyuzin, 2009; Kronestedt, 2015, 2017, 2023; Omelko, 2024). Yet, some species still remain poorly known, being based on a single sex (e.g., Wadicosa prasantae from India or Wadicosa russellsmithi Kronestedt, 2015 from Mauritius) or have never been properly illustrated after the original description.

Wadicosa okinawensis (Tanaka, 1985) is such a taxonomically poorly known species. This species was first described under

Pardosa based on several specimens from Okinawa Island, Japan, with the female holotype collected in Hentona (Yambaru), the northernmost part of the island (Tanaka, 1985). The original description, although well-detailed, mainly focused on somatic characters and relied on relatively simple drawings, thus providing very limited information about genital structures, which are usually critical for accurate species identification in Lycosidae. The only exception regarded the embolus which was subsequently illustrated by Tanaka (2000, Fig. 1). Since then, no further diagnostic information on this species was published until recently when Wang et al. (2021) finally illustrated the palp and epigyne in deeper detail. Yet, in their study, the authors used specimens collected from Hainan Island, China, located over 2000 km away from the type locality of the species. Additionally, they did not examine any original Japanese material nor provide a redescription of the species. The lack of clear illustrations of W. okinawensis from Okinawa has made a proper comparison with other similar species (i.e., W. daliensis) difficult, as noted by Omelko (2024). Detailed illustrations of key diagnostic features of the male palpal bulb and female epigyne and vulva, obtained from the type or topotype material, remain essential to correctly identify this species and avoid future potential misidentifications.

This study aims to fill this taxonomic gap by providing a redescription and diagnostic illustrations of *W. okinawensis* based on specimens from the type locality and surrounding areas, as well as insights into the intraspecific variability of the species. Additionally, we compare *W. okinawensis* with the morphologically similar *W. daliensis* to confirm possible differences between the two species.

MATERIALS AND METHODS

Specimens were hand-collected and preserved in 99% ethanol for morphological and molecular studies. The specimens were examined under an Olympus SZX12 stereomicroscope at the Systematic Zoology

Laboratory, Department of Biological Sciences, Tokyo Metropolitan University (TMU), Japan, and photographed with a mounted Canon EOS Kiss X9 digital camera. To facilitate handling, specimens were placed in small cups with a layer of silicon sand on the bottom. All images were processed using Helicon Focus v.8.2.5 for stacking and Affinity Photo 2 for further refinement. Scale bars were added using ImageJ software.

Genitalia of female (epigyne) and male (left palp) were dissected using a sharp needle and forceps. Epigynes were cleared by keeping them in a hot 20% KOH solution for several minutes until the inner structures were clearly visible. Due to the general dark coloration, palps were soaked in a 20% hydrogen peroxide (H_2O_2) solution for 35 minutes to lighten the palp structures and make them more clearly visible.

The specimens used in the study are preserved in the following collections: FBPC, F. Ballarin personal collection; KTMPC, Kiran Thapa Magar personal collection; MNHAH, Museum of Nature and Human Activities, Hyogo, Japan; NSMT, National Museum of Nature and Science, Tsukuba, Japan. Each voucher specimen was labeled with a unique ID (i.e., KTM20230904-01).

All measurements are reported in millimeters (mm). Leg measurements are given as follows: total length (femur, patella, tibia, metatarsus, tarsus). Eye diameters are taken at the widest point. This work uses the same nomenclature for morphological features as Zyuzin (1985) and Kronestedt & Zyuzin (2009). The abbreviations used in the text and figures are as follows.

Eyes – ALE, anterior lateral eyes; AME, anterior median eyes; PLE, posterior lateral eyes; PME, posterior median eyes. Legs segments – Fe, femur; Mt, metatarsus; Pa, patella; Tr, tarsus; Ti, tibia. Spination –d, dorsal; p, prolateral; r, retrolateral; v, ventral. Copulatory organs - AE, extension of tegular apophysis; AP, anterior retrolateral process of tegulum; Cp, copulatory opening; Cd, copulatory duct; Co, conductor; Ed,

retrolateral edge of tegulum; **Em**, embolus; **Fv**, foveolae; **Fd**, fertilization ducts; **Ho**, hood; **PP**, posterior retrolateral process of tegulum; **Pl**, palea; **SB**, septal base; **Sp**, spermatheca; **Se**, septum; **SS**, septal stem; **St**, subtegulum; **TA**, tegular apophysis; **Te**, tegulum. **a.s.l** - above sea level. **WSC** - World Spider Catalog.

