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Two remarkable new species of *Glaucocharis* (Lepidoptera, Crambidae, Crambinae) from the Ogasawara Islands, Japan

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Abstract

We describe two new species of the genus *Glaucocharis* from the Ogasawara Islands of Japan: *G. triochellaris* Matsui, Yagi & Hirowatari, **sp. nov.** and *G. plumbofascialis* Matsui, Yagi & Hirowatari, **sp. nov.** The new species with remarkable morphological characters are easily distinguished from congeneric species. We provide photographs of adult, male and female genitalia. Molecular phylogenetic analysis revealed a sister relationship between the two new species and the monophyly of *Glaucocharis*.

Key words: Diptychophorini, genitalia, taxonomy, wing venation

Introduction

Glaucocharis Meyrick, 1938, a genus of the tribe Diptychophorini, was established with *G. stella* Meyrick, 1938 from New Zealand as the type species and represents one of the most speciose genera in Crambinae, with 154 described species from the Palaearctic, Oriental, Australian, and Ethiopian regions (Nuss *et al.* 2024). The genus is characterized by the following morphology: head with weak chaetosema and rounded frons; forewing pattern with distinct fasciae, marginal spots, and usually apical marks; forewings with veins Sc and R_1 distinct or distally concurrent; R_3 and R_4 stalked, R_5 arising directly from distal cell; cell opened; termen usually bearing a subapical indentation at the distal extremity of R_5 to M_1, and often a less developed, secondary indentation at M_3; hindwings with sclerotization of CuP, A_{1+2}, A_3 variable, as is the distance between the bases of M_3 and CuA_1 at the cell; and frenulum with three bristles in the female (Gaskin 1985; Park *et al.* 2018). *Glaucocharis* moths are similar to *Roxita* Błeszyński in appearance but can be distinguished by the forewing with a well-developed M_1 and the valva without a ventral fold in the male genitalia; M_1 in the forewing is absent and the ventral fold of the valva is often present in *Roxita* (Li & Li 2012).

Currently, seven species of *Glaucocharis* are known to occur in Japan (Jinbo 2021). Inoue (1982) illustrated four described species from mainland Japan (as *Pareromene* Osthelder, 1941, a synonym of *Glaucocharis*), and Sasaki (2007) described three new species from the Ryukyu Islands. However, no species has been reported from the Ogasawara Islands, the oceanic islands located approximately 1,000 km south of mainland Japan and the only territory in Japan belonging to Oceania. In this study, we describe two new species of *Glaucocharis* with remarkable characters from the Ogasawara Islands and estimate their phylogenetic positions in *Glaucocharis* based on a molecular phylogenetic analysis.

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Materials and Methods

Insect material

All specimens of the new species described here were collected using light trapping, daytime sweeping, and rearing larvae collected from Bryopsida on the Ogasawara Islands. We also collected several *Glaucocharis* species and *Gargela xanthocasis* (Meyrick), which is suggested to be the basal-most lineage of Diptychophorini (Léger et al. 2019), from various localities in Japan by light trapping for use in the following molecular phylogenetic analysis (Table 1). All specimens were deposited in the Entomological Laboratory of Kyushu University, Japan (ELKU).

TABLE 1. List of Crambinae species used for DNA analysis with DDBJ accession numbers.

Tribe	Species	Sample ID	Locality	COI	CAD	MDH	RpS5
Diptychophorini	*Glaucocharis plumbofascialis*	M23-024	JAPAN: Sakaigatake, Hahajima Is., Ogasawara Iss.	LC810429	LC810435	LC810439	LC810445
Diptychophorini	*Glauchocharis triocellalis*	M23-037	JAPAN: Shigureyama, Chichijima Is., Ogasawara Iss.	LC810430		LC810440	
Diptychophorini	*Glaucocharis unipunctalis*	M23-157	JAPAN: Akatsuchiyama, Uken-son, Amamiohshima Is.	LC810431		LC810441	LC810446
Diptychophorini	*Glaucocharis mutuurella*	M23-159	JAPAN: Azo-rindo, Tottori-shi, Tottori Pref.	LC810432	LC810436	LC810442	LC810447
Diptychophorini	*Glaucocharis exsectella*	M23-163	JAPAN: Hiraodai, Kitakyushu-shi, Fukuoka Pref.	LC810433	LC810437	LC810443	LC810448
Diptychophorini	*Gargela xanthocasis*	M23-169	JAPAN: Shidokan, Yamato-son, Amamiohshima Is.	LC810434	LC810438	LC810444	LC810449
Diptychophorini	*Diptychophora harlequinalis*	LEP2783	USA	LR135723	LR134584	LR134849	LR134931
Diptychophorini	*Glaucocharis chrysochyta*	LEP2650	New Zealand	LR135716	LR134576	LR134840	LR134922
Diptychophorini	*Microcausta* sp.	LEP1553	French Guiana	LR214892 & LR135662	LR134558	LR134822	LR134906
Chiloini	*Diatraea saccharalis*	LEP1506	Bolivia	LR135701	LR134551	LR134815	LR134898
Argyriini	*Argyria* sp.	LEP976	Brazil	HG793013	LR134547	LR134811	LR134894
Calamotrophini	*Calamotropha paludella*	LEP1547	Switzerland	LR135703	LR134554	LR134818	LR134902
Crambini	*Chrysoteuchia culmella*	LEP2647	France	LR135714	LR134574	LR134838	LR134920
Euchromiini	*Euchromius vinculellus*	LEP840	Morocco	LR135698	LR134545	LR134809	LR134892
Haimbachiini	*Xubida linearella*	LEP2321	USA	LR135712	LR134569	LR134833	LR134915
Ancylolomiini	*Mesolia incertella*	LEP2319	USA	LR214896	LR134568	LR134832	LR134914
unknown	*Microchilo* cf. *elgrecoi*	ITBC18	Malaysia	LR135730	LR134597	LR134861	LR134944

Morphological investigation

The adult external morphological characteristics were observed using a stereoscopic microscope (S9D; Leica Microsystems GmbH, Wetzlar, Germany). The male and female genitalia were mounted using the following procedure. The abdomen was detached, soaked in 10% potassium hydroxide (KOH) solution, and boiled for 5–10 minutes. The boiled abdomen was transferred to a glass dish containing 70% ethanol and the genitalia were detached from the abdomen using tweezers. The genitalia and abdomen were stained with Chlorazol black E and mounted on a glass slide in Euparal. Images of the genitalia and the abdominal structures were captured using a digital camera (DP25; Olympus, Tokyo, Japan) attached to an upright microscope (CKX41; Olympus). As references for morphological terminology, we followed Gaskin (1985) for wing maculation and genitalia with slight modifications, Maes (1985) for tympanal organs, and Slamka (2008) for other morphological characters.

Molecular analysis

Total DNA was extracted from the legs or abdomen using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The mitochondrial *COI* gene and the nuclear *CAD*, *MDH*, and *RpS5* genes were amplified using PCR and primers described by Wahlberg & Wheat (2008) and Matsui et al. (2021) (Table 2). The composition of the PCR reaction solution was as follows: 5 μL of KOD One® PCR Master Mix -Blue- (TOYOBO CO., LTD., Osaka, Japan), 0.3 μL of forward and reverse primers (each 125 nmol), 3.4 μL of sterilized water, and 1 μL of DNA extract. The *COI* was amplified by the following program: initial denaturation phase at 98°C for 3 min; 35 cycles at 98°C for 10 s, 55°C for 10 s, and 68°C 15 s; and final extension at 68°C for 3 min. The three nuclear genes were amplified using the following program: 98 °C for 3 min; 40 cycles at 98°C for 10 s, 55–62°C (temperature depending on primers, see Table 2) for 5 s with touchdown program, and 68°C for 5 s; and 68°C for 3 min. The PCR products were checked by electrophoresis on a 1% agarose gel and purified using ExoSAP IT Express (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Sequencing was performed by a premixed Sanger sequencing services (Azenta Japan Corp., Tokyo, Japan). The newly obtained sequences were deposited in the DDBJ (https://www.ddbj.nig.ac.jp/), and the accession numbers are listed in Table 1.

