## **PENSOFT**.



## Two new species of the genus *Agramma* (Hemiptera, Heteroptera, Tingidae) from small islands of Japan, with an illustrated key to the Japanese species of the genus

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## Abstract

The present study describes two new species of the monocotyledon-feeding lace bugs of the genus Agramma Stephens, 1829 (Hemiptera, Heteroptera, Tingidae, Tinginae, Tingini) from small islands of Japan. The first is A. (A.) *izuense* **sp. nov.**, which was recorded as A. (A.) *japonicum* (Drake, 1948) from Hachijo Island, the Izu Islands, in a previous study, and is considered an independent species here based on morphological characteristics and molecular data. The second is A. (A.) *keramense* **sp. nov.**, which has a remarkable spineless head and was discovered from Aka and Geruma islands, Kerama Group, the Ryukyu Islands. Consequently, the following four species of Agramma were recognized in Japan: A. (A.) *abruptifrons* Golub, 1990, A. (A.) *izuense* **sp. nov.**, A. (A.) *japonicum*, and A. (A.) *keramense* **sp. nov.** Only dozens of submacropterous morphs were confirmed in these two species in the present study, suggesting that both new species are flightless. In addition, an illustrated key for the identification of the four species from Japan and the host plant relationships of the two new species are provided.

## Key Words

East Asia, host plant, molecular data, new species, phytophagous insect, taxonomy

## Introduction

The true bug family Tingidae (Hemiptera, Heteroptera), known as lace bugs, comprises phytophagous species that are highly host-specific and generally feed on the abaxial side of angiosperm leaves (Schuh and Weirauch 2020). The genus *Agramma* Stephens, 1829 (Tinginae, Tingini), a species-rich Old World taxon with 89 species in three subgenera, includes many species that feed on monocotyledons (cf. Drake and Ruhoff 1965; Péricart and Golub 1996; Aukema et al. 2013; Souma 2020). In Japan, two species belonging to the nominotypical subgenus, namely *A.* (*Agramma*) *abruptifrons* Golub, 1990 and *A.* (*A.*) *japonicum* (Drake, 1948), have been known mainly from Japan proper (Hokkaido, Honshu, Shikoku, and Kyushu) and its surrounding islands (pertaining to the Palaearctic Region) and were reported from *Juncus* sp. (Juncaceae) and *Carex* spp. (Cyperaceae), respectively (Souma 2020). Many previous studies, both older and newer, have reported several localities for these two species in the four main islands of Japan (Drake 1948; Takeya 1953; Tomokuni 1979; Ichita 1988; Yamada and Tomokuni 2012; Souma 2020; Souma and Hisasue 2022; Souma and Iwata 2022; Nozawa and Okuda 2023; Tago 2023; Yamamoto 2023, etc.). In the small islands surrounding Japan proper, however, only *A*. (*A*.) *japonicum* (Drake, 1948) has been recorded from Kunashiri [= Kunashir], Hachijo, Sado and Yakushima islands, which belong to the Palaearctic Region (Kerzhner 1978; Tomokuni 1979; Tomokuni and Ishikawa 2002; Souma 2021). Consequently, *Agramma* species on the small islands of Japan have been rather unevenly investigated.

On the other hand, the general habitus of members of the population from Hachijo Island of the Izu Islands,

Copyright Jun Souma. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. identified as *A*. (*A*.) *japonicum* in the literature, differs from that of *A*. (*A*.) *japonicum* described from Sapporo, Hokkaido, Japan proper (cf. Drake 1948; Tomokuni and Ishikawa 2002; Souma 2020). Additionally, an indeterminate species of *Agramma* was collected from Aka and Geruma islands, Kerama Group, the Ryukyu Islands (pertaining to the Oriental region) by the author and his colleague Reo Ito. Therefore, *Agramma* species distributed on the small islands of Japan require a taxonomic study.

In the present study, the population of A. (A.) japonicum from Hachijo Island and an indeterminate species from Aka and Geruma islands, the latter having a remarkable spineless head, were considered undescribed species based on careful observation of their morphological characteristics, and the monophyly of the former was supported by molecular data from four gene regions (the COI, COII, 16S, and 28S genes). In conclusion, two new species, namely A. (A.) izuense sp. nov. from Hachijo Island and A. (A.) keramense sp. nov. from Aka and Geruma islands, were described. In addition, the possibility of flightlessness for the two new species has been suggested, as only submacropterous morphs have been collected so far. An illustrated identification key and photographs of living individuals of all four species of Agramma occurring in Japan are provided, and the host plant relationships of the two new species were presented.