Molecular analysis

Genomic DNA was extracted from the fourth leg of each specimen following the Chelex-ProK-TE protocol (Ballarin & Eguchi, 2023). Extracted genomic DNA was stored at -20 °C until use. The barcode fragment of the cytochrome c oxidase subunit I (COI) gene (673 bp, the Folmer region) was amplified using a polymerase chain reaction (PCR) following the protocol described in Ballarin & Eguchi (2023).

The following primers were used to amplify the barcode region: LCO1490 (forward) GGTCAACAAATCATCATAAAG ATATTGG (Folmer et al., 1994) and CHR2 (reverse) GGATGGCCAAAAAATCAAAAT AAATG (Barrett & Herbert, 2005). A negative control was included in every PCR reaction to test the presence of contamination. Polymerase chain reaction products were checked for amplified genes using gel electrophoresis. A 2% agarose gel stained with SYBR green was used to run the samples for 15 min, and results were visually checked under an ultraviolet light. Purified PCR products were sent to Fasmac Bio Research Support Division Sequence Service. Kanagawa, Japan for sequencing.

Sequence chromatograms were visually checked and assembled using ChromasPro v.1.7.6 (Technelysium Pty Ltd., Australia). Any ambiguous nucleotides were replaced with N. The sequences were aligned using the software AliView v.1.28 with default parameters (Larsson, 2014). Each amplified region was trimmed to remove the primer regions and overhangs, ensuring all sequences were the same length.

Draposa oakleyi (Gravely, 1924) was selected as outgroup since *Draposa* Kronestedt,

2010 is considered closely related to *Wadicosa* Zyuzin, 1985 within the subfamily Pardosinae Simon, 1898 (Piacentini & Ramírez, 2019). Sequences of *W. fidelis* and *D. oakleyi* were obtained from the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk) and Barcode of Life Data System (BOLD) (https://boldsystems.org), respectively, and included in the dataset.

A maximum likelihood (ML) analysis was performed using IQ-TREE 2.3.2 (Nguyen et al., 2015). Node support was estimated using ultrafast bootstrapping with 1,000 replicates

(Minh et al., 2013) and SH-aLRT with 1,000 replicates (Guindon et al., 2010). All parameters were left as default. The phylogenetic tree was visualized with FigTree v1.4.4 (Rambaut, 2007), and the resulting image was edited with Inkscape 1.3.2 (Bah, 2011). MEGA11 (Tamura et al., 2021) was used to calculate the uncorrected pairwise genetic distances within the COI sequences under a p-distance model leaving all other settings as default. A list of all specimens of *W. okinawensis* used for molecular analysis is in Table 1.

Table 1. Wadicosa okinawensis specimens included in the molecular analyses with related specimen ID, collecting information, and GenBank accession numbers

F								
Specimen ID				GenBank				
(Wadicosa	Gene	Sex	Locations	Accession				
okinawensis)				Number				
KTM20230904-01	COI	8	Ginama, Okinawa-jima Island, Japan	PV241799				
KTM20230904-02	COI	9	Ginama, Okinawa-jima Island, Japan	PV241800				
KTM20240903-01	COI	8	Oku, Okinawa-jima Island, Japan	PV241796				
KTM20240903-02	COI	9	Oku, Okinawa-jima Island, Japan	PV241797				
KTM20240909-03	COI	9	Masana, Okinoerabu-jima Island, Japan	PV241798				

RESULTS

Taxonomy

Family: Lycosidae Sundevall, 1833

Subfamily: Pardosinae Simon, 1898

Genus: Wadicosa Zyuzin, 1985

Type species. *Lycosa fidelis* O. Pickard-Cambridge, 1872, from Palestine and Lebanon

Wadicosa okinawensis (Tanaka, 1985)

Figs. 1A-G, 2A-E, 3A-C, 4A-I, 5A-I, 6A-B; Tables 1-3.

