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原著論文

遠州灘から得られた日本初記録のホウボウ科魚類ミナミソコホウボウ（新称）

First Record of a Gurnard Fish, *Bovitrigla acanthomoplate* (Osteichthyes: Perciformes: Triglidae) from the Enshu-nada Sea, off Shizuoka Prefecture, Japan

矢頭卓児 1)・手良村知功 2)・瀬能　宏 3)

Takuji Yato1), Akinori Teramura2) & Hiroshi Senou3)

Abstract. The monotypic genus *Bovitrigla* established by Fowler in 1938 accommodates *B. acanthomoplate* and is known from the Philippines and the South China Sea. A single specimen of *B. acanthomoplate* was collected from the Enshu-nada Sea off Shizuoka Prefecture, Japan. The species is characterized by having a long rostral spine, a long posttemporal spine and a postocular spine, which are the generic diagnostic characters. This record represents not only the first record from Japan but the northernmost record of this species. Comparing the Japanese specimen with ten specimens of this species collected from the South China Sea, we found the former differs in having the following characters: 52 lateral line scales vs. 54–57; snout length 47.5 % in HL vs. 42.5–46.4 %; and interorbital width 33.8 % in HL vs. 29.7–32.7 %. We redescribe this species in detail and give the new standard Japanese name, Minami-sokohobo for this species and Minami-sokohobo-zoku for the genus.

Key words: color variation, morphological variation, northern most record, redescription

緒　言

ホウボウ科 Triglidae の *Bovitrigla* は、Fowler（1938）によって、ソコホウボウ属 *Pterygotrigla* Waite, 1899 と比べて吻棘が長いこと、後側頭棘が長いこと、眼後棘があることを根拠に、ホロタイプだけに基づき同時に新種として記載された *B. acanthomoplate*（ホロタイプ USNM 98869, Fig.1）をタイプ種として創設された属である。現在、本属に属する種は本種だけである（Fricke *et al.*, 2020）。

2019 年 1 月 14 日、遠州灘における底曳網漁獲物の中から *B. acanthomoplate* に同定される 1 標本が第 2 著者により得られた。この種に言及した研究は、Fowler（1938）の原記載以降では、ホロタイプあるいは原記載を引用した報告（Richards, 1999）やホウボウ科の属の分類学的研究（Richards & Jones, 2002）、南シナ海からの目録的記録（Richards, 2000）、台湾南部からのごく簡単な記載を伴った標本に基づく記録（Yato, 2019）があるだけである。そして遠州灘からの標本は本種の日本からの初記録であり、同時に北限記録でもある。著者らは遠州灘産の標本を詳細に記載し、南シナ海産の標本との比較を行ったのでここに報告する。また、本種と本種を含む属には和名がな

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Fig. 1. Lateral view of *Bovitrigla acanthomoplate*, holotype, USNM 98869, 150 mm TL, in alcohol. Photo by R. Misawa.
図 1. ミナミソコホウボウ（ホロタイプ , アルコ－ル液浸標本 ; 側面），USNM 98869, 全長 150 mm, 三澤　遼撮影.

いため、新標準和名を与える。

材料と方法

本標本は 10 % 中性ホルマリン水溶液で固定した後、70 % エタノ－ル水溶液に置換し、保存した。標本の計数・計測方法は Yato & Heemstra（2019）にしたがった。計測にはデジタルノギスを用い、0.1 mm の精度で測定した。標準体長は SL、頭長は HL と略記した。脊椎骨は軟 X 線写真を撮影して計数した。

本研究で観察または引用した標本は、以下の研究機関に所蔵されている : 台湾中央研究院（ASIZP）; 高知大学理工学部（BSKU）; 神奈川県立生命の星・地球博物館（KPM-NI）; 台湾国立海洋生物博物館（NMMB-P）; スミソニアン自然史博物館（USNM）。なお、神奈川県立生命の星・地球博物館の資料番号は、電子台帳上では桁を埋めるための 0 を付加した 7 桁の数字が用いられているが、本稿では有効数字で表記した。

結　果

ミナミソコホウボウ（新称）
Bovitrigla acanthomoplate Fowler, 1938
（Figs. 2–5, Table 1）

Bovitrigla acanthomoplate Fowler, 1938: 113, fig. 54; Richards & Jones, 2002: 276 (list); Yato, 2019: 544 (brief description).