## Materials and methods

#### Molecular data

A total of 18 operational taxonomic units (OTUs) from four Japanese tingid species were used in the present study (Suppl. material 1). The 16 OTUs consisting of two species of Agramma, A. (Agramma) izuense sp. nov. and A. (A.) japonicum, were used as ingroups because of their strong similarity in terms of morphological characteristics and to judge whether they are independent species or represent geographic variation. The remaining two OTUs consisting of two species, namely Cochlochila (Physodictyon) conchata (Matsumura, 1913) and Limnostatua lewisi (Scott, 1880), were used as outgroups. The genera Cochlochila and Limnostatua are placed into Tingini as well as Agramma but differ from Agramma in their morphological characteristics (cf. Drake and Ruhoff 1965; Souma and Ishikawa 2022). Partial sequences of the following gene markers were used for the present analyses: mitochondrial COI (742 bp), COII (765 bp), 16S (361 bp), and nuclear 28S (444 bp). Fragments of each gene were amplified using the following primers: C1-J-2183 (5'CAA CAT TTA TTT TGA TTT TTT GG 3') and TL2-N-3014 (5'TCC AAT GCA CTA ATC TGC CAT ATT A 3') (Simon et al. 1994) for COI; C1-J-2798 (5'CCW CGW CGW TAY TCW GAY TAT CC 3') and C2-N-3554 (5'GTT CAT GAR TGW ARD ACA TC 3') (Damgaard and Cognato 2005) for COII; Lace16sF (5'ATG ATT TTT AAA TGG CCG CGT 3') and Lace16sR (5'GAA CTC

TCC AAG AAA ATT ACG CTG T 3') (present study) for 16S; and 28sL (5'CCC GTC TTG AAA CAC GGA CCA A 3') (Muraji and Tachikawa 2000) and Lace28sR (5'TCT GAT CCG AGT CCC ACG GCT 3') (present study) for 28S. All sequences used in the present analyses were newly submitted to GenBank by this study or previously registered to GenBank by Souma (2022).

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). All DNA samples were extracted from the abdomens of the specimens using a nondestructive method. The abdomens were preserved in small polyethylene vials containing 50% glycerin and 50% water solution. The other body parts were preserved as dried specimens and pinned. PCR was performed using the following protocols: initial denaturation at 98 °C for 3 min, denaturation at 98 °C for 10 s, annealing at 50 °C (65 °C in 28S) for 5 s, and extension at 68 °C (72 °C in 28S) for 5 s and 35 cycles (33 cycles in COI), with a final extension at 68 °C (72 °C in 28S) for 3 min. The PCR products were purified using an ExoSAP IT kit (Amersham Biosciences, Amersham, United Kingdom).

The edited sequences used in the present study were compared with related sequences from the National Centre for Biotechnology Information (NCBI) (http:// www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al. 1997).

Sequence alignments were performed using Mega 10.1.8 (Kumar et al. 2018). Gaps were treated as missing data. Six OTUs were used in the analysis of concatenated datasets of COI, COII, 16S, and 28S to estimate the phylogenetic relationship between A. (A.) japonicum from three islands of Japan proper (Hokkaido, Honshu, and Kyushu), including the type locality Sapporo (Hokkaido), and A. (A.) izuense sp. nov. from Hachijo Island, the Izu Islands (Suppl. material 1). In addition to the six OTUs, 12 OTUs were used in the analyses of the COI datasets to identify the morphological species. The COI dataset of the 18 OTUs contained four OTUs per locality in A. (A.) izuense sp. nov. and A. (A.) japonicum. The COI, COII, 16S, and 28S sequence datasets were concatenated using Kakusan 4 (Tanabe 2011). The concatenated aligned sequences yielded 2,312 bp. The homogeneity of the base composition of the sequences was tested using the pgtest composition implemented in Phylogears in Kakusan 4. The null hypothesis of homogeneity among the OTUs was rejected for the third codon position of COI. To decrease the saturation and compositional bias, the RY coding dataset of COI for the third codon (Woese et al. 1991) was used for the phylogenetic analyses. The substitution models and partitioning schemes applied in the Bayesian inference analyses were selected using Kakusan 4. Bayesian analyses were performed using MrBayes v.3.2.7 (Ronquist et al. 2012) with two Markov chain Monte Carlo (MCMC) runs of four chains for 2,000,000 generations. The sampled trees and models from the first 1,301,000 generations were discarded as burn-in tree and a majority-rule consensus tree was constructed from the sample trees from the latter 699,000 generations. Convergence of both runs, visualized using Tracer v.1.6 (Rambaut et al. 2014), was determined to have occurred when the effective sample size (ESS) (Kass et al. 1998) increased above 200. The tree was visualized and edited using Figtree v.1.4.4 (Rambaut 2014) and Adobe Photoshop 2023 ver.24.5, respectively.

To identify the morphological species, the pairwise sequence distances of the COI dataset of 18 OTUs were calculated using the Kimura-two parameter (K2P) model in Mega 10.1.8 (Kumar et al. 2018). In a previous study (Jung et al. 2011), the average interspecific and intraspecific genetic distances of the COI gene in Heteroptera were 6.3% and 0.4%, respectively. Therefore, in the present study, interspecific (intraspecific) genetic distances of more than 9% (0.9%) and less than 3% (0.3%) were treated as large and small, respectively.

