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A New Species of *Dicharax* (Caenogastropoda: Cyclophoridae: Alycaeinae) from Amami-Oshima Island, Japan

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Abstract: *Dicharax amamiensis* n. sp. is described based on specimens from Amami-Oshima Island, Japan. The new species has hitherto been identified in the Japanese literature as *Dicharax purus* (as *Chamalycaeus purus*), which was originally described from Tokunoshima Island. However, a molecular phylogenetic analysis using mitochondrial cytochrome oxidase subunit I gene and 16S rRNA region sequences of 10 species of Alycaeinae, as well as those of *D. amamiensis* n. sp., revealed that *D. amamiensis* n. sp. is distinct and separated from the congener *D. purus*. On the basis of morphological observations, *D. amamiensis* n. sp. differs from *D. purus* in possessing a relatively taller shell, a thick, slightly reflexed peristome, a proportionally broader penultimate whorl, and a narrower umbilicus.

Keywords: Alycaeinae, Amami-Oshima, *Dicharax*, new species, taxonomy

Introduction

The subfamily Alycaeinae Blanford, 1864 is a group of terrestrial gastropods generally characterized by a sutural tube on the last whorl (Kobelt, 1902). Although this group was sometimes regarded as an independent family (*e.g.*, Páll-Gergely *et al.*, 2020), recent molecular phylogenetic data showed that it should be treated as a subfamily within Cyclophoridae (Páll-Gergely *et al.*, 2024). Several genera of Alycaeinae (sometimes as Alycaeidae) have recently been revised based on shell morphology (*e.g.*, Páll-Gergely *et al.*, 2017, 2020), and grouped into eight genera (MolluscaBase, 2025). In Japan, 35 taxa of the genus *Dicharax* Kobelt & Möllendorff, 1900 and eight taxa of the genus *Metalycaeus* Pilsbry, 1900 are recognized (Minato, 1988, 2005, 2012; Minato & Yano, 1988, 2000; Yano *et al.*, 2013, 2016; Páll-Gergely & Asami, 2017; Matsuda & Yano, 2024). Some of them are in problematic taxonomic positions (Matsuda & Yano, 2024), and many are yet to be formally described.

Dicharax purus (Pilsbry & Hirase, 1904) is a minute alycaeid species that was originally described as *Alycaeus purus* by Pilsbry & Hirase (1904) from “Tokunoshima, Osumi” (Tokunoshima Island, Nansei Islands, Japan). The species was subsequently reported from Amami-Oshima Island (Kuroda, 1928), although neither a description nor images were given. Since then, *D. purus* (as *Chamalycaeus purus*) has been described as being distributed in the Amami Islands (Kuroda, 1953, 1956, 1963) or on both Tokunoshima Island and Amami-Oshima Island (Yukita, 2003; Minato, 2011). *Dicharax purus* is also designated as an endangered species occurring in both Tokunoshima Island and Amami-Oshima Island in the Red Data Book of Kagoshima Prefecture (Tomiyama & Nishi, 2016) and the Japanese Red Data Book (Minato, 2014).

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To date, no detailed comparisons have been conducted between *D. purus* from Tokunoshima Island and the specimen identified as *D. purus* from Amami-Oshima Island. On the basis of an examination of shell morphology and mitochondrial phylogeny, we concluded that the specimens from Amami-Oshima Island can be distinguished from *D. purus*.

Materials and Methods

Specimens of the new species were collected from Amami-Oshima Island, Kagoshima Prefecture, from October 1, 2016 to November 21, 2018 (Table 1, Fig. 1). *Dicharax purus* were obtained from Tokunoshima Island, Kagoshima Prefecture, from December 23, 2017 to November 23, 2018 (Table 1). Soft parts of live specimens of both species were separated from shells after boiling, following Fukuda *et al*. (2008), and were placed in pure ethanol until DNA extraction. In addition, 16 samples representing six species and subspecies of *Dicharax*, namely *D. tokunoshimanus* (Pilsbry & Hirase, 1904), *D. tokunoshimanus mediocris* (Pilsbry & Hirase, 1909), *D. tokunoshimanus principialis* (Pilsbry & Hirase, 1909), *D. kurozumitaijii* Matsuda & Yano, 2024, *D. oshimanus* (Pilsbry & Hirase, 1904), *D. tanegashimae* (Pilsbry, 1902) and three species of *Metalycaeus*, namely *M. minatoi* Páll-Gergely, 2017, *M. laevis* (Pilsbry & Hirase, 1909), and *M. vinctus* (Pilsbry, 1902), all from the Nansei Islands, were also used for DNA extraction. Locality information, collecting date, and name of the collector for each sample are given in Table 1.

Shells and opercula were observed under an opto-digital microscope (Olympus DSX–100, Tokyo, Japan). The radula and cross-sectional views of the axial ribs along the sutural tube, which is an important structure for classifying species and species groups of Alycaeinae (as Alycaeidae) (Páll-Gergely *et al*., 2016, 2020), were observed with a scanning electron microscope (SEM) (Miniscope TM3030, Hitachi, Tokyo, Japan) without coating. Measurements of the shell were made from digital images taken with an opto-digital microscope, with the aid of Adobe Photoshop CS5 software. The following shell dimensions were measured: shell length (SL), shell width (SW), body whorl length (BL), penultimate whorl width (TW), spire length (SL2), and length of the sutural tube (STL) (Fig. 2). The number of axial ribs along the sutural tube (No. AR along tube) was counted. The diameter, width, and papilla length of the operculum (OD, OW, and PL) were also recorded, but one specimen with a retracted operculum was not included in the operculum measurement data. For *D. purus*, digital images of its lectotype (catalog number 87683) were obtained from the website of the Malacology Collection at the Academy of Natural Sciences of Philadelphia (ANSP), and newly collected specimens were measured for the above shell dimensions.