Pterygotrigla acanthomoplate: Richards, 1999: 2371 (brief description based on holotype); Richards, 2000: 607 (list).

記載標本

KPM-NI 58103、95.8 mm SL、静岡県遠州灘浜松市沖（Fig. 6, 詳細な採集地不明）、水深約 250 m、底曳網、舞阪漁港水揚げ、市場の選別台にて拾得、2019 年 1 月 14 日、手良村知功採集。

記載

頭部と体はやや側扁する。頭部は大きく、標準体長の 41.1 %。吻突起は細長く伸びた扁平な三角形で、先端は鋭く尖り、両突起間隔は前方ほど広がる。鼻骨上に棘は無い。吻は長く、眼窩径の 164.0 %。側面観は眼窩前方で少しくぼむ。眼はやや大きく、頭長の 28.9 %。両眼間隔は広くてややくぼみ、眼窩径の 116.7 %。眼後棘は小さく三角形で、先端は尖る。眼後棘基部の直後には浅い溝がある。後側頭棘は著しく長くて鋭く、後端は背鰭第 4 棘基部に達する。口は端位に近い下位。上顎の後端は眼窩前縁下に達しない。両顎の歯は絨毛状で歯帯を形成する。鋤骨には小さな犬歯状の歯が左右に並ぶ。第 1 鰓弓の鰓耙は上枝にはなく下枝に 5 本あり、細長い。眼下骨板には中央より後方に向かって前鰓蓋骨棘に連なる低い隆起がある。眼下骨板の高さは眼窩径の 83.3 %。前鰓蓋骨棘は 1 本で鋭く、起部下縁に小さな突起をともなう。主鰓蓋骨棘は小さく、棘先端までの主鰓蓋骨の長さは眼窩径の 76.3 %。上膊棘は長大で、後端は背鰭第 4 棘基部をこえる。背鰭基底に沿った骨質板は 10 枚で、左右の最前

Fig. 2. Lateral view of *Bovitrigla acanthomoplate*, KPM-NI 58103, 95.8 mm SL, in fresh condition. Photo by A. Teramura.
図 2. ミナミソコホウボウ（鮮時 ; 側面），KPM-NI 58103, 標準体長 95.8 mm, 手良村知功撮影.

Fig. 3. Lateral view of *Bovitrigla acanthomoplate,* KPM-NI 58103, 95.8 mm SL, in alcohol. Photo by T. Yato.
図 3. ミナミソコホウボウ（アルコ－ル液浸標本 ; 側面），KPM-NI 58103, 標準体長 95.8 mm, 矢頭卓児撮影.

Fig. 4. Dorsal view of *Bovitrigla acanthomoplate*, KPM-NI 58103, 95.8 mm SL, in alcohol. Photo by T. Yato.
図 4. ミナミソコホウボウ（アルコ－ル液浸標本 ; 背面），KPM-NI 58103, 標準体長 95.8 mm, 矢頭卓児撮影.