Pardosa okinawensis: Tanaka (1985: p. 78, f. 41–44, original description \Diamond° ♀).

P. venatrix (Lucas, 1846): Syn. rejected by Yu & Song (1988: p. 117).

P. okinawensis: Chikuni (1989: p. 116, f. 35, $\Diamond \Diamond$).

Wadicosa daliensis: Yin, Peng & Zhang (1997: p. 99, f. 1–6, original description $\Im \diamondsuit$). **Syn. nov.**

W. daliensis: Yin et al. (1997: p. 285, f. 134a–e, $\Diamond \Diamond$). **Syn. nov.**

W. daliensis: Song, Zhu & Chen (1999: p. 346, f. 202B, F, $\Diamond \Diamond$). **Syn. nov.**

W. okinawensis: ♂ removed from syn. with P. venatrix and placed in Wadicosa as an independent species by Tanaka (2000: p. 96, f. 1).

W. okinawensis: Tanaka (2009: p. 229, f. 44–45, $\Im \Im$).

W. okinawensis: Wang et al. (2021: p. 68, f. 2D–F, 69A–H, 70A–E, 71A–D, $\Diamond \Diamond$).

W. daliensis: Omelko (2024: p. 244, f. 32–51, $\Diamond \Diamond$). **Syn. nov.**

Type material. Holotype: female, Hentona, Okinawa Island, Okinawa Prefecture, Japan. **Paratype:** male, same locality as for the holotype. Holotype and paratype specimens were not examined.

Material examined. JAPAN: Okinawa Prefecture: $4 \circlearrowleft \circlearrowleft$, $5 \circlearrowleft \circlearrowleft$, Okinawa-jima Island,

Kunigami district, Ginama, Ryukyu 26.83687°N, University Okuyamaso, 128.27233°E, 280 m a.s.l., sunny and dry meadow, 04.IX.2023, F. Ballarin leg. (NSMT).; 766, 1399, same locality, 26.83687°N, 128.27233°E, 234 m a.s.l., sunny and dry meadow, 03.IX.2024, F. Ballarin leg. $(5 \stackrel{?}{\circ} \stackrel{?}{\circ})$, $8 \stackrel{?}{\circ} \stackrel{?}{\circ}$ FBPC; $2 \stackrel{?}{\circ} \stackrel{?}{\circ}$, $5 \stackrel{?}{\circ} \stackrel{?}{\circ}$ MNHAH).; 1♀, Okinawa-jima Island, Sosu Beach, 26.79163°N, 128.31594°E, 3 m a.s.l., vegetated sandy seashore, 03.IX.2024, F. Ballarin leg. (NSMT).; $3 \circlearrowleft \circlearrowleft$, $6 \hookrightarrow \hookrightarrow$, Okinawajima Island, Uruma City, Yonashirohenza 26.33889°N, 127.95572°E, 7 m a.s.l., dry sunny meadow, 07.IX.24, F. Ballarin leg. (KTMPC).; $2 \circlearrowleft \circlearrowleft$, $1 \circlearrowleft$, Okinawa-jima Island, Uruma City, Hamahiga Island, Katsurenhama 26.32303°N, 127.95262°E, 12 m a.s.l., uncultivated field, 07.IX.24, F. Ballarin leg. (FBPC).; Kogoshima Prefecture: Okinoerabu-jima Island, Oshima district, China Masana, 27.37112°N, City, 128.52504°E, 22 m a.s.l., sunny and open meadow, 09.IX.2024, F. Ballarin leg. (NSMT).