TABLE 2. List of PCR primers used.

Gene	Primer	Direction	Sequence (5' -> 3')	Annealing temperature	Reference
COI	TY-J-1460-Spilo	Forward	TACAATTTATCGCTTAATACTCAGCC	55	Matsui et al. 2021
COI	TL2-N-3014-Spilo	Reverse	TCCATTACATATAATCTGCCATATTA	55	Matsui et al. 2021
CAD	CAD743f	Forward	GGNGTNACNACNGCNTGYTTYGARCC	56	Wahlberg & Wheat 2008
CAD	CAD1028r	Reverse	TTRTTNGGNARYTGNCCNCCCAT	56	Wahlberg & Wheat 2008
MDH	MDHf	Forward	GAYATNGCNCCNATGATGGGNGT	58	Wahlberg & Wheat 2008
MDH	MDHr	Reverse	AGNCCYTCNACDATYTTCCAYTT	58	Wahlberg & Wheat 2008
RpS5	RpS5f	Forward	ATGGCNGARGARAAYTGGAAYGA	62	Wahlberg & Wheat 2008
RpS5	RpS5r	Reverse	CGGTTRGAYTTRGCAACACG	62	Wahlberg & Wheat 2008

For the molecular phylogenetic analysis, we used Crambinae species belonging to all known tribes sequenced by Léger et al. (2019) as outgroups (Table 1). The sequences were manually aligned using MEGA 7.0 (Kumar et al. 2016). Alignments of each gene were concatenated using SeaView 5.0.4 (Gouy et al. 2010). The molecular phylogenetic tree based on maximum likelihood (ML) estimation was constructed using IQ-TREE 2.3.1 (Minh et al. 2020). The concatenated dataset was partitioned for each gene and codon position, and base substitution models

were selected using ModelFinder implemented in IQ-TREE. Branch support was calculated using the standard nonparametric bootstrap (BS) and Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT) with 1,000 replicates each. The resulting tree files were visualized using Figtree v.1.4.3 (Rambaut 2016).

Results

Taxonomy

***Glaucocharis triocellaris* Matsui, Yagi & Hirowatari, sp. nov.**
(Japanese name: nishiki-eguri-tsutoga)
(Figs 1A, 2A, 2B, 3A, 4A, 4C, 5A, 5C, 5E, 6A, 6C, 6D, 7A)

Diagnosis. This new species can be easily distinguished from other *Glaucocharis* species by distinctive morphological characters, such as the three black marginal spots, each enclosing a median silvery-white dot in the forewing; the valva with a sturdy hook-shaped projection emerging from the center of the ventral valva margin and the phallus with a ventrally curved hook-shaped apical thorn in the male genitalia; and the circular spinose lamella antevaginalis in the female genitalia.

Description. Head (Fig. 1A). Frons brown, rounded. Vertex brown, lateral sides ocherous. Maxillary palpus almost as long as compound eye, outer side ocherous basally, pale ocherous apically, inner side white. Labial palpus about 1.6 times longer than compound eye; first palpomere ocherous dorsally, white ventrally; second palpomere with apically expanded scale tuft, ocherous on outer side, white on inner side; third palpomere white basally, brown apically, apex pointed. Maxillary and labial palpi strongly upturned in resting posture (Fig. 7A). Antennae about 3/4 of forewing length, brown, ciliate in male, filiform in female; scape brown dorsally, white ventrally. Proboscis covered with white scales basally.

Thorax and legs. Thorax brown dorsally, white ventrally. Patagium brown, lateral sides ocherous. Tegula ocherous on inner half and whitish silver on outer half. Foreleg femur white; tibia white, dorsal side gray apically; tarsus gray. Midleg femur white; tibia gray dorsally, white ventrally, inner and outer spurs gray, almost of equal length; tarsus gray. Hindleg femur white; tibia gray dorsally, white ventrally, mid and hind spurs gray, almost same length; tarsus gray.

Wings (Fig. 2A, 2B). Forewing length 3.2–4.5 mm (mean 3.9 mm, $n = 10$). Forewing ground color dark brown, tinged with reddish yellow from wing base to medial fascia and apical region; all fasciae and apical marking silvery white with blue luster; basal fascia represented by two oval spots; antemedial and medial fasciae almost straight; postmedial fascia oblique outwardly, ending near termen; submarginal fascia oblique outwardly, disappearing at R_5; apical mark represented by a short stripe vertical to costa; three marginal spots black, comma-shaped, connected, each enclosing silvery-white dot medially; marginal spots surrounded by yellow area with irregular black scaling along inner margin; subapical indentation of termen deep with white cilia; rest of cilia dark brown. Hindwing ground color dark brown, medial area white, with a narrow white band along CuA_2 in male; cilia brown.

Wing venation (Fig. 3A) ($n = 3$). Forewing Sc concurrent with R_1 at 2/3 of length, then diverging as short spur distally; R_2 separate; R_3 stalked with R_4 at 3/5 distance from cell; R_5, M_1, M_2, and M_3 separate, almost parallel; CuA_1 close to M_3 basally; CuA_2 distant from CuA_1; A_{1+2} strong; A_3 weak, not looped; cell opened. Hind wing $Sc+R_1$ stalked with Rs at 4/5 of length; M_1, M_2, M_3, CuA_1, and CuA_2 separate; CuP, A_{1+2} and A_3 well-marked. Female with three frenular bristles.

Abdomen. Dorsal side dark brown, ventral side pale brown, 1st and 2nd sternites tinged white. Tympanal organs (Fig. 4A): bulla tympani small, bean-shaped, anterior margin convex; fornix tympani narrow, recessed under venula prima; pons tympani absent; venula prima developed, extending to about 2/3 from posterior margin of 2nd sternite. Male 8th tergite with a triangular sclerotization on anterior half (Fig. 4C). Male 8th sternite with a long trapezoid sclerotization, posterior margin concaved medially (Fig. 4C). Female eighth sternite wrinkled laterally (Fig. 6A).

Male genitalia (Fig. 5A, 5C, 5E) ($n = 6$). Uncus almost straight, apex pointed. Gnathos beak-shaped, dorsal side sparsely denticulate, apex pointed and curved dorsally. Tegumen long, dorso- and ventrolateral margins forming sclerotized ridges; socii finger-shaped with six petaloid lobes apically. Valva slender, curved ventrally, with sturdy

hook-shaped projection emerging from middle of ventral margin; cucullus slightly enlarging distally, truncated apically, with long setae dorsally; costal margin sclerotized, with thumb-like costal arm at base; sacculus triangular, ventral side setose. Juxta oval, dorsal margin strongly concave medially. Saccus short, rounded. Phallus narrowing posteriorly, distal end pointed and slightly curved ventrally; apical thorn large, hook-shaped, strongly sclerotized, curved ventrally; cornuti absent; ductus ejaculatorius connected at anterior end of phallus.