#### **Systematics**

The morphological characteristics of the dried specimens were examined, drawn, and measured using a stereoscopic microscope (SZ60; Olympus, Tokyo, Japan) equipped with an ocular grid. To observe the parameres, the pygophores were removed from the body after softening the specimens in hot water. The removed pygophores were immersed in a hot 15% KOH solution for 5 min and then soaked in 99% ethanol for the dissection of the paramere. The parameres were observed after fixing the angles with a gel (Museum Gel Clear, Ready America, California, U.S.A.) placed on the microscope slide. The pygophores and parameres were preserved in small polyethylene vials containing a 50% glycerin and 50% water solution. The polyethylene vials were mounted on the pins with the respective specimens. Photographs of the dried specimens and living individuals were taken using a compact digital camera (Tough TG-6, Olympus, Tokyo, Japan) and digital microscopes (VHX-1100, Keyence, Osaka, Japan; Dino-Lite Premier M, Opto Science, Tokyo, Japan). The image stacks were processed using Adobe Photoshop 2023 ver.24.5 when using Dino-Lite Premier M. The host plants were photographed using a smartphone (iPhone 8, Apple, California, U.S.A.). Morphological terms were assigned according to previous monographs (Takeya 1962; Drake and Davis 1960; Drake and Ruhoff 1965; Schuh and Weirauch 2020).

The type specimens of the new species were deposited at the National Museum of Nature and Science, Ibaraki, Japan (NSMT), the Shirakami Research Center for Environmental Sciences, Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan (SIHU), and the Laboratory of Entomology, Faculty of Agriculture, Tokyo University of Agriculture, Kanagawa, Japan (TUA).

Dried specimens of the two known Japanese species of *Agramma*, namely *A*. (*A*.) *abruptifrons* and *A*. (*A*.) *japonicum*, which were compared with the new species described below, were either recorded in previous studies (Souma 2020, 2021; Souma and Hisasue 2022; Souma

and Iwata 2022; Tago 2023) or newly collected (Suppl. material 3). Newly collected specimens comprising only *A*. (*A*.) *japonicum* were deposited at SIHU.

Species distribution records were mapped using SimpleMappr (Shorthouse 2010). Geographical coordinates were obtained from Google Maps (https://www.google. co.jp/maps). The map was edited using Adobe Photoshop 2023 ver.24.5.

### Results

#### Molecular data

The Bayesian tree of COI, COII, 16S, and 28S genes well supported the monophyly of the clade A. (A.) *izuense* sp. nov. + A. (A.) *japonicum*, A. (A.) *japonicum*, and the clade consisting of the populations of A. (A.) *japonicum* from Honshu and Kyushu (all Bayesian posterior probabilities = 100%) (Fig. 1). The phylogenetic analysis of COI well supported the monophyly of the following taxa and populations (all Bayesian posterior probabilities = 100%): the clade A. (A.) *izuense* sp. nov. + A. (A.) *japonicum*; A. (A.) *izuense* sp. nov.; A. (A.) *japonicum*; and the population of A. (A.) *japonicum* from Kyushu.

The inter- and intraspecific distances of 18 individuals of four lace bug species were generated based on the K2P model of substitution of the partial COI gene (742 bp) (Suppl. material 2). The divergence between the ingroups (A. (A.) *izuense* sp. nov. and A. (A.) *japonicum*) and outgroups (*Cochlochila* (*Physodictyon*) conchata and *Limnostatua lewisi*) was in the range of 0.1912–0.2198. The interspecific divergence between A. (A.) *izuense* sp. nov. and A. (A.) *japonicum* was in the range of 0.0916–0.1029 and were considered large. As mentioned above, both species, which can also be distinguished based on morphological characteristics (see the identification key), were monophyletic in the Bayesian trees (Fig. 1). Finally, A. (A.) *izuense* sp. nov. and A. (A.) *japonicum* were considered as independent species in the present study.

The intraspecific divergence of the partial COI gene was 0 in A. (A.) *izuense* sp. nov. and 0–0.0262 in A. (A.) *japonicum*. The interpopulation divergence of A. (A.) *japonicum* between Hokkaido, Honshu, and Kyushu were 0.0220 (Hokkaido and Honshu), 0.0248–0.0262 (Hokkaido and Kyushu), and 0.0095–0.0109 (Honshu and Kyushu), and were considered large. In contrast, the intrapopulation divergence within the three islands was 0–0.0013 and was considered small. Furthermore, the Bayesian trees formed separate clades with high posterior probabilities for the three populations of A. (A.) *japonicum* (Fig. 1). However, no morphological differences were found among the specimens from these three islands, and the three populations of A. (A.) *japonicum* were treated as the same species in the present study.