Type and voucher materials have been deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan.

Molecular phylogeny

Genomic DNA was extracted from the foot tissue or the entire animal using a NucleoSpin Tissue kit (Takara Bio, Shiga, Japan) or the phenol-chloroform extraction method. The DNA extractions were further cleaned with Glassmilk (Qbiogene, Carlsbad, CA, USA).

For phylogenetic analysis, fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene and 16S rRNA region (16S) were sequenced with a standard polymerase chain reaction (PCR) technique using primers LCO1490 and HCO2198 (Folmer *et al*., 1994) for COI and 16Scs1 (Chiba, 1999) and newly designed primer (5′-TTTAATCCAACATCGA-3′) for 16S. The PCR was prepared with a total volume of 10 μL, containing 1 μL of template DNA (10–20 ng/μL), 2.8 μL of dH_2O, 5 μL of 2 × PCR buffer, 0.2 μL of Tks Gflex DNA Polymerase (Takara Bio, Shiga, Japan), and 0.5 μL of each primer (10 pM/μL). The PCR reactions were performed with the following thermal cycling conditions for COI: an initial denaturation step at 94°C for 1 min, followed by 35

Table 1. List of species and subspecies, including collecting localities, locality codes, collecting dates, collectors, voucher ID, and GenBank accession numbers, used for the molecular phylogenetic analysis.

Species	Collecting locality	Locality code	Collecting date	Collector	Voucher ID	GenBank accession No.	
						COI	16S
Dicharax amamiensis n. sp.	Hirata-cho, Naze, Amami, Amami-Oshima Island	a	November 21, 2018	H. Matsuda	NSMT-Mo 79591	LC850278	LC850632
	South of Nagakumo-toge, Tatsugo-cho, Amami-Oshima Island	b	October 1, 2016	S. Yano	NSMT-Mo 79594	LC850281	LC850635
	South of Nagakumo-toge, Tatsugo-cho, Amami-Oshima Island	b	October 1, 2016	S. Yano	NSMT-Mo 79595	LC850285	LC850637
	En, Tatsugo-cho, Amami-Oshima Island	c	October 1, 2016	S. Yano	NSMT-Mo 79596	LC850287	LC850639
	Daimyo Forest Road, Yamato-son, Amami-Oshima Island	d	October 2, 2016	S. Yano	NSMT-Mo 79598	LC850288	LC850640
	Ogamiyama Park, Nazenagata-cho, Amami, Amami-Oshima Island	e	October 4, 2016	S. Yano	NSMT-Mo 79600	LC850289	LC850641
D. purus	East of Mt. Uki-dake, Amagi-cho, Tokunoshima Island	f	December 24, 2017	S. Yano	NSMT-Mo 79603	LC850282	LC850642
	Mt. Amagi-dake to Mt. Sasontsuji-dake, Tokunoshima Island	g	December 23, 2017	S. Yano	NSMT-Mo 79604	LC850283	LC850643
	North of Mt. Sasontsuji-dake, Tokunoshima-cho, Tokunoshima Island	h	November 23, 2018	S. Yano	NSMT-Mo 79605	LC850284	LC850636
	North of Mt. Sasontsuji-dake, Tokunoshima-cho, Tokunoshima Island	h	November 23, 2018	H. Kawano	NSMT-Mo 79602		
D. tokunoshimanus	Kenpuku, Isen, Tokunoshima Island	i	November 23, 2018	H. Matsuda	M-TO3	LC850270	LC850625
	North of Mt. Sasontsuji-dake, Tokunoshima-cho, Tokunoshima Island	h	November 23, 2018	H. Kawano	K-TO3	LC850271	LC850624
	North of Mt. Uki-dake, Amagi-cho, Tokunoshima Island	f	December 22, 2017	S. Yano	Y-TO4	LC850272	LC850626
D. tokunoshimanus mediocris	Ikeji, Uke Island	j	January 30, 2017	H. Minato	M-YO1	LC850266	LC850622
	Ikeji, Uke Island	j	January 30, 2017	H. Minato	M-YO2	LC850269	LC850623
D. tokunoshimanus principialis	Akina, Tatsugo-cho, Amami-Oshima Island	k	October 1, 2016	S. Yano	Y-OO1	LC850273	LC850627
	Hirata-cho, Naze, Amami, Amami-Oshima Island	a	November 21, 2018	H. Matsuda	M-OO1	LC850274	LC850628
D. kurozumitaijii	Uehara Cave, Uehara, Taketomi, Iriomote Island	l	March 26, 2017	Y. Nakahara	NSMT-Mo 79505	LC850275	LC850633
	Komi, Taketomi, Iriomote Island	m	March 4, 2015	Y. Nakahara	NSMT-Mo 79503	LC850276	LC850634
D. oshimanus	Nishinakama, Sumiyou, Amami, Amami-Oshima Island	n	October 3, 2016	S. Yano	Y-AM4	LC850280	LC850629
	North of Adachi, Setouchi-cho, Kakeroma Island	o	March 23, 2022	O. Masuda	M-OO7	LC850279	LC850630
D. tanegashimae	Kumano, Nakatane-cho, Tanegashima Island	p	December 14, 2015	Y. Nakahara	N-TA2	LC850268	LC850620
	Kumano, Nakatane-cho, Tanegashima Island	p	December 14, 2015	Y. Nakahara	N-TA3	LC850267	LC850621
Metalycaeus minatoi	Matsubara, Minamitane-cho, Tanegashima Island	q	December 14, 2015	B. Páll-Gergely	B-MI1	LC850265	LC850617
M. laevis	Kamimura, Toshima-mura, Akuseki Island, Tokara Islands	r	March 5, 2019	T. Miyai	M-BE1	LC850264	LC850618
M. vinctus	Iseki, Nishinoomote, Tanegashima Island	s	December 15, 2015	B. Páll-Gergely	B-KU1	LC850263	LC850619