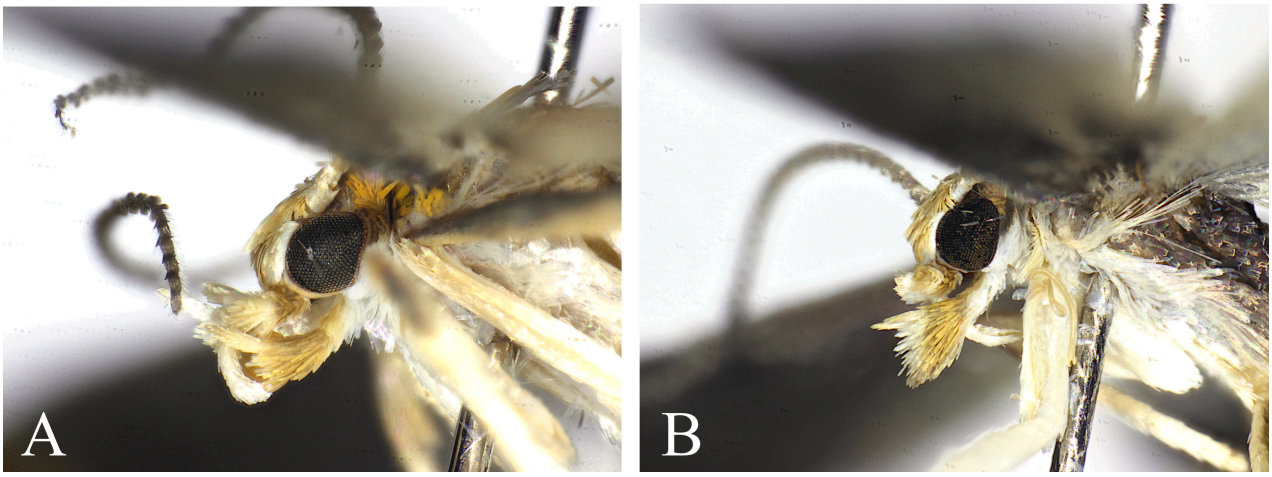

FIGURE 1. *Glaucocharis* spp. head in lateral view. A: *G. triocellaris* male, paratype. B: *G. plumbofascialis* male, holotype.

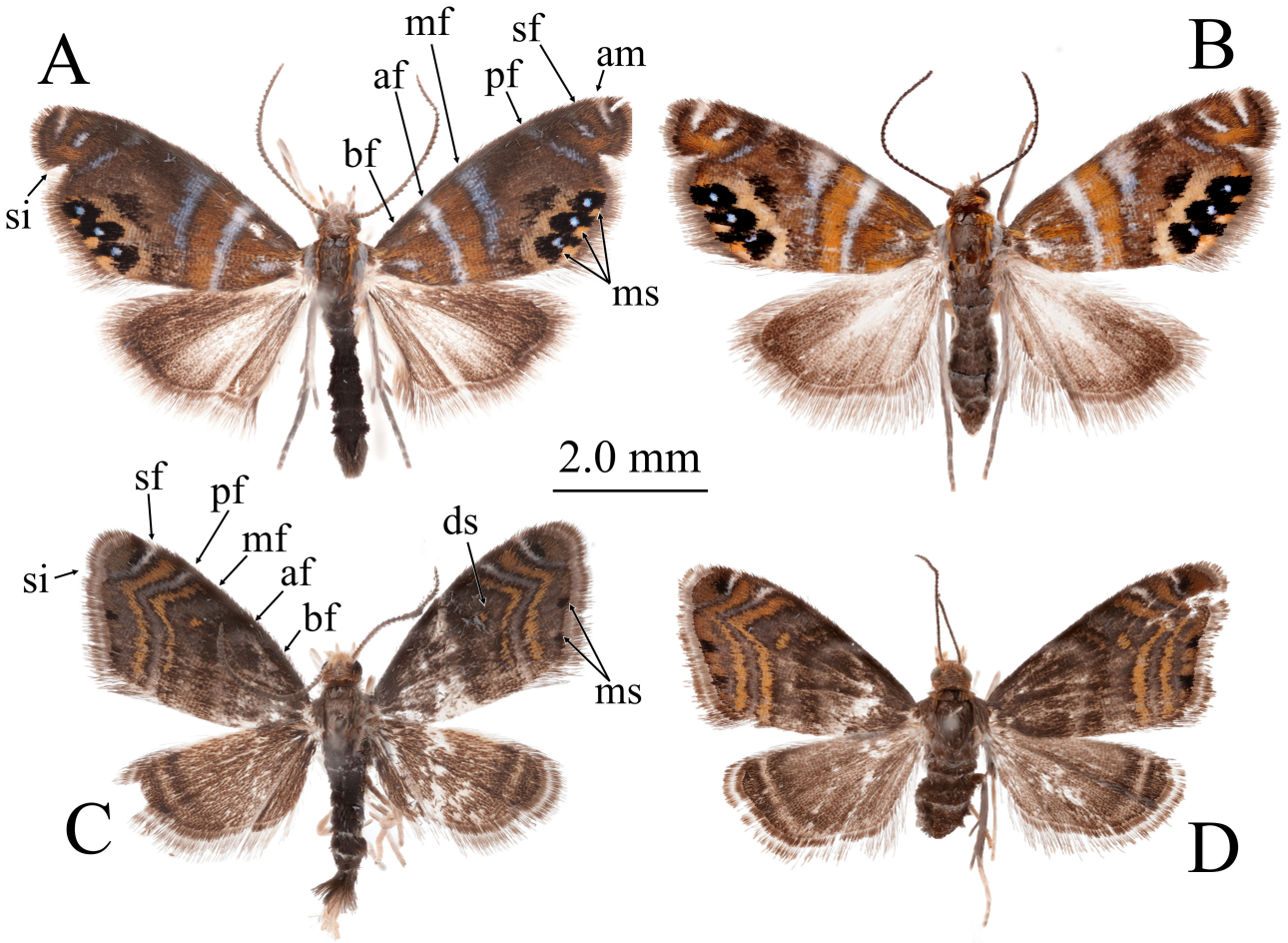

FIGURE 2. *Glaucocharis* spp. adults in dorsal view. A: *G. triocellaris*, holotype male. B: ditto, paratype female. C: *G. plumbofascialis*, holotype male. D: ditto, paratype female. af: antemedial fascia, am: apical mark, bf: basal fascia, mf: medial fascia, ms: marginal spot, pf: postmedial fascia, sf: submarginal fascia, si: subapical indentation.

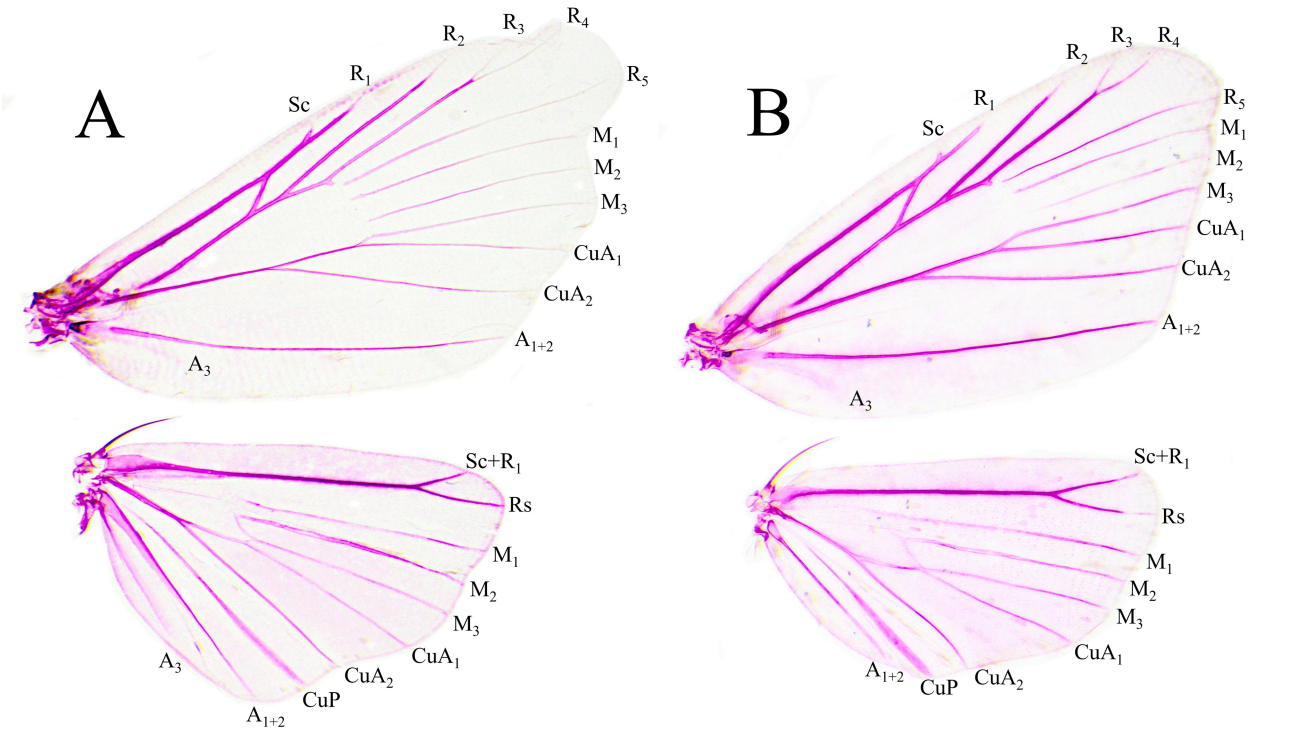

FIGURE 3. *Glaucocharis* spp., male wing venations. A: *G. triocellaris*, paratype (wing slide no. YM-W-10). B: *G. plumbofascialis*, paratype (wing slide no. YM-W-8).