Table 1. Meristic and morphometric characters of *Bovitrigla acanthomoplate*
表 1. ミナミソコホウボウ *Bovitrigla acanthomoplate* の計数値および計測値

specimens	NMMB-P 12093	NMMB-P 12094	NMMB-P 18099	NMMB-P 20411	NMMB-P 20432	NMMB-P 21040	NMMB-P 21041	NMMB-P 21172-1	NMMB-P 21172-2	NMMB-P 25536	KPM-NI 58103
Counts											
1st Dorsal-fin spines	8	8	8	8	7	8	8	8	8	7	7
2nd Dorsal-fin rays	11	11	11	11	11	11	11	10	11	11	11
Anal-fin rays	12	11	12	12	12	12	12	12	12	12	12
Pectoral-fin rays (connected)	12	12	12	12	12	12	12	12	12	12	12
Pelvic-fin rays	I,5	I,5	I,5	I,5	I,5	I,5	I,5	I,5	I,5	I,5	I,5
Caudal-fin rays (branched)	9	9	9	9	9	9	9	9	9	9	9
LL Scales	56	55	54	55	55	54	56	57	55	55	52
Bony plates	10	10	10	10	10	10	10	10	10	10	10
Gill-rakers(right side)	1+7	1+6	-	1+8	1+6	-	-	-	1+8	1+7	1+5
Vertebrae with urostyle	-	-	10+17	-	10+17	-	-	10+17	10+17	-	10+17
Measurements (mm)											
Total length	106.1	106.2	151.5	109.5	190	110.4	111.8	136.6	131	163.8	121.8
Standard length	84.5	85.8	120	88.4	150.3	88.9	91.4	107.5	106	130.9	95.8
% of Standard length											
Distance from snout to 1st dorsal fin	47.8	47.2	46.3	41.4	46.0	47.9	46.3	44.3	45.4	43.9	45.2
Distance from snout to 2nd dorsal fin	65.4	64.0	66.7	63.5	64.7	65.5	64.2	64.3	63.4	63.8	63.8
Distance from snout to pectoral fin	61.4	37.9	39.8	34.6	38.7	40.5	40.3	41.0	41.4	39.0	40.6
Distance from snout to pelvic fin	34.6	33.1	34.4	30.5	33.8	34.9	36.1	35.7	34.7	32.7	34.8
Distance from snout to anal-fin	61.5	58.5	58.1	57.9	61.0	60.7	58.2	59.6	58.9	60.1	59.7
Body depth in front of 1st dorsal fin	22.6	22.6	26.3	19.5	25.3	24.4	24.5	25.7	25.8	22.5	24.4
Body width at cleithral base	18.8	16.7	19.6	13.8	21.0	18.3	18.9	18.5	17.7	16.7	18.3
Caudal peduncle length	15.4	16.1	16.4	11.3	14.6	14.6	15.9	16.0	15.1	14.4	16.3
depth	5.9	5.7	6.4	6.0	6.1	6.3	6.2	6.0	5.9	5.7	5.9
1st Dorsal-fin base length	16.8	17.6	19.3	21.3	18.4	18.3	20.2	20.6	20.2	17.8	18.7
2nd Dorsal-fin base length	21.5	22.6	23.0	26.2	22.3	22.5	23.2	23.4	22.9	21.8	23.7
Anal-fin base length	24.7	25.8	27.6	28.1	25.9	27.7	26.9	27.3	27.2	27.0	26.6
Head length	43.4	41.4	43.1	41.9	41.0	42.7	42.1	40.9	41.6	41.4	41.1
Length of 1st detached pectoral-fin ray	31.5	31.5	30.9	33.7	28.9	34.4	31.7	32.7	32.3	30.9	30.2
Length of 2nd detached pectoral-fin ray	28.6	28.1	26.9	31.0	26.3	30.5	29.2	28.7	29.2	28.5	26.4
Length of 3rd detached pectoral-fin ray	24.1	22.7	22.3	25.1	21.6	27.0	27.1	22.5	22.9	21.4	22.2
Pectoral fin length	38.1	36.5	38.2	34.6	38.4	37.6	36.3	37.4	37.2	36.4	38.0
Pelvic fin length	26.5	25.2	27.6	27.5	23.8	28.7	29.1	26.0	25.8	23.8	27.0
Length of 1st spine of first dorsal fin	11.0	-	11.8	-	8.6	10.1	11.2	10.1	9.7	9.6	9.7
Length of 2nd spine of first dorsal fin	16.9	15.4	18.2	17.8	15.7	17.1	17.0	15.9	-	16.2	15.4
Length of 3rd spine of first dorsal fin	17.3	18.5	18.3	18.0	-	17.1	18.3	17.2	-	17.6	16.5
Length of 4th spine of first dorsal fin	13.0	14.3	17.0	12.7	-	14.3	16.4	13.7	12.9	13.1	11.9
% of Head length											
Head depth at middle of orbit	58.3	59.7	57.6	56.2	55.2	57.9	56.9	53.9	57.8	56.5	56.9
Head width at preopercle	43.1	42.0	51.5	45.7	52.3	46.6	46.5	48.9	47.2	44.8	46.7
Snout length	43.3	42.5	45.5	45.4	46.4	43.4	43.4	45.9	43.5	46.3	47.5
Height of suborbital stay	24.3	23.4	26.9	23.8	26.0	24.5	24.7	25.7	24.5	26.2	24.1
Distance between rostral projection tips	67.8	63.2	60.1	65.4	63.8	62.7	68.9	67.1	72.5	61.4	50.4
Length of Rostral projection	51.7	50.2	39.6	46.4	29.8	45.9	40.7	41.5	50.3	36.0	50.2
Upper jaw length	34.6	35.5	33.8	38.1	37.5	38.4	34.0	36.1	36.3	34.5	36.0
Lower jaw length	42.5	43.1	43.5	43.2	42.4	43.4	41.0	40.0	41.0	40.2	41.9
Orbit diameter	32.7	34.4	28.0	30.5	28.7	32.4	31.7	30.2	30.4	29.0	28.9
Interorbital width	29.7	31.8	30.2	31.9	31.5	31.8	31.7	32.7	30.2	31.7	33.8
Opercular spine length	20.2	19.7	-	18.4	22.4	22.1	20.3	22.3	22.2	19.2	22.1
% of Orbit diameter											
Opercular spine length	61.7	57.4	-	60.2	78.0	68.3	63.9	73.7	73.1	66.2	76.3