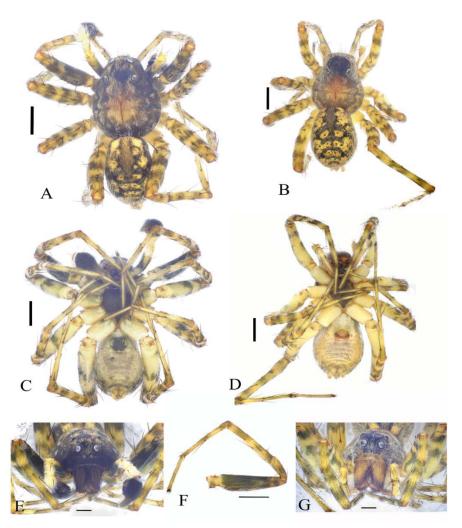


Figure 1. Male and female of Wadicosa okinawensis from Okinawa-jima Island. A, C, E, F, sample code: KTM20230904-01; B, D, G, KTM20230904-02. A, habitus of male, dorsal view; B, habitus of female, dorsal view; C, habitus of male, ventral view; D, habitus of female, ventral view; E, male cephalic area, frontal view; F, right leg I of male, prolateral view; G, female cephalic area, frontal view. Scale bars: A–E, 1 mm; F, 0.5 mm; G, 0.2 mm

Diagnosis. *W. okinawensis* is most similar to *W. fidelis* in general appearance and genital morphology. Males of *W. okinawensis* differ from males of *W. fidelis* by having a tegular apophysis (TA) strongly curved, widened medially with a conspicuous pointed extension (AE), and ending with a stockier, rounded tip (vs. less sharply curved, lacking a well-developed, pointed AE and ending with a more lanceolate tip in *W. fidelis*) (cf. Figs. 2D, 4G–I vs. fig. 1 in Kronestedt & Zyuzin, 2009 and figs. 66C, E; 67C in Wang et al., 2021) and a thicker process on the posterior retrolateral side (PP) (vs. thinner or absent in *W. fidelis*) (cf.

Figs. 2D, E; 4A–D vs. fig. 1 in Kronestedt & Zyuzin, 2009 and figs. 67A, B in Wang et al., 2021). Females of *W. okinawensis* can be distinguished from females of *W. fidelis* by having kidney-shaped spermathecae (Sp), elongated horizontally ca. 1.3–2 times wider than long (vs rounded, ca. as wide as long in *W. fidelis*) (cf. Figs. 3C; 5C, F, I vs. fig. 17 in Kronestedt & Zyuzin, 2009 and 66H in Wang et al., 2021); and more oval foveolae, ca. 2–2.5 times longer than wide (vs. foveolae more elongated, ca. 3–4 times longer than wide in *W. fidelis*) (cf. Figs. 5A, B, D, G vs. fig. 15 in Kronestedt & Zyuzin, 2009 and fig. 66G in Wang et al. 2021).

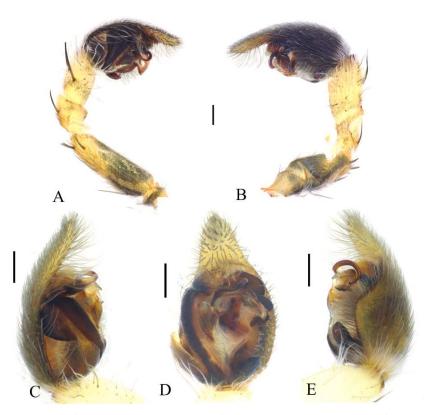


Figure 2. Male palp of Wadicosa okinawensis from Okinawa-jima Island. A–B, sample code: KTM20230904-01; C–E, KTM20240903-01. A, whole palp, prolateral view; B, same, retrolateral view; C, tip of palp, prolateral view; D, same, ventral view; E, same, retrolateral view. Scale bars: A-E, 0.2 mm

Redescription. Male (KTM20230904-01; Figs. 1A, C, E; Table 2). Total length, 5.46; carapace, 2.88 long, 2.21 wide; opisthosoma, 2.58 long, 1.54 wide. Carapace.

Cephalic area blackish brown, median stripe yellowish, lateral side brownish-black with patches of short, white setae; fovea distinct (Fig. 1A). Clypeus blackish; chelicera greyish brown, blackish near fangs (Fig. 1E); sternum dark brown (Fig. 1C). Habitus in life

as in Figure 6A. Colors and pattern more vivid in life.