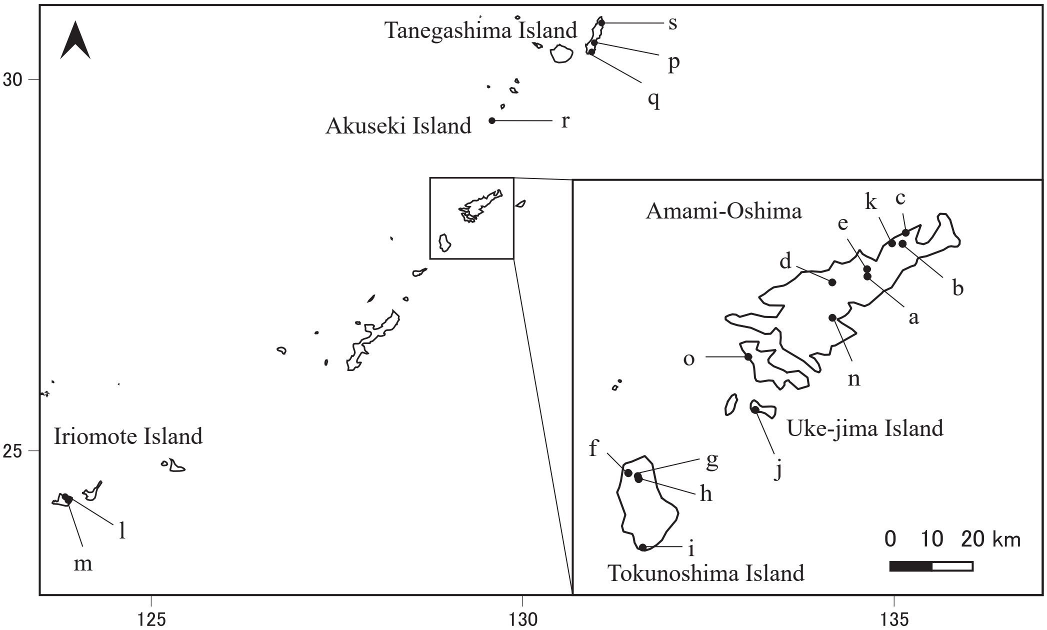

Fig. 1. Map showing the collecting localities of *Dicharax* and *Metalycaeus* species. See Table 1 for the details of collecting localities.

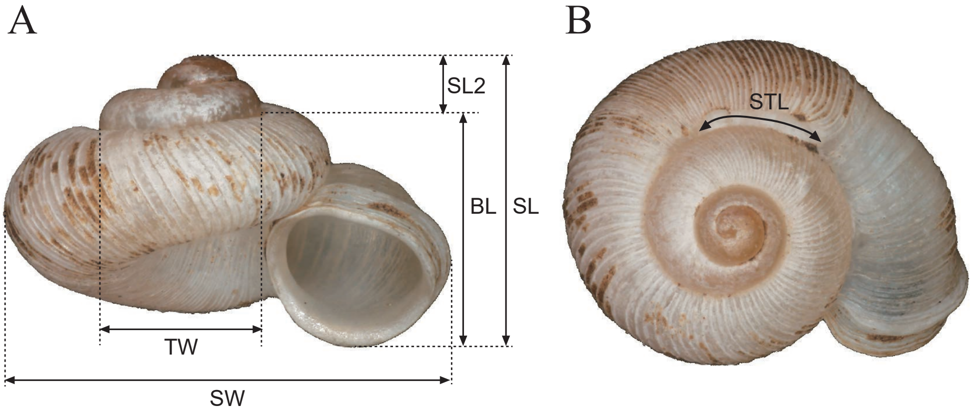

Fig. 2. Shell dimensions measured in this study. **A.** Apertural view of shell. **B.** Apical view of shell.

cycles at 98°C for 10 s, 43–44°C for 20 s, and 68°C for 45 s, with a final extension at 72°C for 10 min. For 16S, reactions were cycled at 94°C for 1 min, followed by 30 cycles at 98°C for 10 s, 50°C for 20 s, and 68°C for 45 s, with a final extension at 72°C for 10 min. The PCR products were purified using ExoSAP-IT (ThermoFisher Scientific, Waltham, MA, USA) and sequenced using the FASMAC sequencing service (FASMAC, Kanagawa, Japan) or the Eurofins sequencing service (Eurofins Genomics K.K., Tokyo, Japan). All the sequences obtained in this study have been deposited in the GenBank database under the accession number shown in Table 1.

The sequencing results were visually checked using Chromas v2.6.6 software (Technelysium Pty Ltd., South Brisbane, Australia). The 16S sequences were aligned using the online version of MAFFT v.7 (Katoh *et al.*, 2019) with default settings, and ambiguous sites were removed with the online version of Gblocks ver. 0.91b (Castresana, 2002) with the options “Allow smaller final blocks”, “Allow gap positions within the final blocks”, and “Allow less strict flanking positions”; alignments of COI sequences lacked indels. The two sequences consisting of COI and 16S were combined and analyzed via maximum likelihood (ML) using MEGA 11 (Tamura *et al*., 2021). The Hasegawa–Kishino–Yano model with gamma distributed rates and invariant sites (HKY+G+I) was selected for the best-fit model (Hasegawa *et al*., 1985). Statistical support for each node was evaluated using bootstrapping with 1,000 replications. Three *Metalycaeus* species were selected as the outgroup. Pairwise genetic distances between sequences were calculated using MEGA 11.