の骨質板は融合して、背鰭起部直前に位置する。第 2 背鰭基底には骨質板がない。

背鰭は 7 棘 11 軟条で、各棘条の前縁は円滑。第 3 棘条が最も長い。第 2 背鰭起部は臀鰭第 3 軟条基部上方にある。また、第 4 軟条より後方の鰭条は分枝する。臀鰭鰭条数は 12 で、第 8 軟条より後方の鰭条は分枝する。尾鰭の後縁はやや湾入し、上葉 5 軟条、下葉 4 軟条は分枝する。胸鰭は下方 3 本の遊離軟条を含めて 15 軟条、うち第 2–8 軟条は分枝する。胸鰭の後縁は円くて、その後端は臀鰭第 7 軟条基部に達する。最も上の遊離軟条が一番長く、後端は腹鰭後端をこえて、臀鰭第 3 軟条基部に達する。腹鰭は 1 棘 5 軟条で、第 2 軟条が最も長く、後端は臀鰭起部に達する。

体表の鱗は後端が尖る涙型で、体上半部ではそれぞれの鱗の縁辺がやや皮膚下に埋没して互いに重ならず、明瞭な列をつくらない。下半部の鱗は覆瓦状。胸鰭と腹鰭の基底部周辺は無鱗だが、項部は鱗で密に覆われ、胸鰭前方、胸部、両腹鰭間にも鱗がある。側線鱗数は 52 で、体前方の鱗上には複数の小顆粒がある。側線管は分枝しない。