To the best of the author's knowledge (Suppl. material 3) and in accordance with the findings of a previous study (Souma 2020), the submacropterous and macropter-



**Figure 1.** Bayesian trees constructed using COI, COII, 16S and 28S genes (2,312 bp) and COI gene (742 bp). Bayesian posterior probabilities are indicated near nodes. Scale bars represent number of expected substitutions per site. Each sample ID is followed by species name and collection locality.

ous morphs of A. (A.) *japonicum* are considered common and rare, respectively. Thus, opportunities for long-distance flight dispersal could be rare in A. (A.) *japonicum*. Although further studies are needed, the hypothesis of the low dispersal ability could explain why the interpopulation and intrapopulation divergences of the partial COI gene in A. (A.) *japonicum* were large and small, respectively.

#### **Systematics**

#### Genus Agramma Stephens, 1829

Japanese name: Naga-gunbai-zoku

*Agramma* Stephens, 1829: 64. Type species: *Tingis laeta* Fallén, 1807, by monotypy.

**Note.** For synonyms and detailed descriptions of the genus see Péricart (1983), Péricart and Golub (1996) and Souma (2020).

**Remarks.** The genus *Agramma* comprises 88 extant and one fossil species in three subgenera from the Old World. Among them, two species belonging to the nominotypical subgenus, namely *A.* (*Agramma*) abruptifrons (Figs 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, B) and *A*. (*A*.) *japonicum* (Figs 2D, 3C, 4C, 5E, 6C, 7C, 8C, 9C, 10D), have been known from Japan to date (cf. Souma 2020). Here, two new species of the nominotypical subgenus, namely *A*. (*A*.) *izuense* sp. nov. and *A*. (*A*.) *keramense* sp. nov., are described from Japan. A key to the four species is provided.

#### Agramma (Agramma) izuense sp. nov.

https://zoobank.org/23E9D009-B96B-4905-91B8-D783B3BF0ECD Figs 2B, C, 3B, 4B, 5B–D, 6B, 7B, 8B, 9B, 10C Japanese name: Hachijonaga-gunbai

- *Agramma nexile* (non Drake, 1948): Tomokuni and Ishikawa (2002: 170) (distribution); Yamada and Tomokuni (2012: 188) (distribution: part); Yamada and Ishikawa (2016: 429) (distribution: part). Misidentifications.
- Agramma japonicum (non Drake, 1948): Souma (2020: 532) (distribution: part). Misidentification.

**Type series.** *Holotype* (submacropterous ♂, SIHU), "[JAPAN]: Izu Isls., Hachijo Is., Mitsune, Mt. Hachijo-Fuji" [=JAPAN: IZU ISLANDS: Hachijo Island:



**Figure 2.** Habitus of four *Agramma* species from Japan, dorsal view: *A.* (*Agramma*) *abruptifrons* from Honshu (**A**); *A.* (*A.*) *izuense* sp. nov. from Hachijo Island, Izu Islands, male (**B**) and female (**C**); *A.* (*A.*) *japonicum* from Yakushima Island, Osumi Group, Ryukyu Islands (**D**); *A.* (*A.*) *keramense* sp. nov. from Geruma Island, Kerama Group, Ryukyu Islands, male (**E**) and female (**F**). Scale bar: 1.0 mm.

 5.vii.2001, leg. M. Tomokuni (submacropterous 5 332 2 99, NSMT); Ohkago, 19.v.2021, leg. J. Souma (submacropterous 7 335 999, SIHU); Western foothills of Mt. Hachijo-Fuji, 21.v.2021, leg. J. Souma (submacropterous 4 332 2 999, SIHU). Eight specimens collected in 2001 were recorded as "*Agramma nexile* (Drake, 1948)" by the previous study (Tomokuni and Ishikawa 2002).

Additional material examined. Non-types (1 nymph, SIHU), JAPAN: IZU ISLANDS: Hachijo Island: as holotype but 16.v.2021. The single nymph recorded above was in poor condition and was thus not described in the present study.



Figure 3. Pronota of four *Agramma* species from Japan, dorsal view: *A.* (*Agramma*) *abruptifrons* (A); *A.* (*A.*) *izuense* sp. nov. (B); *A.* (*A.*) *japonicum* (C); *A.* (*A.*) *keramense* sp. nov. (D). Scale bars: 0.2 mm.

**Diagnosis.** Agramma (Agramma) izuense sp. nov. is recognized among other species of Agramma by a combination of the following characters: pubescence on body less than 0.5 times as long as diameter of compound eye; antennal segment IV brown (Fig. 2B, C); posterior process in apical part and hemelytron sometimes irregularly dark (Fig. 5C); thoracic sterna, pygophore and female terminalia black (Figs 4B, 6B, 7B); head with a pair of frontal spines (Figs 3B, 8B); rostrum reaching middle part of mesosternum; pronotum without paranotum; median carina of pronotum distinct on posterior process; anterior margin of hemelytron gently curved outward (Fig. 5B, D); apices of hemelytra separated from each other at rest; R+M (radiomedial) vein of hemelytron present in apical part, carinate throughout its length; costal area usually with 2 rows of areolae at widest part; discoidal-sutural area with 7–8 rows of areolae at widest part; outer and inner margins of paramere angularly curved in middle part (Fig. 9B); and female terminalia hexagonal in ventral view, with posterior margin protruding posteriad in middle part.