Female genitalia (Fig. 6A, 6C, 6D) (n = 6). Papillae anales ovate, membranous, weakly fused. Anterior and posterior apophyses thin and straight, almost equal in length. Lamella postvaginalis a square plate. Lamella antevaginalis a circular spinose sclerotization, covering ostium. Ductus bursae membranous, almost as long as corpus bursae. Ductus seminalis located at anterior 2/3 of ductus bursae. Corpus bursae membranous, anterior half slightly swollen, posterior half granulate; signa absent.

Type material. Holotype. ♂ [JPN: Tokyo-Pref.] Shigureyama, Chichijima Is., Ogasawara-mura, N27.0629, E142.2214, alt. 252 m, 9.iii.2023, LT, Shunsuke TOMURA leg., gen. slide no. YM-853, deposited in ELKU. **Paratypes. [Chichijima Is.]** 2♂7♀, same locality as holotype, 18.vi.2022, LT, T. Hirowatari et al. leg., gen. slide no. YM-534, Wing slide no. YM-W-10, DNA sample id. M23-037; 1♂, same locality, 26.vi.2022, LT, Shunsuke TOMURA leg.; 1♀, same locality, 13.xi.2022, LT, T. Hirowatari et al. leg.; 12♂1♀, same locality, 9.iii.2023, LT, T. Hirowatari et al. leg., gen. slide no. YM-533, YM-851, wing slide no. YM-W-7; 1♂1♀, same locality, 13.iii.2023, LT, T. Hirowatari et al. leg.; 1♂1♀, Hatsuneura Observatory, 12.iii.2022, SW, T. Hirowatari et al. leg., gen. slide no. YM-735, YM-835; 1♂, Higashidaira, 26.vi.2022, Shunsuke TOMURA leg.; 1♂, same locality, 13.vii.2024, Y. Matsui leg.; 1♀, same locality, 10.iii.2023, SW, T. Hirowatari et al. leg., gen. slide no. YM-737; 1♂, Higashidaira ~ Mt. Hatsune-yama, 13.vi.2023, J.-H. PARK leg.; 1♀, Mt. Mikazuki-yama, 13.vi.2023, SW, J.-H. PARK leg., gen. slide no. YM-855; 1♀, Kitafukurozawa ~ Nishikaigan, 13.iii.2023; T. Hirowatari et al. leg., gen. slide no. YM-856, DNA sample id. M23-180; 1♂, Kuwanokiyama, 14.xi.2022, LT, T. Hirowatari et al. leg.; 1♂, Ogaguwa-no-mori, 12.iii.2023, SW, T. Hirowatari et al. leg.; 1♂, same data except LT. **[Hahajima Is.]** 2♂2♀, Mt. Funaki-yama, 25.ix.2023 (larva), 2-26.xi.2023 em. (host: Bryopsida spp.), J. Hamaguchi leg., gen. slide no. YM-857, YM-836, DNA sample id. M23-181; 1♂, same locality, 14.vii.2024, Y. Matsui leg.; 3♂, Mt. Kuwanoki-yama, 8.xi.2022, LT, T. Hirowatari et al. leg., wing slide no. YM-W-6; 1♂, same locality, 9.xi.2022, T. Hirowatari leg.; 2♂, Tamagawa-dam, 25.ix.2023, light trap, Yuki MATSUI leg., gen. slide no. YM-749, DNA sample id. M23-161; 1♂, Mt. Chôki-yama, 15.vi.2023, LT, Sadahisa YAGI leg.; 1♂, Sakaigatake, 22.vi.2022, SW, S. Yagi leg.; 1♀, same locality and collecting date, Shunsuke TOMURA leg., gen. slide no. YM-858, DNA sample id. M23-182.

Biology (Fig. 7A, 7D–F). Although larvae were not found, the adults emerged from various Bryopsida species collected on Hahajima Island. The larvae made a cocoon using the leaf pieces of Bryopsida spp. (Fig. 7E) and then

pupated therein. The pupal exuviae were not exposed from the cocoon after emergence (Fig. 7F). Adults fly both during the day and night, but most specimens were collected by light trapping at night. The species is presumed multivoltine.

Distribution. Japan: Ogasawara Islands (Chichijima and Hahajima Islands).

Etymology. The name of the new species is derived from the three marginal spots on the forewing.