色彩 : 鮮時（Fig. 2）では、頭部と体の背面は淡い赤色で、暗い鶯色の不定形の小斑が散在する。体の腹面は白色。第 1 背鰭第 1–4 棘の鰭膜上半と第 2 背鰭上方は淡い黒色。尾鰭後縁近くも淡い黒色。胸鰭内面は第 1–9 軟条では縁辺を除いて基底まで濃淡にむらのある黒色で、後縁と第 9–12 軟条の鰭膜は白色、第 8 軟条に沿って不明瞭な小白色斑が 4 個ある。胸鰭の遊離軟条は淡い朱色。腹鰭は白色。

アルコール液浸標本（Figs. 3–5）では、頭部と体全体は薄茶色で，背面に黒色の不定形の小斑が散在する。第 1 背鰭第 1–4 棘の鰭膜上半と第 2 背鰭上方は黒色。尾鰭後縁近くも黒色。胸鰭内面では第 1–9 軟条は縁辺を除いて基底まで濃淡にむらのある黒色で、後縁と第 9–12 軟条の鰭膜は白色、第 8 軟条に沿って不明瞭な小白色斑が 4 個ある。胸鰭の遊離軟条と腹鰭は白色。

Fig. 5. Inner surface of the pectoral fin of *Bovitrigla acanthomoplate*, KPM-NI 58103, 95.8 mm SL, in alcohol. Photo by T. Yato.

図 5. ミナミソコホウボウの胸鰭内面（アルコ－ル液浸標本），KPM-NI 58103, 標準体長 95.8 mm, 矢頭卓児撮影.

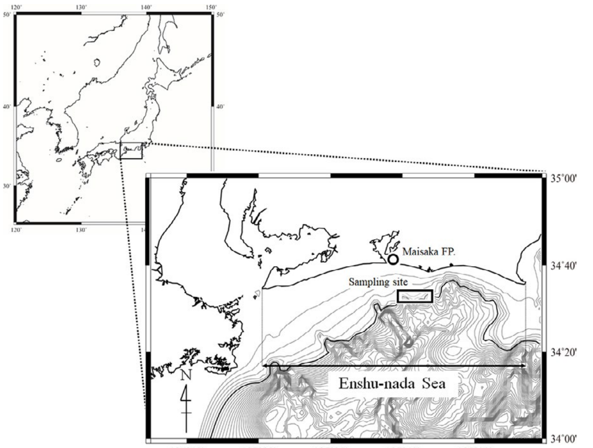

Fig. 6. Map showing the capture locality of the *Bovitrigla acanthomoplate* specimen from the Enshu-nada Sea. Open square: estimated fishing location where the specimen was collected; open circle: Maisaka Fishing Port where the specimen was landed; solid and bold lines of isobaths indicating 50 m and 300 m respectively. Drawn by A. Teramura.

図 6. ミナミソコホウボウの標本（KPM-NI 58103）の採集場所と遠州灘の地図. 白抜きの四角 : 推定漁獲場所; 白抜きの円 : 舞阪漁港 ; 細い実線は 50 m，太い実線は 300 m の等深線を示す．手良村知功作図.

分布

フィリピン（Fowler, 1938）、南シナ海（Yato, 2019）、遠州灘（本研究）。

備考

本標本は、吻突起が長大な三角形であること、吻長が頭長の 47.5 % であること、眼後棘があること、後側頭棘が長大であること、項部と胸部に隣があること、体側の鱗は涙形で先端が尖っていること、第 1 背鰭上半が黒色であること、第 2 背鰭縁辺が黒色であることが Fowler（1938）による *B. acanthomoplate* の原記載やホロタイプによく一致する（Fig. 1, 2, 3）。吻長について原記載では 44.4 %（頭長は吻長の 2.125 倍）とされているが、計測方法が明記されていない。そこで、改めて原記載のホロタイプのスケッチを計測したところ、48.2 % と計算され、本標本と大きな差はないと判断された。