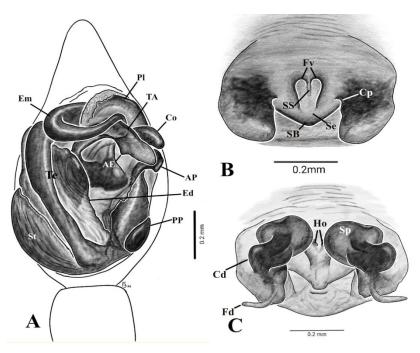


Figure 3. Male and female genitalia of *Wadicosa okinawensis* from Uruma area, Okinawa-jima Island, Japan. A, male palp, ventral view; B, epigyne, ventral view; C, vulva, dorsal view. Abbreviations: AE, extension of tegular apophysis; AP, anterior retrolateral process of tegulum; Cd, copulatory duct; Co, conductor; Cp, copulatory opening; Ed, retrolateral edge of tegulum; Em, embolus; Fd, fertilization duct; Fv, foveolae; Ho, hoods; Pl, palea; PP, posterior retrolateral process of tegulum; SB, septal base; Se, septum; Sp, spermatheca; SS, septal stem; St, subtegulum; TA, tegular apophysis; Te, tegulum. Scale bar: A-C, 0.2 mm

Eye sizes and interdistances: AME 0.12, ALE 0.10, PME 0.32, PLE 0.24, AME-AME 0.12, AME-ALE 0.03, PME-PME 0.33, PME-PLE 0.35, AME-PME 0.11, ALE-PME 0.11.

Legs. Coxae I–II grey, III–IV yellowish; femora I dark greyish II–IV light yellow with grey annulation; patellae, tibiae, and metatarsi I–IV light yellow with faint greyish annulation; tarsi I–IV light yellow. Greyness ventrally reduced in femora from leg I to leg IV (Figs. 1A, C). Legs measurements and spination as in Tables 2 and 3, respectively. Leg I as in Figure 1F.

Opisthosoma. Dorsal side blackish grey with yellowish marks, cardiac mark dark greyish followed by pairs of yellowish marks each with internal black dot in middle (Fig. 1A). Ventral side greyish yellow with black marking near anterior part (Fig. 1C). Spinnerets pale brown (Fig. 1C).

Palp as in Figures 2A–E, 3A. Femur dark brown with yellow apical part, patella and tibia uniformly yellow. Patella, tibia, and basal part of cymbium dorsally covered with numerous white setae. Cymbium dark brown, distally lighter. Tegulum (Te) with well-developed, large, ridge-like process (PP) on posterior retrolateral side, coneshaped process (AP) on anterior retrolateral side (AP) (Figs. 3A, 4B, C); retrolateral edge of tegulum (Ed) serrated (Fig. 4C). Tegular apophysis (TA) strongly curved, ending wide and rounded, widened medially

with 1 conspicuous, pointed, triangular extension (AE) on ventral border (Figs. 4G–I). Embolic division as in Figures 4E, F. Conductor (Co) strongly sclerotized, broad and inclined at axial part, ending slightly

hooked (Fig. 4F). Embolus (Em) elongated, curving over the upper part of TA, and heading retrolaterally; distal part enlarged in a flat, transparent extension, ending with pointed tip (Figs. 4A–F).

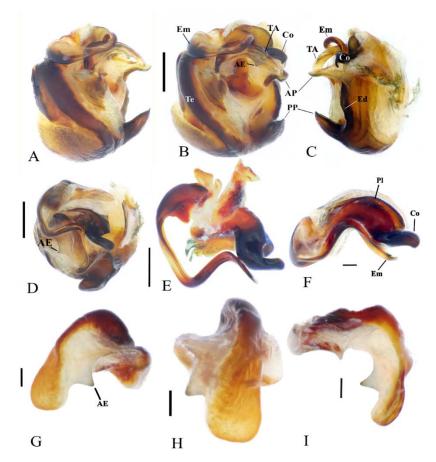


Figure 4. Details of male palp of Wadicosa okinawensis from Okinawa-jima Island. A–D, sample code: KTM20240903-01; E–I, KTM20230904-01). A, bulb, prolateral view; B, same, ventral view; C, same, retrolateral view; D, same, anterior view; E, embolic division, posterior view; F, same, anterior view; G, tegular apophysis, dorsal view; H, same, retrolateral view; I, same, ventral view. Abbreviations: AE, extension of tegular apophysis; AP, anterior retrolateral process of tegulum; Co, conductor; Ed, retrolateral edge of tegulum; Em, embolus; Pl, palea; PP, posterior retrolateral process of tegulum; TA, tegular apophysis; Te, tegulum. Scale bars: A–D, 0.2 mm; E–I, 0.05 mm