Results

Molecular phylogeny

In the present study, a total of 25 sequences of COI and 16S from 11 taxa, namely 6 sequences from *D. amamiensis* n. sp., 3 sequences from *D. purus*, 3 sequences from *D. tokunoshimanus*, 2 sequences from *D. tokunoshimanus mediocris*, *D. tokunoshimanus principialis*, *D. kurozumitaijii*, *D. oshimanus*, *D. tanegashimae*, and 1 sequence from each of the three outgroup species, *Metalycaeus minatoi*, *M. laevis*, and *M. vinctus*, were used. The final sequences had lengths of 614 bp (COI) and 841 bp (16S).

Phylogenetic analyses showed that all nominal and candidate species were clustered together, with *Dicharax* species divided into two clades (namely clade A and clade B) (Fig. 3). In clade A, *D. tokunoshimanus* and its subspecies, *D. tokunoshimanus principialis* and *D. tokunoshimanus mediocris*, were segregated with strong support (= subclade a) (97% bootstrap support [BS]). *D. tanegashimae* appeared as a sister group to subclade a.

In clade B, *D. amamiensis* n. sp. was confirmed to be monophyletic (100% BS). The genetic distances of the COI between the specimens of *D. amamiensis* n. sp. were 0.01–0.05. *Dicharax amamiensis* n. sp. clustered together with *D. kurozumitaijii*, *D. oshimanus*, and *D. purus* with low statistical support (66% BS). *Dicharax purus* and *D. amamiensis* n. sp. were genetically closely related. The genetic similarities of the COI were rather low (0.02–0.12) among *D. purus*, and the specimen from north of Mt. Sasontsuji-dake exhibited a distance of 0.12 from the specimens from other two localities (east of Mt. Uki-dake and the region between Mt. Amagi-dake to Mt. Sasontsuji-dake). However, no apparent correlation was found with the distance between sampling locations. Clade A was composed of *Dicharax* spp. from Amami-Oshima Island, Uke Island, Tokunoshima Island, and Tanegashima Island, and clade B was composed of *Dicharax* spp. from Amami-Oshima Island, Tokunoshima Island, and Iriomote Island.

Systematic Description

Family **Cyclophoridae** Gray, 1847
Subfamily **Alycaeinae** Blanford, 1864

Genus ***Dicharax*** Kobelt & Möllendorff, 1900

***Dicharax amamiensis* n. sp.**
(Figs 4, 5)
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Type material: Holotype, NSMT-Mo 79591 (Fig. 4A–G), November 21, 2018, Hirata-cho, Naze, Amami, Amami-Oshima Island, Kagoshima Prefecture (type locality), *leg*. H. Matsuda; paratype #1, NSMT-Mo 79592, collection site and date same as for holotype, *leg*. H. Kawano;

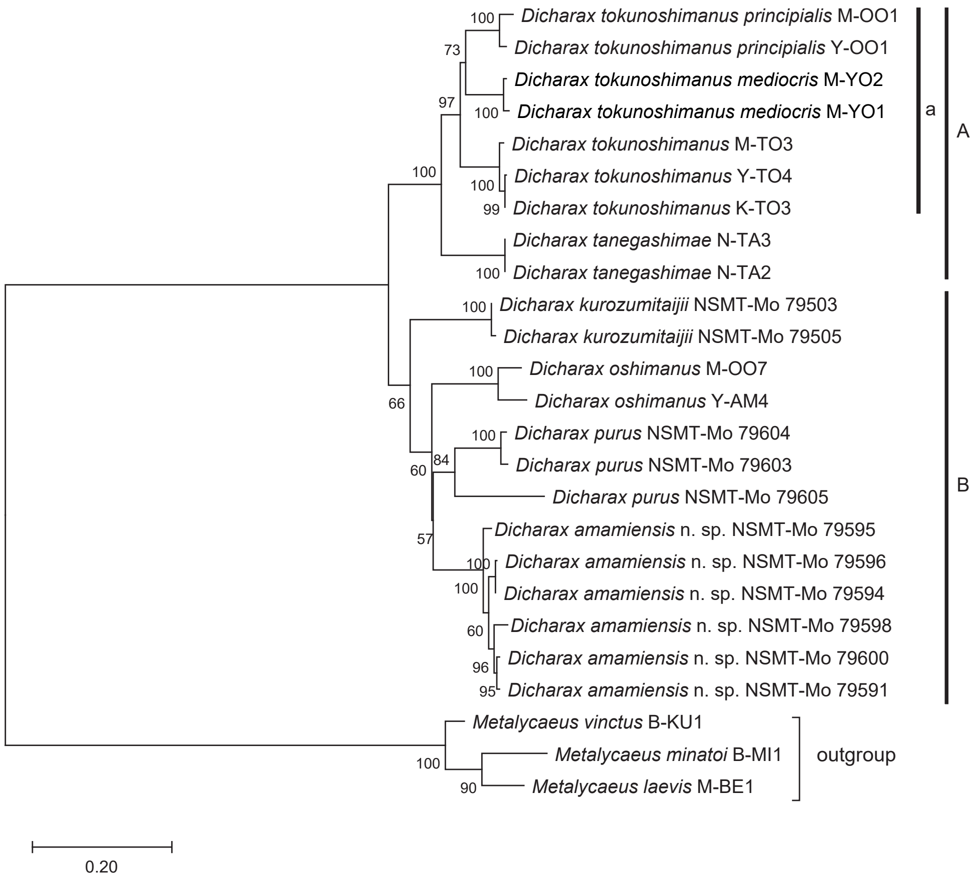

Fig. 3. Phylogenetic trees of *Dicharax* species using *Metalycaeus* species as the outgroup, based on the mitochondrial cytochrome subunit I gene (COI) and 16S rRNA (16S).