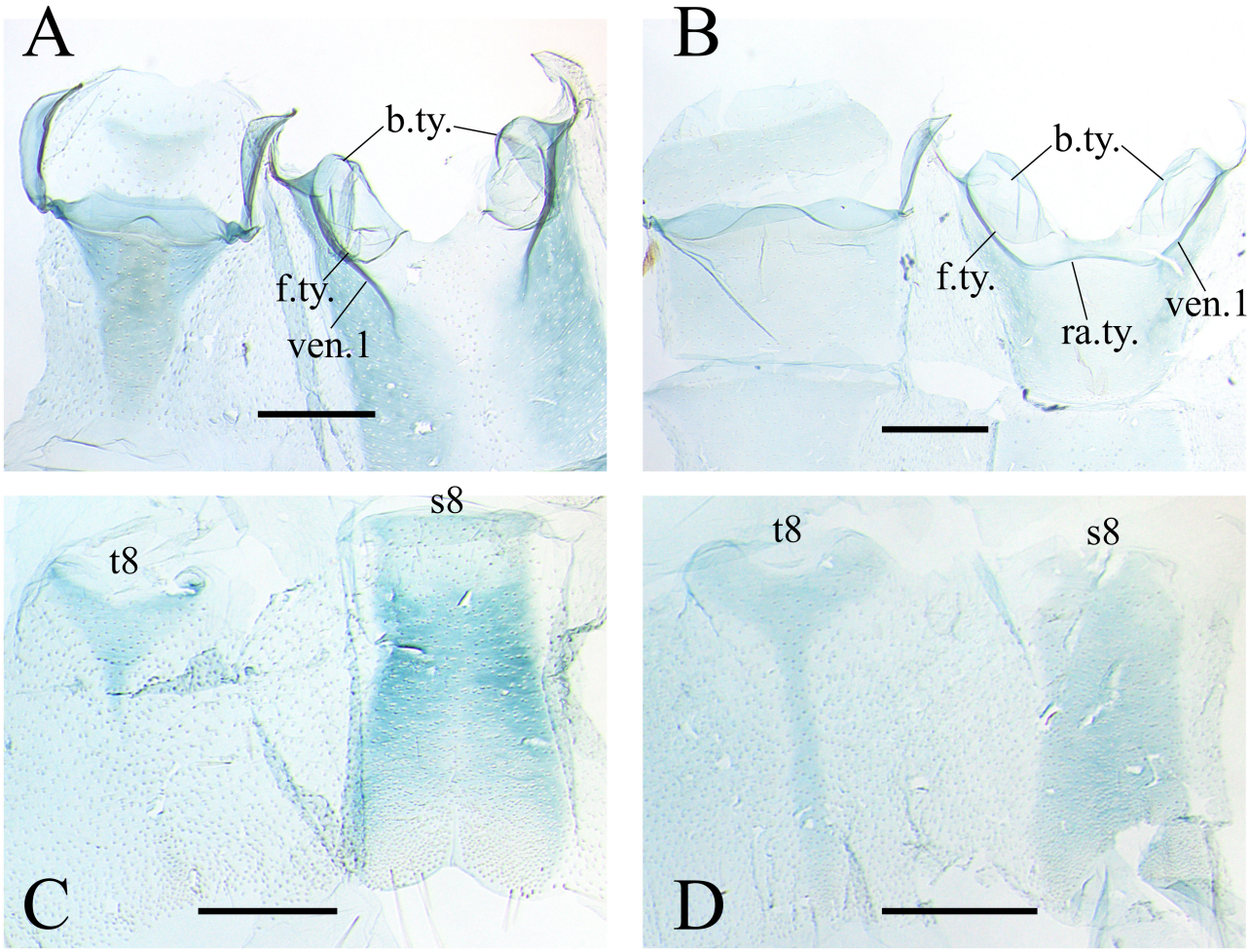

FIGURE 4. *Glaucocharis* spp. abdominal structures. A: *G. triocellaris* tympanal organ (gen. slide no. YM853). B: *G. plumbofascialis*, ditto (gen. slide no. YM837). C: *G. triocellaris* male tergite and sternite 8 (gen. slide no. YM853). D: *G. plumbofascialis*, ditto (gen. slide no. YM837). b.ty.: bulla tympani, f.ty.: fornix tympani, ra.ty.: ramus tympani, s8: sternite 8, t8: tergite 8, ven.1: venula prima. Scale bars = 0.2 mm.

***Glaucocharis plumbofascialis* Matsui, Yagi & Hirowatari, sp. nov.**
(Figs 1B, 2C, 2D, 3B, 4B, 4D, 5B, 5D, 5F, 6B, 6E, 7B, 7C)
(Japanese name: munin-eguri-tsutoga)

Diagnosis. This new species can be easily distinguished from other *Glaucocharis* species by its distinctive morphological characters, such as the plumbeous postmedial and submarginal fasciae sharply angled outwards at M_1 in the forewing; the valva cucullus with a strongly sclerotized prong and the phallus with a sheath-shaped apical thorn in the male genitalia; and the long square plate-like lamellae postvaginalis and antevaginalis in the female genitalia.

Description. Head (Fig. 1B). Frons pale ocherous, anterior margin white. Vertex brown, lateral sides ocherous. Maxillary palpus about 0.7 times longer than compound eye, outer side ocherous basally, pale ocherous apically, inner side white. Labial palpus about 1.6 times longer than compound eye; first palpomere ocherous dorsally, white ventrally; second palpomere with apically expanded scale tuft, ocherous on outer side, white on inner side; third

palpomere pale ocherous, with apex pointed. Maxillary and labial palpi strongly upturned in resting posture (Fig. 7B). Antennae about 3/4 of forewing length, brown, ciliate in male, filiform in female; scape brown dorsally, white ventrally. Proboscis covered with white scales basally.

Thorax and legs. Thorax brown dorsally, white ventrally. Patagium and tegula brown. Foreleg entirely white. Midleg femur white, ventral side basally gray; tibia gray dorsally, cream white ventrally, inner and outer spurs cream white, inner one slightly longer than outer one; tarsus cream white. Hindleg femur cream white, ventral side black at 2/3 from base; tibia gray dorsally except basal 1/3, cream white ventrally, mid and hind spurs gray, respective inner and outer spurs almost same length; tarsus cream white.

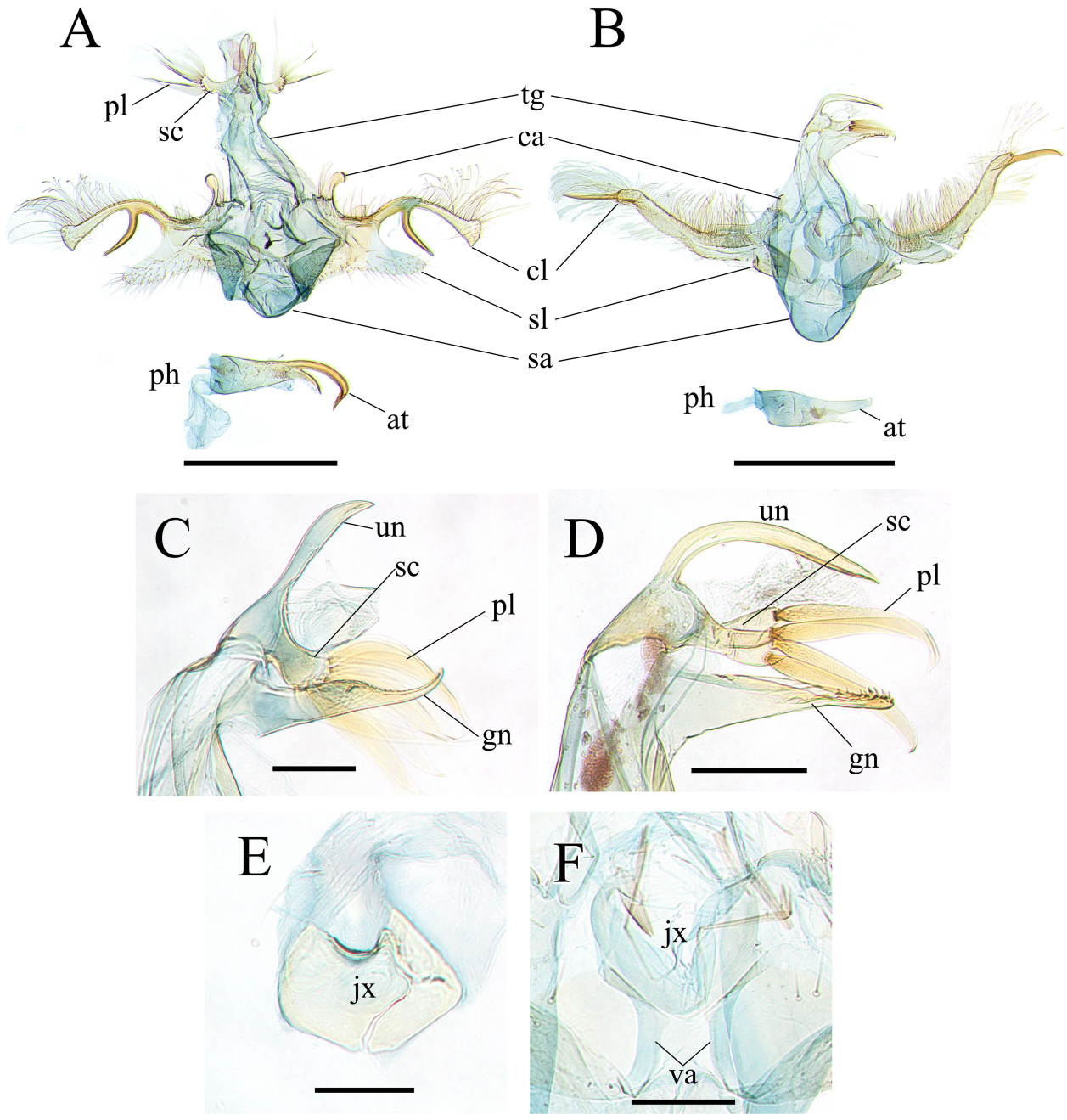

FIGURE 5. *Glaucocharis* spp. male genitalia. A: *G. triocellaris* whole genitalia (gen. slide no. YM853). B: *G. plumbofascialis*, ditto (gen. slide no. YM837). C: *G. triocellaris* uncus, tegumen, and gnathos lateral view (gen. slide no. YM857). D: *G. plumbofascialis*, ditto (gen. slide no. YM738). E: *G. triocellaris* juxta (gen. slide no. YM857). F: *G. plumbofascialis*, ditto (gen. slide no. YM837). at: apical thorn, ca: costal arm, cl: cucullus, gn: gnathos, jx: juxta, ph: phallus, pl: petaloid lobes, sa: saccus, sc: socius, sl: sacculus, tg: tegumen, un: uncus, va: ventral arms of juxta. Scale bars: A, B = 0.5 mm, C–F = 0.1 mm.