南シナ海から得られ NMMB に所蔵されている 10 個体の *B. acanthomoplate* と比較すると（Table 1）、基本的にはよく一致しているが、遠州灘産の標本は南シナ海産の標本に比べて側線鱗数が 52 とやや少ない（南シナ海産の 10 個体では 54–57）、頭長に対して吻長がやや長い 47.5 % in HL（南シナ海産 10 個体 : 42.5–46.4 %）、両眼間隔幅はやや広い 33.8 % in HL（南シナ海産 10 個体 : 29.7–32.7 %）といった点で異なる。本研究で得られた日本産の標本は 1 個体だけであり、いずれの値も南シナ海産の標本の値からわずかに外れているだけであることから、ここではこれらの違いを暫定的に個体変異とみなしておく。胸鰭内面の白色小斑は日本産の個体では 4 個、NMMB-P 18099 では 7 個、NMMB-P 21040 では 2 個が認められるが、他の NMMB の標本では斑が認められなかった。白色斑については原記載にも示されておらず、本種の胸鰭内面の斑紋には変異が多いと考えられる。

本種の外観はリュウキュウソコホウボウ *Pterygotrigla ryukyuensis* Matsubara & Hiyama, 1932 によく似ている（Fig. 7）が、本種は項部と胸部に鱗があること（リュウキュウソコホウボウではどちらも無麟）、体表の鱗は後端が尖る涙型であること（円鱗）、体前方の側線鱗上には小顆粒があり表面が凸凹であること（顆粒は無く表面は円滑）、吻突起は少し外側に開き左右の突起間隔は前方でやや広がること（吻突起は大きく外側に開き左右の突起間隔は前方で大きく広がる）で容易に区別できる。

本種はこれまでフィリピンと南シナ海から記録されているが（Fowler, 1938; Yato, 2019）、これまで日本からの報告はなかった。遠州灘から得られた標本は日本からの初記録になると同時に本種の北限記録である。

また、本種と本種を含む *Bovitrigla* には和名がない。したがって本種に対しては本標本に基づいて新標準和名ミナミソコホウボウを提唱する。属については日本魚類学会（2020）の項 4.2.1 に従い、*Bovitrigla* のタイプ種である本種の標準和名に基づきミナミソコホウボウ属を提唱する。

Fig. 7. Dorsal view of *Pterygotrigla ryukyuensis*, ASIZP 73756, 96.0 mm SL, in alcohol. Photo by T. Yato.
図 7. リュウキュウソコホウボウ（アルコ－ル液浸標本 ; 背面）, ASIZP 73756, 標準体長 96.0 mm, 矢頭卓児撮影.

ミナミソコホウボウなる名称はこれまでに採集されている標本の多くが南シナ海から得られていることに由来する。

比較標本

ミナミソコホウボウ *Bovitrigla acanthomoplate*: USNM 98869，holotype，150 mm TL; NMMB-P 12093，84.5 mm SL; NMMB-P 12094，85.8 mm SL; NMMB-P 18099，120.0 mm SL; NMMB-P 20411，88.4 mm SL; NMMB-P 20432，150.3 mm SL; NMMB-P 21040，88.9 mm SL; NMMB-P 21041，91.4 mm SL; NMMB-P 21172-1，107.5 mm SL & NMMB-P 21172-2，106.0 mm SL; NMMB-P 25536，130.9 mm SL. リュウキュウソコホウボウ *Pterygotrigla ryukyuensis*: ASIZP 73756，96.0 mm SL; BSKU 17656，166.7 mm SL; BSKU 104265，186.5 mm SL.

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引用文献

Fowler, H. W., 1938. Description of new fishes obtained by the United States Bureau of Fisheries steamer “Albatross”, chiefly in Philippine seas and adjacent waters. Proceedings of the United States National Museum, 85(3032): 31–135.

Fricke, R., W. N. Eschmeyer & R. Van der Laan (eds.), 2020. Catalogue of fishes: genera, species, references. Online. Available from internet: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (Accessed on 2021-03-05).

日本魚類学会 , 2020. 魚類の標準和名の命名ガイドライン . Online. Available from internet: http://www.fish–isj.jp/iin/standname/guideline/guidelines2020.pdf (Accessed on 2021-07-11).