**Description.** *Submacropterous male.* Head, calli, pronotal disc, basal part of posterior process, thoracic pleura, thoracic sterna, sternal laminae, apical part of tarsi and abdomen black; antenna, frontal spine, buccula, rostrum, collar, apical part of posterior process, hemelytron and legs except apical part of tarsi brown; apical part of poste-



**Figure 4.** Rostra of four *Agramma* species from Japan, ventral view: *A.* (*Agramma*) *abruptifrons* (**A**); *A.* (*A.*) *izuense* sp. nov. (**B**); *A.* (*A.*) *japonicum* (**C**); *A.* (*A.*) *keramense* sp. nov. (**D**). Scale bars: 0.2 mm.

rior process and hemelytron sometimes irregularly dark; compound eyes dark red; pubescence on body yellowish (Figs 2B, 3B, 4B, 5B, C, 6B).

Body (Fig. 2B) oblong; pubescence on body less than 0.5 times as long as diameter of compound eye. Head (Figs 3B, 8B) glabrous, with a pair of frontal spines; frontal spines separated from each other at apices, not

reaching apex of clypeus; antenniferous tubercles obtuse, slightly curved inward; clypeus smooth; vertex coarsely punctate. Compound eye round in dorsal view. Antenna densely covered with pubescence throughout its length and tiny tubercles in segments I to II; segment I cylindrical; segment II cylindrical, shortest among antennal segments; segment III longest among antennal segments;



Figure 5. Hemelytra of four *Agramma* species from Japan, dorsal view: *A.* (*Agramma*) *abruptifrons* (**A**); *A.* (*A.*) *izuense* sp. nov., male (**B**, **C**) and female (**D**); *A.* (*A.*) *japonicum* (**E**); *A.* (*A.*) *keramense* sp. nov. (**F**). Scale bars: 0.2 mm.

segment IV cylindrical, longer than segment I. Bucculae contiguous with each other at anterior ends, with 3 rows of areolae throughout their length. Rostrum (Fig. 4B) reaching middle part of mesosternum.

Pronotum (Figs 3B, 8B) unicarinate, without paranotum. Pronotal disc coarsely punctate. Hood absent. Collar coarsely punctate; anterior margin gently curved inward. Calli smooth. Median carina ridge-like, distinct on posterior process. Posterior process well-developed, flattened, triangular. Thoracic pleura coarsely punctate. Ostiolar peritreme oblong. Mesosternum (Fig. 4B) as wide as metasternum at widest part. Sternal laminae nearly straight throughout their length. Legs smooth, covered with pubescence.

Hemelytron (Fig. 5B, C), extending beyond apex of abdomen; anterior margin gently curved outward; apices



Figure 6. Male terminalia of four *Agramma* species from Japan, ventral view: *A. (Agramma) abruptifrons* (A); *A. (A.) izuense* sp. nov. (B); *A. (A.) japonicum* (C); *A. (A.) keramense* sp. nov. (D). Scale bars: 0.2 mm.

separated from each other at rest; C (costal) and R+M (radiomedial) veins present, carinate throughout their length; Cu (cubital) vein indistinct; costal area usually with 2 rows of areolae at widest part, rarely with a single row throughout its length; subcostal area with 3–4 rows of areolae at widest part; discoidal-sutural area with 7–8 rows of areolae at widest part; hypocostal lamina with a single row of areolae throughout its length.

Abdomen oblong in dorsal and ventral views. Pygophore (Fig. 6B) compressed dorsoventrally, semicircular in ventral view, covered with pubescence. Paramere (Fig. 9B) slender, expanded in middle part; outer and inner margins angularly curved in middle part, covered with pubescence in middle part.

Measurements (n = 20). Body length with hemelytra 2.1–2.4 mm; maximum width across hemelytra 0.8-0.9 mm; length of antennal segments I to IV 0.2 mm, 0.1 mm, 0.5 mm, and 0.3 mm, respectively; pronotal length 0.8–0.9 mm; pronotal width across humeri 0.6 mm; hemelytral length 1.4–1.6 mm; maximum width of hemelytron 0.4–0.5 mm.

*Submacropterous female.* General habitus very similar to that of male (Figs 2C, 5D, 7B) except for the following characters: subcostal area of hemelytron wider



Figure 7. Female terminalia of four *Agramma* species from Japan, ventral view: *A*. (*Agramma*) *abruptifrons* (**A**); *A*. (*A*.) *izuense* sp. nov. (**B**); *A*. (*A*.) *japonicum* (**C**); *A*. (*A*.) *keramense* sp. nov. (**D**). Scale bars: 0.2 mm.

than in male, with 4–5 rows of areolae at widest part; apical part of abdomen hexagonal in ventral view; posterior margin of terminalia protruding posteriad in middle part; and ovipositor with well-developed ovivalvula at base.