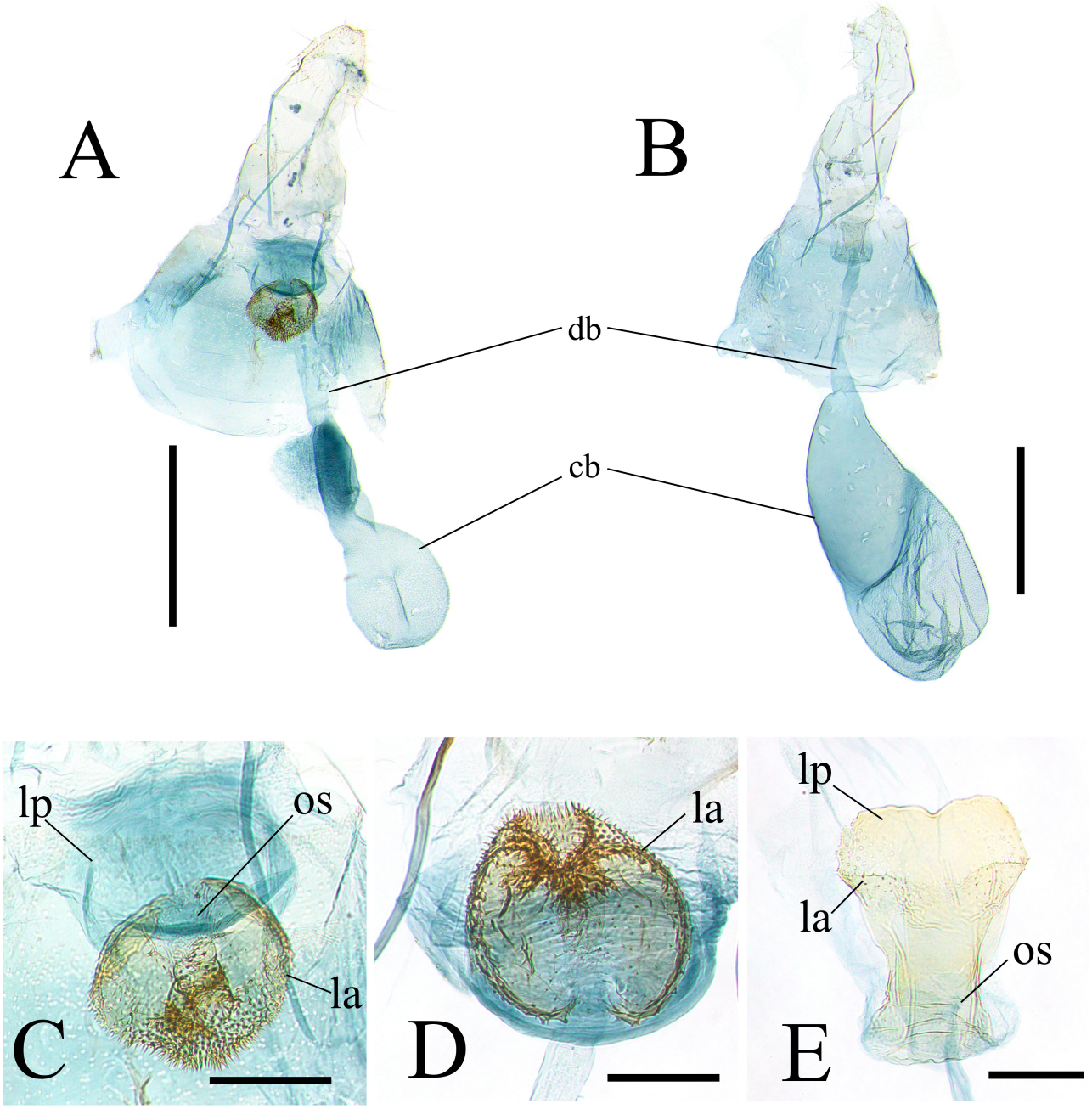

FIGURE 6. *Glaucocharis* spp. female genitalia. A: *G. triocellaris* whole genitalia (gen. slide no. YM836). B: *G. plumbofascialis*, ditto (gen. slide no. YM838). C: *G. triocellaris*, around ostium (lamella antevaginalis opened anteriorly, gen. slide no. YM836). D: ditto, lamella antevaginalis normal position (gen. slide no. YM735). E: *G. plumbofascialis*, ditto (gen. slide no. YM750). cb: corpus bursae, db: ductus bursae, la: lamella antevaginalis, lp: lamella postvaginalis, os: ostium. Scale bars: A, B = 0.5 mm, C–E = 0.1 mm.

Wings (Fig. 2C, 2D). Forewing length 3.1–3.9 mm (mean 3.4 mm, *n* = 10). Forewing ground color dark brown, outer area of medial fascia ocherous; basal, antemedial, medial, postmedial, and submarginal fasciae plumbeous with blue luster, both sides of each fascia margined with black lines; basal, antemedial, and medial fasciae obscure, angled outwards at M_1; postmedial and submarginal fasciae distinct, sharply angled outwards at M_1, whitish near costa; discal spot ocherous, oval, located at inner angle of medial fascia; two marginal spots irregular, black, located at M_3 and CuA_2 on termen; subapical indentation on termen vestigial; cilia plumbeous basally, dark brown distally. Hindwing ground color brown, distally darker, with whitish fascia subapically; in male, basal area covered with black lustered scales, and a whitish gray fascia along CuA_2; cilia as in forewing.

Wing venation (Fig. 3B) (*n* = 4). Almost as in *G. triocellaris*, but hindwing lacking A_3, and M_3 and CuA_1 occasionally stalked basally (as in Fig. 3B).

Abdomen. Dorsally dark brown. Ventrally brown, with posterior margin of each segment white. Tympanal organs (Fig. 4B) as in *G. triocellaris*, but ramus tympani present as horizontal bar connected to venula prima laterally. Male 8th tergite with Y-shaped sclerotization medially (Fig. 4D). Male 8th sternite with long square sclerotization medially, posterior margin slightly concave (Fig. 4D).

Male genitalia (Fig. 5B, 5D, 5F) (*n* = 9). Uncus long, curved ventrally, apex pointed. Gnathos subtriangular, at right angle from tegumen, dorsal side denticulate apically. Tegumen long, dorso- and ventrolateral margins forming sclerotized ridges; socii finger-shaped with two petaloid lobes apically. Valva slender; cucullus with a strongly sclerotized thorn apically; costal margin with dense long setae, with a short finger-like costal arm at base; sacculus short, triangular. Juxta heart-shaped, lateral sides extended as a pair of ventral arms. Saccus rounded. Phallus tapering posteriorly; apical thorn sheath-shaped, apex truncated; cornuti absent; ductus ejaculatorius connected at anterior end of phallus.

Female genitalia (Fig. 6B, 6E) (*n* = 7). Papillae anales, anterior and posterior apophyses as in those of *G. triocellaris*. Lamella postvaginalis a long square plate, posterior margin slightly concave medially. Lamella antevaginalis a shorter square plate than lamella postvaginalis, anterior margin folded postero-internally, then strongly constricted and connected to ductus bursae. Ductus bursae membranous, short and thin. Ductus seminalis located at anterior 2/3 of ductus bursae. Corpus bursae ovate, approximately 2–3 times longer than ductus bursae, posterior half lightly sclerotized with smooth surface, anterior half membranous, with weak granules; signa absent.