Female (KTM20230904-02; Figs. 1B, D, G; Table 2). Total length: 5.59; carapace: 2.77 long, 2.14 wide; opisthosoma: 2.82 long, 1.91 wide. Carapace. General coloration and dorsal pattern as in male but lighter (Figs. 1B, D, G); cephalic area blackish but slightly lighter than

male; clypeus light brown; chelicerae light brownish, dark brownish near fangs; sternum brown. Habitus in life as in Figure 6B.

Eye sizes and interdistances: AME 0.15, ALE 0.11, PME 0.32, PLE 0.24, AME-AME 0.13, AME-ALE 0.03, PME-PME 0.34,

PME-PLE 0.41, AME-PME 0.12, ALE-PME 0.12.

Legs. Coxae I–IV yellow; femora I–IV dorsally and laterally light yellowish with grey annulation, ventrally side yellow; patellae, tibiae and metatarsi I–IV light yellow

with faint annulation; tarsi I–IV light yellow. Legs measurement and spination as in Tables 2 and 3, respectively.

Opisthosoma. Dorsal pattern and coloration as in male, lateral and ventral sides light yellowish (Fig. 1B).

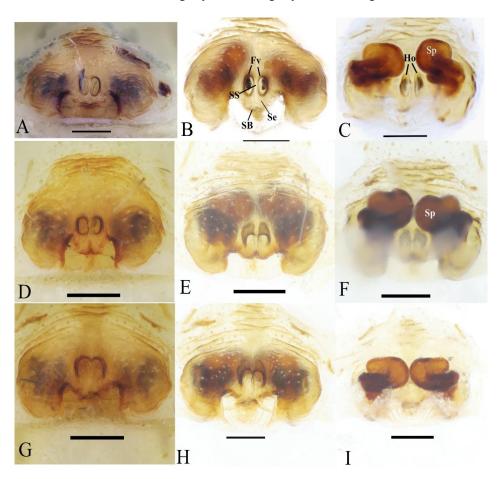


Figure 5. Variation of epigynes of Wadicosa okinawensis from Okinawa-jima Island. A–C, sample code: KTM20230904-02; D–F, KTM20240903-02; G–I, KTM20240909-03. A, D, G, epigyne before dissection, ventral view; B, E, H, same after maceration, ventral view; C, F, I, vulva after maceration, dorsal view. Abbreviations: Fv, foveolae; Ho, hood; SB, septal base; Se, septum; Sp, spermatheca; SS, septal stem. Scale bars: A–B, 0.02 mm; C–L, 0.2 mm

Epigyne as in Figures 3B, 5A, B, D, E, G, H. Foveolae (Fv) oval, ca. 2.5 times longer than wide, clearly separated from each other by a narrow septal stem (SS), open posteriorly in some individuals. Copulatory opening (Cp) at sides of basal part of septum. Septum (Se) more or less triangular, tapering backward with a flat

septal base (SB). Vulva as in Figures 3C, 5C, F, I. Oval foveolae. Fertilization ducts (Fd) narrow, comma-like. Copulatory ducts (Cd) wide, bent with ca. 90° angle before reaching spermathecae. Spermathecae (Sp) wide, kidney-like, slightly depressed at center, horizontally elongated, ca. 1.3 times wider than long.

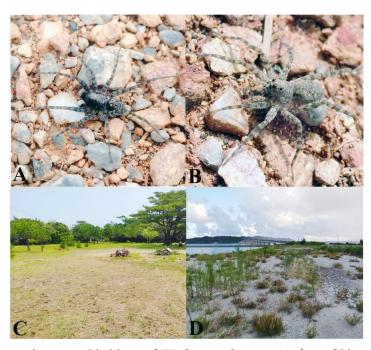


Figure 6. Live specimens and habitats of Wadicosa okinawensis from Okinawa-jima Island. A, habitus of male in life; B, habitus of female in life; C-D, examples of habitats where the species was collected