paratype #2, NSMT-Mo 79593, collection site and date same as for holotype, *leg*. S. Yano; paratypes #3, 4, NSMT-Mo 79594, 79595, October 1, 2016, south of Nagakumo-toge, Tatsugo-cho, Amami-Oshima Island, Kagoshima Prefecture, *leg*. S. Yano; paratypes #5, 6, NSMT-Mo 79596, 79597, October 1, 2016, En, Tatsugo-cho, Amami-Oshima Island, Kagoshima Prefecture, *leg*. S. Yano; paratype #7, NSMT-Mo 79598, October 2, 2016, Daimyo Forest Road, Yamato-son, Amami-Oshima Island, Kagoshima Prefecture, *leg*. S. Yano; paratype #8, NSMT-Mo 79599, October 3, 2016, mountain side of Nazekaneku-cho, Amami, Amami-Oshima Island, Kagoshima Prefecture, *leg*. S. Yano; paratype #9, NSMT-Mo 79600, October 4, 2016, Ogamiyama Park, Nazenagata-cho, Amami, Amami-Oshima Island, Kagoshima Prefecture, *leg*. S. Yano.

Other material examined: NSMT-Mo 79601, 1.91 mm SL, collection site, date and collector same as for paratypes #3, 4.

Description: Shell small for genus (SL = 1.93–2.37 mm; SW = 3.13–3.75 mm), relatively long (SL/SW = 0.59–0.66), spire elevated (SL2 = 0.32–0.47 mm); conical in shape, semi-translucent, white to pale pink in color (Fig. 4A–D, Table 2). Protoconch of 1.8–2.0 whorls, elevated, yellowish white to pale red in color, glossy, smooth, demarcated from teleoconch by indistinct incremental scar. Teleoconch comprises 1.8–2.0 whorls, ornamented with axial ribs that are ambiguous on first half of teleoconch, becoming visible and dense and subsequently more widely spaced towards the whorl of adapical end of sutural tube (Fig. 4A). Axial ribs become slightly denser on region along

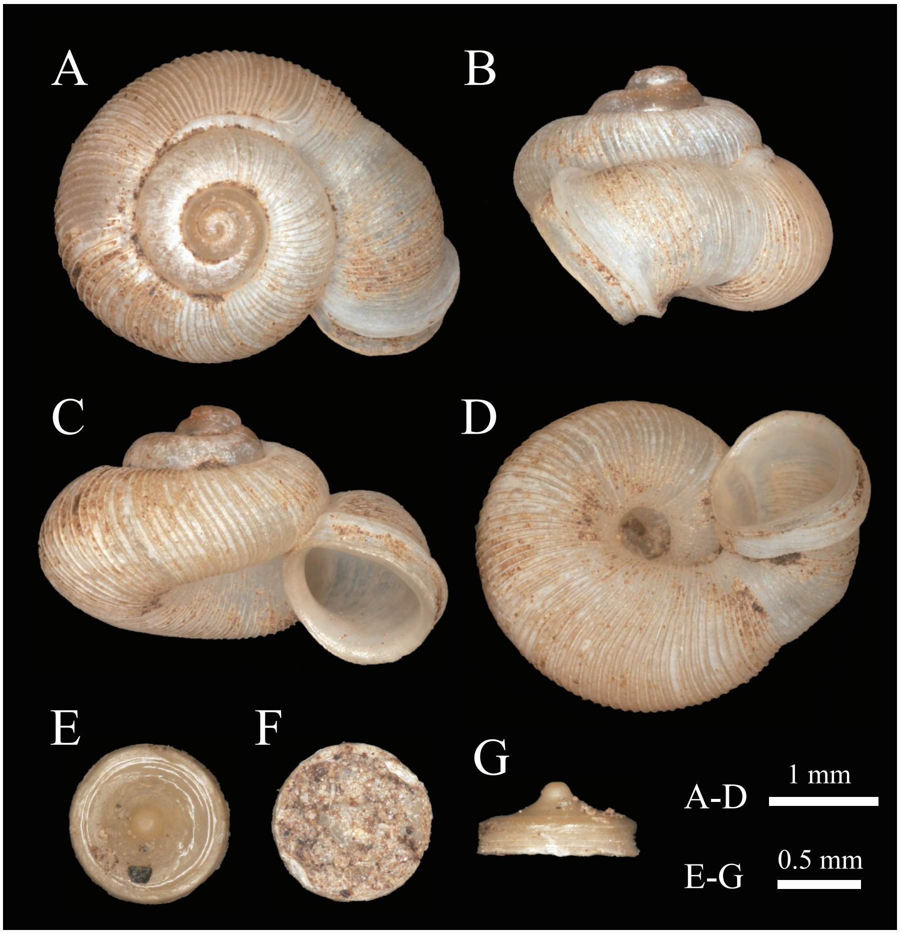

Fig. 4. *Dicharax amamiensis* n. sp. **A–G.** Holotype, NSMT-Mo 79591, SL = 2.37 mm; A–D, apical (A), lateral (B), apertural (C), and basal (D) views of shell. **E–G.** Inner (E), outer (F), and lateral (G) views of operculum.