Type material. Holotype. ♂ [JPN: Tokyo-Met.] Mt. Funaki-yama, Haha-jima Is., Ogasawara-mura, 25.ix.2023 (larva), 26.xi.2023 em. (host: Bryopsida spp.), J. Hamaguchi leg., gen. slide no. YM-854. **Paratypes. [Chichijima Is.]** 2♀, Asahiyama, 11.vi.2023, T. Hirowatari leg.; 1♀, same locality and collector, 18.vi.2022; 2♂, Asahiyama-tenbodai, Sakaiura, 26.vi.2022, SW, S. Yagi leg., gen. slide no. YM-535, YM-789, wing slide no. YM-W-8; 1♂, Higashidaira, 18.vi.2022, Shunsuke TOMURA leg., gen. slide no. YM-861, DNA sample id. M23-185; 1♀, same locality, 26.vi.2022, T. Hirowatari leg.; 1♂, Higashi-machi, 11.iii.2023, T. Hirowatari et al. leg.; 1♂, Kuwanokiyama, 19.vi.2022, LT, T. Hirowatari et al. leg.; 3♀, same locality, 23.vi.2022, M. Kimura leg.; 1♂, Mikazukiyama, 13.vi.2023, T. Hirowatari leg., gen. slide no. YM-792; 1♀, Mt. Chûô-san, 19.vi.2023, SW, J.-H. Park leg.; 1♀, Ogamiyama, 25.vi.2022, LT, Shunsuke TOMURA leg., gen. slide no. YM-537; 1♂, same locality, 11.vii.2024, Y. Matsui leg.; 1♂, Mt. Akahata-yama; 11.vi.2023, light trap, Yuki MATSUI leg., gen. slide no. YM-738; 1♂1♀, Shigureyama, 18.vi.2022, LT, Shunsuke TOMURA leg., gen. slide no. YM-862, DNA sample id. M23-186; 1♀, Yoakeyama, 12.iii.2023, T. Hirowatari leg., gen. slide no. YM-736. **[Anijima Is.]** 1♀, Takinoura, 20.vi.2022, beating: dead leaf *Livistona chinensis* var. *boninensis*, Shunsuke TOMURA leg., gen. slide no. YM-750, DNA sample id. M23-162. **[Otôtojima Is.]** 5♂, 12-13.vii.2024, SW, S. Yagi leg. **[Mukohjima Is.]** 1♂, 16.vii.2024, Y. Matsui leg. **[Hahajima Is.]** 1♂, same locality as holotype, 24.vi.2022, Shunsuke TOMURA leg.; 1♀, same locality, 17.iii.2023, LT, T. Hirowatari et al. leg., gen. slide no. YM-859, DNA sample id. M23-183; 2♂, same locality, 14.vii.2024, Y. Matsui leg.; 2♂1♀, Choukiyama, 15.vi.2023, LT, T. Hirowatari et al. leg., gen. slide no. YM-837; 1♂, Mt. Chibusa-yama, 16.vi.2023 (larva), 11.vii.2023 em. (host: Bryopsida spp.), J. Hamaguchi leg.; ♀1, same locality and collecting date, LT, Sadahisa YAGI leg.; 1♀, Mt. Kuwanoki-yama, 8.xi.2022, LT, T. Hirowatari et al. leg.; 1♀, Mt. Sakaigatake, 16.iii.2023, Toshiya Hirowatari leg.; 1♂2♀, same locality, 17.vi.2023, LT, J.-H. Park & I. KAWASHIMA leg., DNA sample id. M23-024; 2♀, same locality, 24.ix.2023 (larva), 8.xi.2023 em. (host: Bryopsida spp.), J. Hamaguchi leg., gen. slide no. YM-838; 1♀, Nishiura, 15.iii.2023, LT, T. Hirowatari et al. leg.; 2♂, Higashikô, 24.ix.2023, flower of *Bidens pilosa*, Masaaki Kimura leg., gen. slide no. YM-791, YM-790; 1♂, Shinyûhigaoka, 21.ix.2023 light trap; Yuki MATSUI leg., gen. slide no. YM-860, DNA sample id. M23-184.

Biology (Fig. 7B–F). Similar to *G. triocellaris*. We observed that some adults visited the flowers of *Bidens pilosa* var. *radiata* (Sch. Bip.) J.A. Schmidtthe during daytime. We also observed many adults swarming around *Euonymus boninensis* Koidz. at twilight: all individuals were males as far as we could confirm.

Distribution. Japan: Ogasawara Islands (Chichijima, Anijima, Otôtojima, Mukohjima, and Hahajima Islands).

Etymology. The name of the new species is derived from the plumbeous (dull-gray colored) fasciae of the forewing.

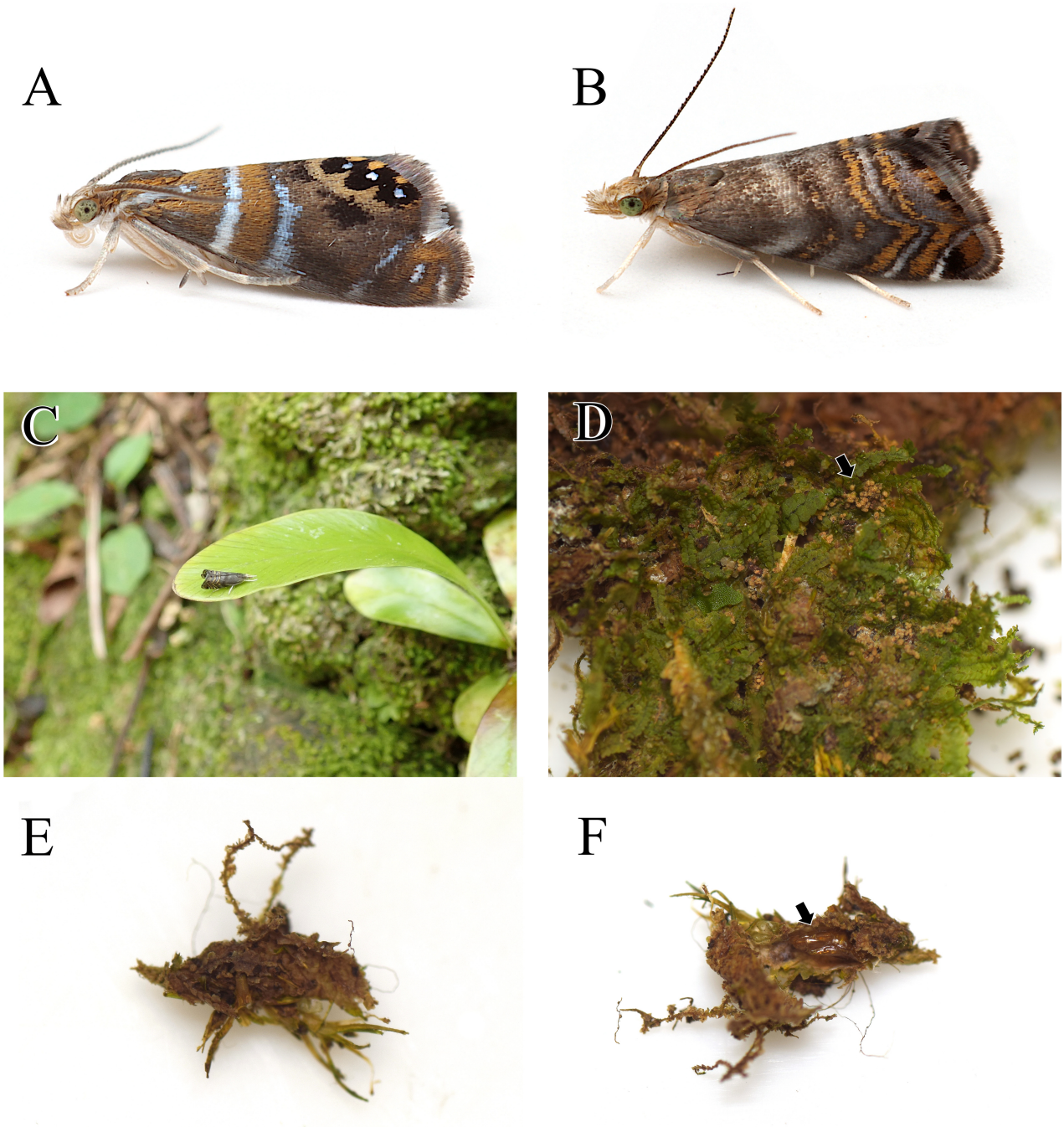

FIGURE 7. Biology of *Glaucocharis* spp. A: *G. triocellaris* resting posture. B: *G. plumbofascialis*, ditto. C: ditto, resting posture on the leaf. D: Bryopsida spp. collected on Mt. Funakiyama, Hahajima Island, where the adults of *G. triocellaris* and *G. plumbofascialis* emerged (the arrow indicates the frass of them). E: a cocoon of *G. triocellaris* or *G. plumbofascialis.* E: ditto, opened view with pupal exuviae (arrow).