Richards, W. J., 1999. Triglidae. In Carpenter, K. E. & V. H. Niem (eds.), FAO species identification guide for fisheries purposes: the living marine resources of the western central Pacific, Vol. 4, Bony fishes part 2 (Mugilidae to Carangidae), pp. 2359–2382. FAO, Rome.

Richards, W. J., 2000. Triglidae. In Randall, J. E. & K. K. P. Lim (eds.), A checklist of the fishes of the South China Sea, The Raffles Bulletin of Zoology, Supplement, No. 8, pp. 606–607. Department of Biological Sciences, National University of Singapore, Singapore.

Richards, W. J. & D. L. Jones, 2002. Preliminary classification of the gurnards (Triglidae: Scorpaeniformes). Marine and Freshwater Research, 53(2): 275–282.

Yato, T., 2019. Triglidae. In Koeda, K. & H.-C. Ho (eds.), Fishes of southern Taiwan. I, pp. 544–556. National Museum of Marine Biology & Aquarium, Pingtung, Taiwan.

Yato, T. & E. Heemstra, 2019. A new deepwater gurnard of the genus *Pterygotrigla* (Scorpaeniformes: Triglidae) from the southern Indian Ocean. Zootaxa, 4706: 189–200.

摘　要

矢頭卓児・手良村知功・瀬能　宏, 2022. 遠州灘から得られた日本初記録のホウボウ科魚類ミナミソコホウボウ（新称）. 神奈川県立博物館研究報告（自然科学）, (51): 1–7. [Yato, T., A. Teramura & H. Senou, 2022. First Record of a Gurnard Fish, *Bovitrigla acanthomoplate* (Osteichthyes: Perciformes: Triglidae) from the Enshu-nada Sea, off Shizuoka Prefecture, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 1–7.]

ホウボウ科のミナミソコホウボウ（新称）*Bovitrigla acanthomoplate* Fowler, 1938 の 1 標本が遠州灘から得られた。この種は日本初記録種であり、遠州灘における出現はこの種の北限記録となる。南シナ海産の 10 個体と比較するとともに本種を詳細に再記載した。

Bull. Kanagawa Pref. Mus. (Nat. Sci.), no. 51, pp. 9-34, Mar. 2022

Original Article

A New Species with Two New Subspecies of *Rhinogobius* (Teleostei: Gobiidae) from Yaeyama Group, the Ryukyu Islands, Japan

Toshiyuki Suzuki[1], Naoharu Oseko[2], Yo Y. Yamasaki[3], Seishi Kimura[4] & Koichi Shibukawa[5]

Abstract. A new freshwater species with two new subspecies of the gobiid fish genus *Rhinogobius* is described from the Yaeyama Group of the Ryukyu Islands, Japan. One of the subspecies, *Rhinogobius aonumai aonumai* (29 specimens, 35.9–70.5 mm SL) known only from Iriomote-jima Island, is distinguished from all congeneric species-group taxa (species and subspecies) by having the following combination of features: 9–15 predorsal scales; 32–37 longitudinal scales; 11+15–17=26–28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of 10th vertebra; fifth segmented pelvic-fin ray divided into 3–4 (usually four) branches at the position where proximal-most segment of each branch alignes transversely; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with vertical rows of dark spots or forming dark zigzag bands. The other subspecies, *Rhinogobius aonumai ishigakiensis* (12 specimens, 33.3–56.5 mm SL) known only from Ishigaki-jima Island, is distinguished from all congeneric species-group taxa by having the following combination of features: 10–14 predorsal scales; 33–38 longitudinal scales; 10+16–18=26–28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of 9th vertebra; fifth segmented pelvic-fin ray divided into 2–3 (usually two) branches at the position where the proximal-most segment of each branch alignes transversely; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with dark zigzag bands on the caudal fin.