Measurements (n = 20). Body length with hemelytra 2.4–2.6 mm; maximum width across hemelytra 0.9-1.0 mm; length of antennal segments I to IV 0.2 mm, 0.1 mm, 1.5 mm, and 0.3 mm, respectively; pronotal length 0.9–1.0 mm; pronotal width across humeri 0.6-0.7 mm; hemelytral length 1.6–1.8 mm; maximum width of hemelytron 0.5–0.6 mm.

**Remarks.** In a previous study, *Agramma (Agramma) izuense* sp. nov. was misidentified as A. (A.) *japonicum* 

from the latter by the following characters: posterior process in apical part and hemelytron sometimes irregularly dark (brown in A. (A.) *japonicum*) (Figs 2B, D, 5C, E); apices of hemelytra separated from each other at rest (close to each other in A. (A.) *japonicum*) (Figs 2C, 5B, D); R+M (radiomedial) vein carinate throughout its length (carinate in basal part and not carinate in apical part in A. (A.) *japonicum*); and costal area usually with 2 rows of areolae at widest part (a single row in A. (A.) *japonicum*). Morphological differences between

(Tomokuni and Ishikawa 2002), because both species are

the most similar among the Asian species of the genus

Agramma. However, the former is easily distinguished

the new species and the other two Japanese species are provided in the identification key below.

On the other hand, the new species is similar in general appearance to A. (A.) ruficorne (Germar, 1835), which is widely distributed in the Palaearctic Region (Péricart and Golub 1996; Aukema et al. 2013). Nevertheless, A. (A.) ruficorne shares the morphological features mentioned in the above paragraph with A. (A.) japonicum so that it is easily distinguished from A. (A.) izuense sp. nov.

**Distribution.** Japan (Izu Islands: Hachijo Island) (Fig. 12) (Tomokuni and Ishikawa 2002; Yamada and Tomokuni 2012; Yamada and Ishikawa 2016; present study). *Agramma (Agramma) izuense* sp. nov. inhabits the forest floor in the warm-temperate climate of the Izu Islands in the Palaearctic Region.

**Etymology.** The specific epithet refers to its occurrence in the Izu Islands, Japan; an adjective.

**Host plants.** *Carex* sp. (Cyperaceae) (Fig. 11B) (present study). Although the host plant species could not be identified, *Agramma (Agramma) izuense* sp. nov. feeds only on this cyperaceous herb and appears to be monophagous.

**Biology.** Agramma (Agramma) izuense sp. nov. feeds on the abaxial surface of the leaves of the abovementioned cyperaceous plant (present study). Dozens of type materials consisting of only submacropterous morphs were collected, suggesting that this new species is flightless. Adults and nymphs were collected in May and July (Tomokuni and Ishikawa 2002; present study).

#### Agramma (Agramma) keramense sp. nov.

https://zoobank.org/5A41CB70-F8B5-4B30-8B29-A0B7CCFB6605 Figs 2E, F, 3D, 4D, 5F, 6D, 7D, 8D, 9D, 10E–I Japanese name: Hosonaga-gunbai

**Type series.** *Holotype* (submacropterous 3, SIHU), "[JA-PAN]: the Ryukyus, Okinawa Isls., Aka Is., Aka" [=JA-PAN: RYUKYU ISLANDS: Kerama Group: Aka Island: Aka (approximate coordinates:  $26^{\circ}11'43.7"N$ ,  $127^{\circ}17'07.9"E$ )], 3.xi.2022, leg. J. Souma. *Paratypes* (submacropterous 13  $3^{\circ}3$  19  $9^{\circ}9$ ), JAPAN: RYUKYU ISLANDS: Kerama Group: Aka Island: as holotype (submacropterous 2  $3^{\circ}3$  5  $9^{\circ}9$ , SIHU); as holotype but 4.v.2021, leg. R. Ito (submacropterous 1  $9^{\circ}$ , SIHU); as holotype but 5.v.2021, leg. R. Ito (submacropterous 3  $3^{\circ}3$  3  $9^{\circ}9$ , SIHU); as holotype but 17.vii.2021, leg. R. Ito (submacropterous 3  $3^{\circ}3$  9  $9^{\circ}9$ , SIHU). Geruma Island: Geruma, 3.xi.2022, leg. J. Souma (submacropterous 5  $3^{\circ}3$   $9^{\circ}9$ , SIHU).

Additional material examined. Non-types (7 nymphs), JAPAN: RYUKYU ISLANDS: Kerama Group: Aka Island: as holotype (6 nymphs, SIHU); as holotype but 5.v.2021, leg. R. Ito (1 nymph, SIHU). All 7 nymphs recorded above were in poor condition and are thus not described here.