Molecular phylogeny

The final sequence dataset contained 1,518 bp of COI, 792 bp of CAD, 732 bp of MDH, and 558 bp of RpS5 (3,600 bp in total). The ML tree (Fig. 8) recovered the monophyly of *Glaucocharis* with moderate support (SH-aLRT = 87, BS = 56). The two new species, *G. triocellaris* and *G. plumbofascialis* were recovered as sister species with maximum support (SH-aLRT and BS = 100), and they were sisters to *G. mutuurella* (Błeszyński) without support (SH-aLRT = 27.9, BS = 33).

The monophyly of Diptychophorini was strongly supported (SH-aLRT = 96, BS = 97), and intra-tribal relationships were congruent to the results of Léger et al. (2019): *Gargela* is the basalmost linage, and *Diptychophora* is sister to *Glaucocharis* + *Microcausta*. *Microchilo*, placed by Gaskin (1971) in the Diptychophorini, was excluded from this tribe by Léger et al. (2019); it is recovered as sister to Diptychophorini in our study but without good support (SH-aLRT = 79, BS = 40).

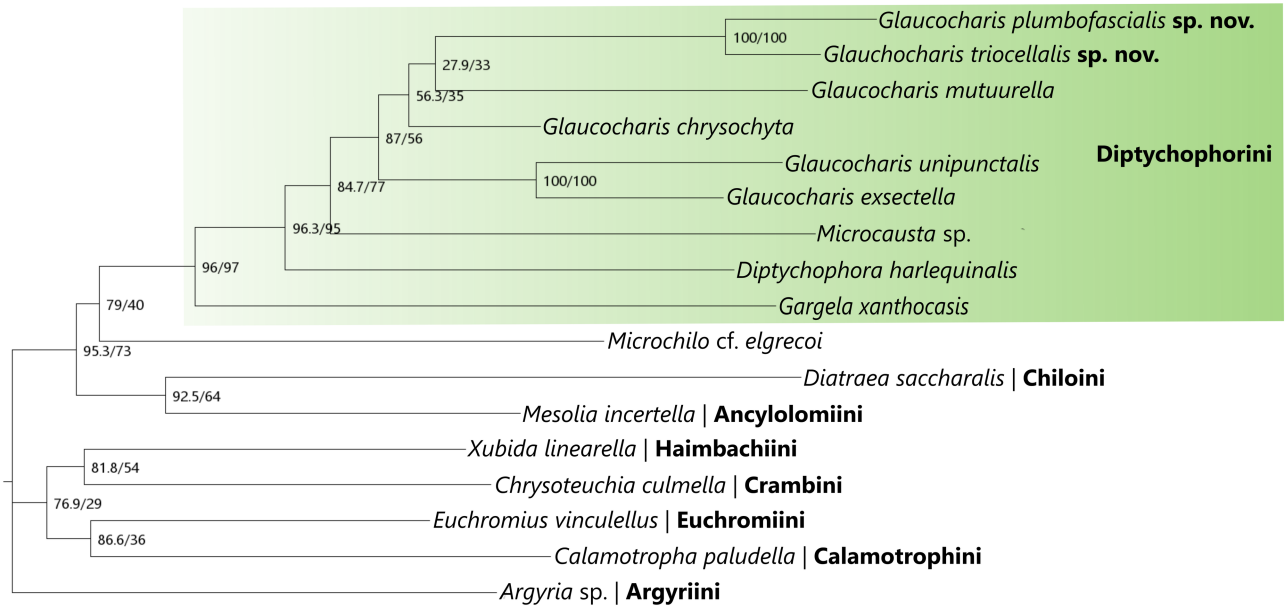

FIGURE 8. Maximum likelihood (ML) phylogenetic tree of *Glaucocharis* and related Crambinae species. The number on each node is the Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT) / standard nonparametric bootstrap (BS).

Discussion

Inoue (1996) conducted a comprehensive taxonomic study of the Pyraloidea on the Ogasawara Islands, but he recorded no *Glaucocharis* species. Because the two new species described here, *G. triocellaris* and *G. plumbofascialis* are commonly found on these islands, they may have been overlooked due to their small size.

Glaucocharis triocellaris and *G. plumbofascialis* were recovered as sister species in our molecular phylogenetic analysis (Fig. 8), despite their remarkable differences in wing maculation and the shapes of both the male and female genitalia. Careful evaluation of both species' morphologies suggested that they share striking male genital characteristics: the socius with two or six petaloid lobes, the valva with a finger-shaped costal arm, and the posteriorly tapered phallus with a developed apical thorn (Fig. 5). To the best of our knowledge, these characteristics are not found in other *Glaucocharis* species; therefore, we initially questioned whether these two species truly belong to *Glaucocharis*. However, our molecular evidence and the wing venation confidently placed these two species in *Glaucocharis*. In particular, the wing venation is likely a reliable character for diagnosing the genus, although it can vary even within species in other crambid taxa (e.g. Maes 1995).

The larvae of *Glaucocharis* species are known to be moss (Bryopsida) feeders (Gaskin 1971; Yoshiyasu 2011; Sasaki 2013), although the records are sparse. *Glaucocharis triocellaris* and *G. plumbofascialis*, which emerged from various Bryopsida species collected on Hahajima Island in this study, stress the possibility that the moss-feeding habits are common in this genus. Interestingly, these two species emerged from the same piece of Bryopsida spp. collected from Mt. Funakiyama on Hahajima Island (Fig. 7D). They may have speciated due to factors other than host shifts or geographic isolation on the Ogasawara Islands, or speciated in geographic isolation but later became sympatric.

The components of the forewing maculation found in *Glaucocharis triocellaris* including the three marginal spots and the silvery-white fasciae are reminiscent of jumping spiders (Fig. 7A). Such jumping spider-like appearance is convergently acquired in various insect taxa (e.g. Rota & Wagner 2006, and references therein). Few studies demonstrated the effectiveness of the jumping spider mimicry, but certain tephritid flies and metalmark moths that show jumping spider-like appearance have a lower predation rate from jumping spiders than sibling non-mimetic

ones (Greene *et al.* 1987; Mather & Roitberg 1987; Whitman *et al.* 1988; Rota & Wagner 2006; Wang *et al.* 2017). In the Ogasawara Islands, 10 species of jumping spiders have been recorded (Ono 2011; Suguro & Nagano 2015) and so members of this spider family might have driven the evolution of the jumping spider-like maculation in *G. triocellaris*. It is also interesting that the sympatric sister species (*G. plumbofascialis*) do not have such maculation. The two new *Glaucocharis* species would be fascinating material to study the evolution of the jumping spider mimicry.

Endemic organisms in the Ogasawara Islands originated from various geographic areas, such as mainland Japan, Southeast Asia, and Micronesia (e.g. Ito 1998). In the present study, the two species endemic to the Ogasawara Islands were recovered as sisters to *G. mutuurella* (distributed in mainland Japan and China; Sasaki 2013) but the relationship was poorly supported (Fig. 8). We constructed a preliminary barcode tree, including sequence data from the BOLD (https://www.boldsystems.org/) database, to further infer the origin of the two species; however, most nodes lacked significant support (data not shown). The origin of *G. triocellaris* and *G. plumbofascialis* is still unknown, and further phylogenetic analyses using additional species and more gene regions are needed to investigate this.

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