Table 2. Length of leg segments (I–IV) for the male (KTM20230904-01) and female (KTM20230904-02) of *Wadicosa okinawensis*

	Fe	Pa	Ti	Mt	Ta	Total				
	Male									
I	2.14	0.82	1.72	1.63	1.1	7.41				
II	2.57	0.69	2.13	3.26	1.36	10.01				
III	1.89	0.68	1.3	1.74	1.07	6.68				
IV	1.78	0.63	1.48	1.59	0.99	6.47				
Female										
I	2.16	0.74	1.93	1.92	1.29	8.04				
II	1.99	0.7	1.8	1.82	1.14	7.45				
III	1.93	0.76	1.44	2.01	1.04	7.18				
IV	2.58	0.79	2.18	3.42	1.02	9.99				

Table 3. Leg I spination for male and female of Wadicosa okinawensis

	Fe	Pa	Ti	Mt			
Male							
Leg I	2p 3d 3(4)r	1p 1d 1r	2p 2r 2-2-2v	2p 2r 2-2-3v			
Female							
Leg I	2p 3d 3r	1p 2d	2p 2r 2-2-2v	2p 1d 2r 2-2-3v			

Size and somatic intraspecific variation (based on specimens from Okinawa). Males (n = 5): total length, 4.69–5.99; carapace,

2.31–2.99 long, 2.21–2.36 wide; opisthosoma, 1.81–2.36 long, 1.38–1.76 wide. Females (n = 5): total length, 5.1–6.43; carapace, 2.49–3.16

long, 1.98–2.38 wide; opisthosoma, 2.61–3.59 long, 1.88–2.12 wide.

Wadicosa okinawensis exhibits high intraspecific variability, even among individuals from the same locality. Variations are particularly evident in female genitalia, e.g., foveolae more or less elongated with posterior side open or closed, 0.44-0.67 in width/height ratio; septum more or less triangular, more or less elongated with narrower or wider septal base, 2.5-4 in width/height ratio; spermathecae more or less elongated and kidney-shaped, 1.18-1.46 in width/height ratio (Figs. 5A-I).

Habitat. The species seems to prefer sunny, open, and dry meadows (Figs. 6C, D). It was also observed wandering in crops and

uncultivated fields. During night collections individuals have been frequently observed resting inactive on low vegetation. See also Tanaka (1985, p. 80) for additional information.

Remarks distribution. on distribution of Wadicosa okinawensis extends from the central Ryukyus to southern China and Laos (Wang et al., 2021; Omelko, 2024). In Japan the species is recorded from numerous islands of the central and southern including Ryukyus, Amami-Oshima, Okinoerabu-jima (new record), Okinawa-jima, Kume-jima, Miyako-jima, Kakeroma-jima, Tokuno-shima, Yoron-jima, Tokashiki-jima and Iriomote-jima (Tanaka, 1985; Shinkai et al., 2024) (Fig. 7).

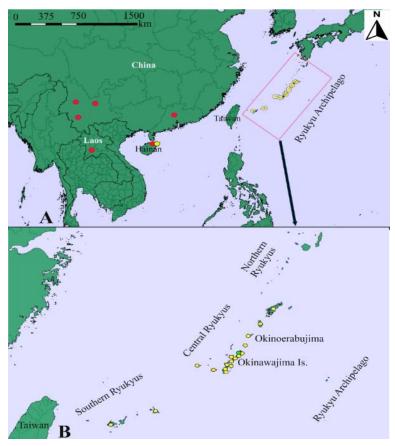


Figure 7. Known distribution of Wadicosa okinawensis. A, general distribution of the species; B, distribution of species in Ryukyu Archipelago (Japan). Legend: red circle, former distribution of W. daliensis in China and Laos; yellow circle, distribution of W. okinawensis; green circle, type locality of W. okinawensis