**Diagnosis.** Agramma (Agramma) keramense sp. nov. is recognized among other species of Agramma by a combination of the following characters: pubescence on body less than 0.5 times as long as diameter of compound eye; antennal segment IV light brown (Fig. 2E, F); posterior process in apical part and hemelytron light brown (Fig. 5F); thoracic sterna, pygophore and female terminalia dark brown (Figs 4D, 6D, 7D); head without spine (Figs 3D, 8D); rostrum reaching posterior part of prosternum; pronotum without paranotum; median carina of pronotum indistinct on posterior process; anterior margin of hemelytron nearly straight; apices of hemelytra separated from each other at rest; R+M (radiomedial) vein of hemelytron present in apical part, carinate throughout its length; costal area with a single row of areolae throughout its length; discoidal-sutural area with 5 rows of areolae at widest part; outer and inner margins of paramere gently curved in middle part (Fig. 9D); and female terminalia hexagonal in ventral view, with posterior margin protruding posteriad in middle part.

**Description.** *Submacropterous male.* Head black; calli, pronotal disc, basal part of posterior process, thoracic pleura, thoracic sterna, sternal laminae and abdomen dark brown; antenna, buccula, rostrum, collar, apical part of posterior process, hemelytron and legs light brown; compound eye dark red; pubescence on body yellowish (Figs 2E, 3D, 4D, 5F, 6D).

Body (Fig. 2E) oblong; pubescence on body less than 0.5 times as long as diameter of compound eye. Head (Figs 3D, 8D) glabrous, without spine; antenniferous tubercles obtuse, slightly curved inward; clypeus smooth; vertex coarsely punctate. Compound eye round in dorsal view. Antenna densely covered with pubescence throughout its length and tiny tubercles in segments I to II; segment I cylindrical, as long as segment II; segment II cylindrical; segment III longest among antennal segments; segment IV cylindrical, longer than segment I. Bucculae contiguous with each other at anterior ends, with 3 rows of areolae throughout their length. Rostrum (Fig. 4D) reaching posterior part of prosternum.

Pronotum (Figs 3D, 8D) without carina, without paranotum. Pronotal disc coarsely punctate. Hood absent. Collar coarsely punctate; anterior margin gently curved inward. Calli smooth. Posterior process well-developed, flattened, triangular. Thoracic pleura coarsely punctate. Ostiolar peritreme oblong. Mesosternum (Fig. 4D) as wide as metasternum at widest part. Sternal laminae nearly straight throughout their length. Legs smooth, covered with pubescence.

Hemelytron (Fig. 5F), extending beyond apex of abdomen; anterior margin nearly straight; apices separated from each other at rest; C (costal) and R+M (radiomedial) veins present, carinate throughout their length; Cu (cubital) vein indistinct; costal area with a single row of areolae throughout its length; subcostal area with 3 rows of areolae at widest part; discoidal-sutural area with 5 rows of areolae at widest part; hypocostal lamina with a single row of areolae throughout its length.

Abdomen oblong in dorsal and ventral views. Pygophore (Fig. 6D) compressed dorsoventrally, semicircular in ventral view, covered with pubescence. Paramere (Fig. 9D) slender, expanded in middle part; outer and inner margins gently curved in middle part, covered with pubescence in middle part.

Measurements (n = 14). Body length with hemelytra 2.2–2.4 mm; maximum width across hemelytra 0.5–0.6 mm;



**Figure 8.** Line drawings of pronota of four *Agramma* species from Japan, dorsal view: *A.* (*Agramma*) *abruptifrons* (**A**); *A.* (*A.*) *izuense* sp. nov. (**B**); *A.* (*A.*) *japonicum* (**C**); *A.* (*A.*) *keramense* sp. nov. (**D**). Abbreviations: ca, calli; co, collar; fs, frontal spine; mc, median carina; pd, pronotal disc; pp, posterior process. Scale bars: 0.2 mm.



Figure 9. Line drawings of parameres of four *Agramma* species from Japan, dorsal view: *A. (Agramma) abruptifrons* (A); *A. (A.) izuense* sp. nov. (B); *A. (A.) japonicum* (C); *A. (A.) keramense* sp. nov. (D). Scale bars: 0.1 mm.



**Figure 10.** Living individuals of four *Agramma* species from Japan: *A.* (*Agramma*) *abruptifrons* from Honshu, female (**A**) and teneral male (**B**); *A.* (*A.*) *izuense* sp. nov. from Hachijo Island, Izu Islands, female (**C**); *A.* (*A.*) *japonicum* from Honshu, female (**D**); *A.* (*A.*) *keramense* sp. nov. from Aka Island, Kerama Group, Ryukyu Islands, male (**E**), mature (**F**) and teneral (**G**) females, and fifth (**H**) and fourth (**I**) instar nymphs.

length of antennal segments I to IV 0.1 mm, 0.1 mm, 0.4 mm, and 0.2 mm, respectively; pronotal length 0.7 mm; pronotal width across humeri 0.4 mm; hemelytral length 1.6–1.7 mm; maximum width of hemelytron 0.3 mm.