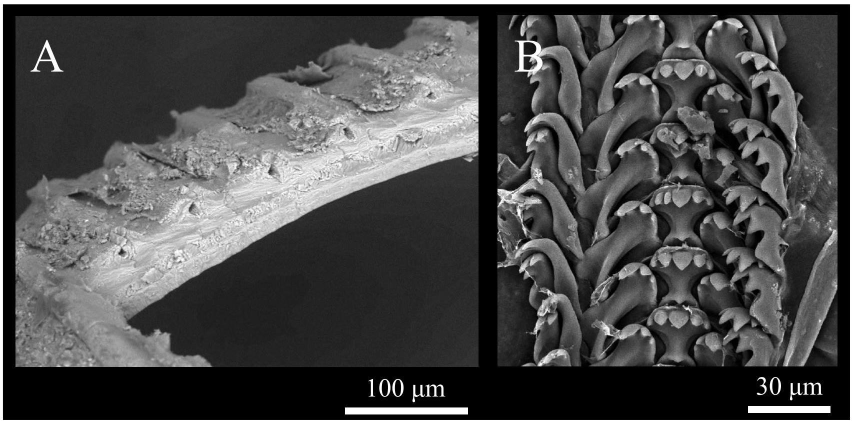

Fig. 5. *Dicharax amamiensis* n. sp., SEM of shell and radula. **A.** Cross section of axial ribs along the sutural tube, NSMT-Mo 79601, SL = 1.91 mm. **B.** Radula, specimen was collected from south of Nagakumo-toge, Tatsugo-cho, Amami-Oshima Island.

Table 2. Shell demensions for *Dicharax amamiensis* n. sp.

	Holotype NSMT-Mo 79591	Paratypes NSMT-Mo 79592	79593	79594	79595	79596	79597	79598	79599	79600	mean		SD	n	Min		Max
SL (mm)	2.37	2.26	2.08	2.06	2.08	1.93	2.05	2.07	2.11	1.97	2.10	±	0.12	10	1.93	–	2.37
SW (mm)	3.75	3.59	3.13	3.13	3.39	3.25	3.34	3.23	3.25	3.20	3.33	±	0.19	10	3.13	–	3.75
SL/SW	0.63	0.63	0.66	0.66	0.61	0.59	0.61	0.64	0.65	0.62	0.63	±	0.02	10	0.59	–	0.66
BL (mm)	1.90	1.87	1.71	1.68	1.73	1.61	1.68	1.64	1.72	1.60	1.71	±	0.10	10	1.60	–	1.90
TW (mm)	1.32	1.28	1.10	1.09	1.12	1.03	1.15	1.11	1.17	1.08	1.15	±	0.09	10	1.03	–	1.32
TW/SW	0.35	0.36	0.35	0.35	0.33	0.32	0.34	0.34	0.36	0.34	0.34	±	0.01	10	0.32	–	0.36
SL2 (mm)	0.47	0.38	0.37	0.38	0.35	0.32	0.37	0.42	0.39	0.37	0.38	±	0.04	10	0.32	–	0.47
STL (mm)	1.03	1.15	1.08	0.87	1.19	0.95	1.07	1.09	1.04	0.92	1.04	±	0.09	10	0.87	–	1.19
No. AR along tube	28	23	29	26	30	30	33	31	36	28	29.40	±	3.41	10	23	–	36
No. protoconch whorl	1.9	1.8	1.8	1.9	1.9	1.9	2.0	2.0	1.9	1.9	1.90	±	0.06	10	1.8	–	2.0
No. teleoconch whorl	2.0	2.0	2.0	1.8	1.9	1.9	2.0	1.9	1.9	1.8	1.92	±	0.07	10	1.8	–	2.0
No. of whorl	3.9	3.8	3.8	3.7	3.8	3.8	4.0	3.9	3.8	3.7	3.82	±	0.09	10	3.7	–	4.0
OD (mm)	0.96	–	0.84	0.92	0.95	0.93	0.95	0.94	0.94	0.89	0.93	±	0.04	9	0.84	–	0.96
OW (mm)	0.44	–	0.24	0.36	0.36	0.36	0.36	0.33	0.35	0.27	0.34	±	0.05	9	0.24	–	0.44
PL (mm)	0.22	–	0.09	0.17	0.16	0.16	0.15	0.14	0.17	0.15	0.16	±	0.03	9	0.09	–	0.22

the sutural tube (STL = 0.87–1.19 mm; No. AR along tube = 23–36). In cross sectional view of whorl along sutural tube, axial ribs and microtunnels consisting of anterior and posterior crusts (terminology following Páll-Gergely *et al*., 2016); anterior crust folds over posterior crust and forms blunt, not elevated ribs (Fig. 5A). In apertural view, axial ribs on body whorl prosocline (Fig. 4C). In apical view, shell oval in appearance, spire weakly constricted at abapical part of anterior end of sutural tube (Fig. 4A). Section between constriction and aperture long; swelling of this part blunt with dense axial ribs. Umbilicus narrow, slightly oblong by expanded spire (Fig. 4D). Aperture large, peristome double, outer margin expanded and slightly reflexed, inner one protruding anteriorly from outer one (Fig. 4B), with large variation in expansion of outer margin and protrusion of inner margin.

Operculum calcareous, orbicular (OD = 0.84–0.96 mm, OW = 0.24–0.44 mm), with groove around periphery (Fig. 4E–G). Inner side smooth, glossy with central papilla (PL = 0.09–0.22 mm). Operculum thick in lateral view but outer side concave and covered by horny membrane.

Radula of taenioglossate type, with one rachidian tooth flanked by one pair of lateral teeth and two pairs of marginal teeth on each side (Fig. 5B). Rachidian tooth with one triangular main denticle flanked by two smaller cusps on each side, as in the majority of species in Alycaeinae. Lateral teeth longer than rachidian tooth, with one large triangular main denticle flanked by one smaller cusp at outer side and two cusps at inner side. Inner and outer marginal teeth possess four cusps.

Distribution: Central to northeastern regions of Amami-Oshima Island, Kagoshima Prefecture.

Habitat: The new species was found in leaf litter.