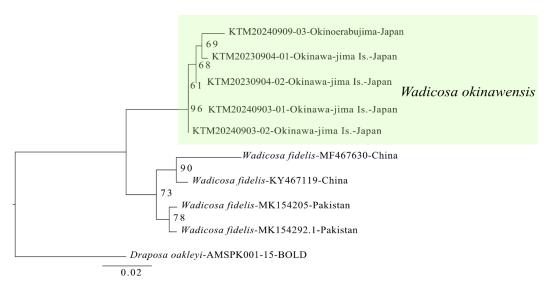


Figure 8. Phylogenetic tree generated by maximum likelihood (ML) analysis based on the COI sequences data of Wadicosa okinawensis and Wadicosa fidelis. Numbers at nodes represent bootstrap support values. Branch lengths are scaled to the number of substitutions per site. The tree is rooted with Draposa oakleyi. Accession numbers of the samples harvested from ENA and BOLD are reported in the tree, for the newly amplified sequences of Wadicosa okinawensis refer to Table 1

DISCUSSION

Synonymization of Wadicosa daliensis under Wadicosa okinawensis

Wadicosa daliensis was first described by Yin et al. (1997) based on specimens collected in Yunnan Province, China, and later reported from the provinces of Hainan and Guangdong (Song et al., 1999). The original description compared the new species with W. fidelis while a diagnosis with the similar W. okinawensis, described over a decade earlier, was missing. Since then, W. daliensis has not been properly illustrated until recently when Omelko (2024) filled this gap by redescribing the species in detail using samples from northern Laos. Omelko also compared W. daliensis with W. okinawensis. However, due to the absence of any useful figures of the topotype specimens (type locality: Okinawa-jima) and the other specimens from the Ryukyus, his diagnosis was based on the pictures of samples from Hainan Island provided by Wang et al. (2021). A direct comparison with topotype specimens

from Okinawa-jima, as well as any consideration of the intraspecific variability of the species, has never been conducted.

Although we were unable to directly examine specimens of W. daliensis, a comparison with the highly detailed photos by Omelko (2024) failed to find any clear differences between the two species. Differences in genitalia reported by Omelko (2024, p. 244) such as a serrated retrolateral edge of the male palpal tegulum and oval foveolae, were also commonly observed in our W. okinawensis samples from Okinawajima and appear to fall within the high intraspecific variation of the Okinawa-jima populations (Figs. 4B, C; 5A, B, D). Additionally, different individuals within the same populations from the islands display significant variability in the width/height ratio of the epigynal septal base (2.5-4) as visible in Figures 5A–I. This includes ratios near 3 as reported by Omelko for the Laotian samples of W. daliensis (see notes on variation of the species above). A darker body coloration,

similar to the specimens from China and Laos, is also commonly observed in the Okinawajima populations (Figs. 1A, B; 6A, B) although lighter-colored individuals like those from Hainan Island illustrated by Wang et al. (2021) are also occasionally found in the Okinawa-jima populations. It is also worth noting that Song et al. (1999) recorded W. daliensis from Hainan Island, while Wang et al. (2021) recorded only W. okinawensis from the same location without mentioning the previous record of W. daliensis. Although additional data are needed to confirm this hypothesis, such discrepancy suggests possible misidentifications between the two putative species on the island, which further underlines their close similarities.

Based on the reasons mentioned above, and in the absence of any clear morphological differences between the two species, we propose that *W. daliensis* be treated as a junior synonym of *W. okinawensis*.

DNA barcoding

The phylogenetic tree resulting from the maximum likelihood analyses is illustrated in Fig. 8. This represents the first molecular phylogenetic analysis to include sequences of *W. okinawensis*. All *W. okinawensis* specimens (Table 1) cluster together forming a monophyletic clade clearly distinct from *W. fidelis*, thus supporting the separation of the two species as suggested by the morphological analysis.

The intraspecific genetic distance of W. okinawensis is calculated between 0% to 1.05%, while the interspecific genetic distance between W. okinawensis and W. fidelis is from 4.12% to 6.67%. The interspecific threshold for DNA-based species delimitation in Lycosidae has been estimated to range from 1.2% to 3.6% (Čandek & Kuntner, 2015). Therefore, these results provide further support W. okinawensis and W. fidelis are distinct species. The lack of fresh samples of W. daliensis did not allow us a molecular confirmation of its synonym with W. okinawensis, for that we rely on morphological results only. Future research integrating

ecological studies, expanding geographic sampling, and conducting comparative DNA analyses of these species will be crucial for further supporting the synonym.

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