*Submacropterous female.* General habitus very similar to that of male (Figs 2F, 7D) except for the following characters: subcostal area of hemelytron wider than in male, with 4 rows of areolae at widest part; apical part of abdomen hexagonal in ventral view; posterior margin of terminalia protruding posteriad in middle part; and ovipositor with well-developed ovivalvula at base.

Measurements (n = 19). Body length with hemelytra 2.4–2.5 mm; maximum width across hemelytra 0.6 mm; length of antennal segments I to IV 0.1 mm, 0.1 mm, 0.4 mm, and 0.2 mm, respectively; pronotal length 0.7 mm; pronotal width across humeri 0.4 mm; hemelytral length 1.7-1.8 mm; maximum width of hemelytron 0.3–0.4 mm.

**Remarks.** Agramma (Agramma) keramense sp. nov. does not completely match the diagnosis of the genus Agramma provided by Souma (2020) because of the lack of spines on the head. However, the new species can be provisionally placed into Agramma based on the general similarity. Among the Asian species of Agramma, A.(A.) keramense sp. nov. is most similar to A.(A.) vicinale (Drake, 1927) in its general habitus. However, based on a comparison between the type materials of the new species and the photographs of the holotype (United States National Museum of Natural History 2023), together with the original description (Drake 1927) of A.(A.) vicinale, two main characters were recognized to easily differentiate A.(A.) keramense sp. nov. from A.(A.) vicinale: head without spine (with a pair of frontal spines in A.(A.) vicinale) (Fig. 3D, 8D); and median carina of pronotum indistinct on posterior process (distinct in A.(A.) vicinale). Morphological differences between the new species and the three other Japanese species are provided in the identification key below.

**Distribution.** Japan (Ryukyu Islands: Kerama Group: Aka Island, Geruma Island) (Fig. 12). *Agramma (Agramma) keramense* sp. nov. inhabits grasslands in the subtropical climate of Kerama Group of the Ryukyu Islands in the Oriental Region.

**Etymology.** The specific epithet refers to its occurrence in Kerama Group, the Ryukyu Islands, Japan; an adjective.

Host plants. Poaceae gen. et sp. indet. (present study) (Fig. 11D). Although the host plant genus and species



**Figure 11.** Monocotyledonous host plants of four *Agramma* species from Japan: *Juncus* sp. (**A**) from Honshu, damaged by *A*. (*Agramma*) *abruptifrons*; *Carex* sp. (**B**) from Hachijo Island, Izu Islands, damaged by *A*. (*A*.) *izuense* sp. nov.; *Carex* sp. (**C**) from Honshu, damaged by *A*. (*A.*) *japonicum*; Poaceae gen. et sp. indet. (**D**) from Aka Island, Kerama Group, Ryukyu Islands, damaged by *A*. (*A.*) *keramense* sp. nov.



**Figure 12.** Collection sites of two species of *Agramma* from Japan used in present study. Red circles = A. (*Agramma*) *izuense* sp. nov.; green squares = A. (A.) *keramense* sp. nov.

could not be identified, *Agramma* (*Agramma*) keramense sp. nov. only feeds on this poaceous herb and appears to be monophagous.

**Biology.** Agramma (Agramma) keramense sp. nov. feeds on the abaxial surface of the leaves of the aforementioned poaceous plant (present study). Dozens of type materials consisting of only submacropterous morphs were collected, suggesting that this new species is flightless. Adults were collected in May, July, and November, whereas nymphs were collected in May and November (present study).

## Key to species of Agramma from Japan

- 1 Head without spine (Figs 3D, 8D); rostrum reaching posterior part of prosternum (Fig. 4D); anterior margin of hemelytron nearly straight (Figs 2E, F, 5F); collected from Poaceae (Fig. 11D)...... Agramma (Agramma) keramense sp. nov.
- Head with a pair of frontal spines (Figs 3A–C, 8A–C); rostrum reaching middle part of mesosternum (Fig. 4A–C); anterior margin of hemelytron gently curved outward (Figs 2A–D, 5A–E); collected from Cyperaceae or Juncaceae (Fig. 11A–C)...2

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  2. [A distributional record of Agramma abruptifrons Golub, 1990 (Heteroptera, Tingidae) from Shiga Prefecture, Honshu, Japan] [in Japanese]

## Supplementary material 1

## Sample IDs and GenBank Accession numbers for 18 individuals of four tingid species used for DNA extraction

Authors: Jun Souma

Data type: xlsx

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## Supplementary material 2

## Pairwise genetic divergence (Kimuratwo parameter) for 18 individuals of four tingid species based on partial sequences of mitochondrial COI gene

Authors: Jun Souma

Data type: xlsx

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## Supplementary material 3

## Newly collected specimens of *Agramma* (*Agramma*) *japonicum* from Japan deposited at SIHU

Authors: Jun Souma

Data type: xlsx

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