Etymology: The specific name of the new species refers to its type locality, Amami-Oshima

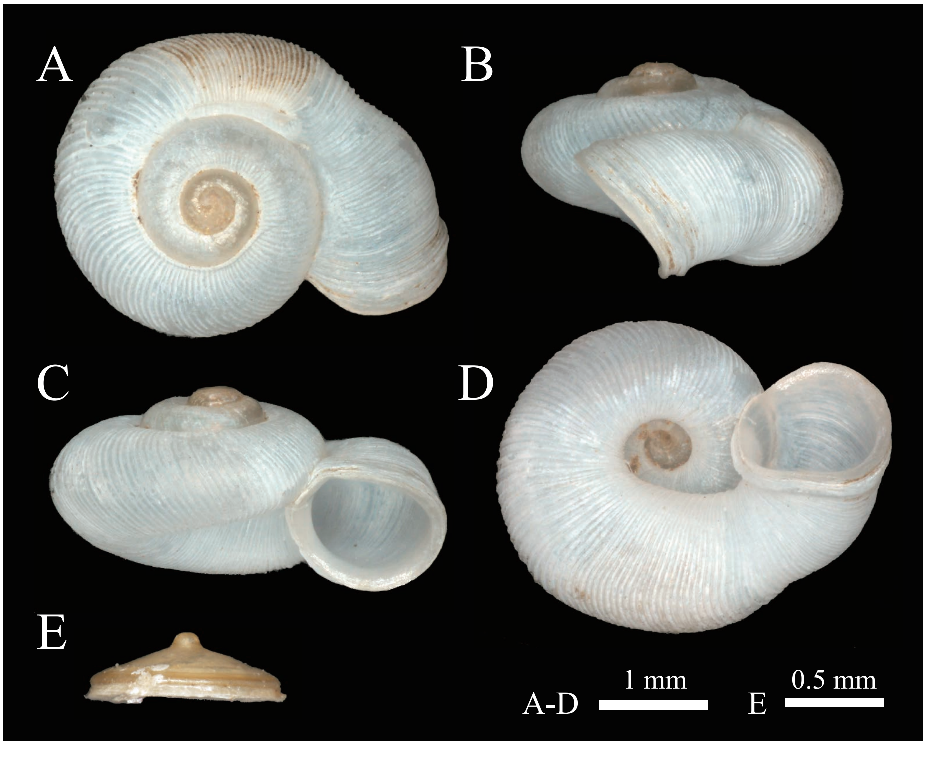

Fig. 6. *Dicharax purus* (Pilsbry & Hirase, 1904). **A–E.** NSMT-Mo 79605, SL = 1.88 mm; A–D, apical (A), lateral (B), apertural (C), and basal (D) views of shell; E, lateral view of operculum.

Table 3. Shell demensions for *Dicharax purus* (Pilsbry & Hirase, 1904).

	Lectotype ANSP 87683	Material examined										
		NSMT-Mo				mean		SD	n	Min		Max
		79602	79603	79604	79605							
SL (mm)	1.63	1.83	1.87	2.06	1.88	1.91	±	0.09	4	1.83	–	2.06
SW (mm)	3.27	3.52	3.50	3.79	3.69	3.62	±	0.12	4	3.50	–	3.79
SL/SW	0.50	0.52	0.53	0.54	0.51	0.53	±	0.01	4	0.51	–	0.54
BL (mm)	1.36	1.53	1.54	1.74	1.61	1.61	±	0.08	4	1.53	–	1.74
TW (mm)	1.01	1.05	1.09	1.18	1.16	1.12	±	0.05	4	1.05	–	1.18
TW/SW	0.31	0.30	0.31	0.31	0.31	0.31	±	0.01	4	0.30	–	0.31
SL2 (mm)	0.26	0.29	0.33	0.32	0.27	0.30	±	0.02	4	0.27	–	0.33
STL (mm)	–	1.03	1.00	1.03	1.05	1.03	±	0.02	4	1.00	–	1.05
No. AR along tube	–	36	35	35	35	35	±	0.43	4	35	–	36
No. of whorl	–	3.5	3.7	3.7	3.7	3.65	±	0.09	4	3.5	–	3.7
OD (mm)	–	0.95	0.95	1.00	1.02	0.98	±	0.03	4	0.95	–	1.02
OW (mm)	–	0.35	0.30	0.34	0.34	0.34	±	0.02	4	0.30	–	0.35
PL (mm)	–	0.26	0.19	0.21	0.20	0.21	±	0.02	4	0.19	–	0.26

Island.

Remarks: The generic position of the new species is supported by the absence of spiral striation on the entire shell (Páll-Gergely *et al.*, 2020). The new species closely resembles *D. purus* in its small shell (1.7 mm SL in the original description), oval shape in apical view, tinted apical whorl, closely settled axial ribs on the last whorl, relatively long sutural tube, and oblique, circular aperture (Pilsbry & Hirase, 1904). However, *D. purus* differs from the new species by its depressed shell, thin peristome, and delicate collar (Pilsbry & Hirase, 1904). On the basis of the collected specimens, *D. purus* is similar to the new species in shell appearance in apical view (Figs 4A, 6A) and shell size (SL = 1.83–2.06 mm [*purus*, *n* = 4] vs. 1.93–2.37 mm [*amamiensis*, *n* = 10]), but the shell is depressed compared with the new species (SL/SW = 0.51–0.54 [*purus*, *n* = 4] vs. 0.59–0.66 [*amamiensis*, *n* = 10]), and has fewer whorls (3.5–3.7 [*purus*, *n* = 4] vs. 3.7–4.0 [*amamiensis*, *n* = 10]) (Tables 2, 3). Furthermore, the penultimate whorl is proportionally narrower than in *D. amamiensis* n. sp. (TW/SW = 0.30–0.31 [*purus*, *n* = 4] vs. 0.32–0.36 [*amamiensis*, *n* = 10]) (Tables 2, 3). The umbilicus of the new species is narrower than that of *D. purus* (Figs 4D, 6D).

Another congener, *D. oshimanus*, resembles the new species in having a double peristome, a similar SL/SW ratio (0.55–0.65 [*oshimanus*, *n* = 10, in Matsuda & Yano, 2024] vs. 0.59–0.66 [*amamiensis*, *n* = 10]), and an operculum with a large papilla. However, *D. oshimanus* can be distinguished by a larger shell (SW = 3.34–4.00 mm [*oshimanus*, *n* = 10, in Matsuda & Yano, 2024] vs. 3.13–3.75 mm [*amamiensis*, *n* = 10]), nearly round shape in apical view, and a relatively shorter whorl between the constriction and the aperture. The new species clearly differs from other Japanese *Dicharax* species by the combination of a small shell, oval shape in apical view, and long whorl between the constriction part to the aperture.

Discussion

In the present study, mitochondrial sequences (COI and 16S) of *D. amamiensis* n. sp. were compared with 10 named species and subspecies of Alycaeinae, including three *Metalycaeus* species. The new species were found to comprise a well-supported monophyletic clade (Fig. 3). Although *D. amamiensis* n. sp. had been identified as *D. purus* (Kuroda, 1953, 1956, 1963; Yukita, 2003; Minato, 2011, 2014; Tomiyama & Nishi, 2016), our comparison of the specimens and molecular analysis reveal that they are a distinct species. While *D. purus* has been reported from both Tokunoshima Island and Amami-Oshima Island, the record from Amami-Oshima Island is likely to be the new species. *Dicharax purus* has been listed as a critically endangered species in the Japanese Red List and a vulnerable species in the local Red Data Book of Kagoshima Prefecture because of its limited distribution and low population density (Minato, 2014; Tomiyama & Nishi, 2016; Ministry of the Environment, Japan, 2020). The extinction risk of *D. purus* may be higher than previously estimated, because on the basis of the precise identification in this study, their habitat is even more restricted (*i.e.*, to Tokunoshima Island). Because the habitat of the new species is also limited, it should be designated as threatened.

Molecular phylogenetic investigations showed some specific relationships within alycaeid species in the Nansei Islands. The three species and subspecies of *Dicharax*, *i.e.*, *D. tokunoshimanus*, *D. tokunoshimanus principialis*, and *D. tokunoshimanus mediocris*, were confirmed to be closely related. These taxa share the synapomorphic characteristic of having a reddish shell with orthocline axial ribs on the body whorl in apertural view (based on the photographs of the lectotype, ANSP 87505 for *D. tokunoshimanus*, the lectotype, ANSP 95830 for *D. tokunoshimanus principialis*, the lectotype, ANSP 89926 for *D. tokunoshimanus mediocris*, from the website of the ANSP malacology collection, accessed on January 28, 2025). To determine their taxonomic status, however, further analyses incorporating additional undescribed taxa, such as the large-shelled population of *D. tokunoshimanus principialis* (as "*Chamalycaeus principialis*") (Minato, 2009) and *Dicharax* sp. from Amami-Oshima Island (Matsuda & Yano, 2024), are required.

We found that *D. purus* shows relatively large genetic distances in our molecular analysis. All specimens analyzed in the present study were collected from the northern mountain area of Tokunoshima Island (Fig. 1) and no conchological differences were detected. Because our molecular data and sample size are insufficient to evaluate this difference, additional sampling and analysis are required.

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奄美大島から採集された *Dicharax* 属（新生腹足上目：ヤマタニシ科：ムシオイ亜科）の 1 新種記載

松田春菜・矢野重文

要　約

奄美群島の徳之島を模式産地とするヒメムシオイ *Dicharax purus*（Pilsbry & Hirase, 1904）は，奄美大島にも分布すると報告されてきたが，これまで徳之島産の標本と奄美大島産の標本との比較検討は行われてこなかった。そこで本研究では，徳之島で採集したヒメムシオイと奄美大島から採集した標本を用い，*Dicharax* 属 6 種と *Metalycaeus* 属 3 種とともにミトコンドリア DNA の COI 領域及び 16S 領域の塩基配列に基づく分子系統解析を行った。その結果，それぞれ単系統性が認められ，徳之島のヒメムシオイと奄美大島でヒメムシオイに同定されてきた個体とは遺伝的に異なることが判明した。つまり，ヒメムシオイは徳之島にのみ分布する種となる。フィラデルフィア自然科学アカデミーのヒメムシオイのレクトタイプ標本を確認するとともに，採集した 2 種の形態比較を行い，両種の識別点を明らかにし，混同されてきた種を新種として記載した。

Dicharax amamiensis n. sp. アマミヒメムシオイ（新種・新称）

殻高 2.37 mm，殻幅 3.75 mm（ホロタイプ）。殻は螺塔が張り出した円錐形で，半透明の白色～赤褐色を呈する。胎殻は光沢があり，滑らかで，黄白色～赤褐色を呈する。後生殻の成長肋は最初は不明瞭だが，徐々に密にはっきりと現れ，虫様管に向かって次第にその間隔が広くなり，虫様管横で再び密になる。殻口はやや大きい。殻口縁は二重になり，外側の口縁は拡張してやや反曲し，内側はわずかに前方に突出する。正面から見ると体層部分の成長肋は斜めになる。背面から見ると殻形は楕円形で，頸部で収縮し，殻口に向かってわずかに広がる。臍孔は狭く，体層の膨らみによって扁圧される。蓋は直径約 1 mm の円形で，内側は中央が突起状にふくらむ。

本種はヒメムシオイに近似するが，殻幅に対する殻高の比率がより高いこと，殻口縁が厚く，わずかに反曲すること，殻幅に対する次体層の殻幅がより広いこと，臍孔が狭いことによって区別できる。