Key words: description, fish taxonomy, freshwater resident, *Rhinogobius* sp. YB

Introduction

Rhinogobius Gill, 1859 comprises medium-sized freshwater gobies (reaching up to 30–100 mm in standard length) and is known from the East and Southeast Asian regions, including the Russia Far East, Japan, Korea, China, Taiwan, the Philippines, Vietnam, Laos, Cambodia, and Thailand (Chen & Miller, 2014). At least in the insular habitats, a majority of the species of the genus are amphidromous; namely, adults spawn in the freshwater habitats, larvae just after hatching immediately go to the coastal marine waters, and after that, the juveniles enter the inland waters (Mizuno, 1960a). On the other hand, in the continental areas, many species of the genus are non-diadromous, freshwater inhabitants (e.g., lakes, ponds, and rivers) throughout their life cycle (Huang & Chen, 2007).

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Rhinogobius, originally described as a monotypic genus based on *Rhinogobius similis* Gill, 1859, is currently known as the most species-rich freshwater gobiid genus, comprising 86 described, valid species worldwide (Suzuki *et al.*, 2020; Wanghe *et al.*, 2020), although several additional species remain unnamed. In Japanese waters, 13 valid species hitherto have been confirmed: *Rhinogobius biwaensis* Takahashi & Okazaki, 2017, *R. brunneus* (Temminck & Schlegel, 1845), *R. flumineus* (Mizuno, 1960b), *R. fluviatilis* Tanaka, 1925, *R. kurodai* (Tanaka, 1908), *R. mizunoi* Suzuki, Shibukawa & Aizawa, 2017, *R. nagoyae* Jordan & Seale, 1906, *R. ogasawaraensis* Suzuki, Chen & Senou, 2012, *R. simillis*, *R. telma* Suzuki, Kimura & Shibukawa, 2019, *R. tyoni* Suzuki, Kimura & Shibukawa, 2019, *R. yaima* Suzuki, Oseko, Kimura & Shibukawa, 2020 and *R. yonezawai* Suzuki, Oseko, Kimura & Shibukawa, 2020. In addition, Akihito *et al.* (2013) reported five undescribed species from Japanese waters, all of which are distinguished by specific abbreviations as follows (each vernacular name in Japan is in parenthesis): *Rhinogobius* sp. BB (Aobara-yoshinobori), *Rhinogobius* sp. KZ (Kazusa-yoshinobori), *Rhinogobius* sp. MO (Aya-yoshinobori), *Rhinogobius* sp. OM (Oumi-yoshinobori) and *Rhinogobius* sp. YB (Kibara-yoshinobori).

Yamasaki *et al.* (2020) reported that the speciation of *Rhinogobius* sp. YB from its amphidromous ancestor, *R. brunneus*, occurred in parallel across five islands groups (Amami-oshima Island, Tokuno-shima + Okinoerabu-jima islands, Okinawa-jima Island, Kume-jima Island, and Ishigaki-jima + Iriomote-jima islands in the Ryukyu Islands, Japan) using population genetic analysis.

We describe one of these populations from Ishigaki-jima and Iriomote-jima islands as a new species with two new subspecies of *Rhinogobius*. Namely, these new species/subspecies were known and confused under the name "*Rhinogobius* sp. YB" or "Kibara-yoshinobori" in the previous studies (e.g., Iwata, 1989; Akihito *et al.*, 2013; see "Discussion", below). The taxonomic status of the other populations of "*Rhinogobius* sp. YB" will be resolved elsewhere.

Materials and Methods

Yamasaki *et al.* (2020) reported that the populations of "*Rhinogobius* sp. YB" in the upper streams above the dam lakes in Ishigaki-jima Island contained individuals that have hybridized with the other congener *Rhinogobius* brunneus. For selecting type specimens in Ishigaki-jima Island, we thus used non-hybridized specimens with *R. brunneus* in the upper streams of the dam lakes, judged from microsatellite markers and STRUCTURE analysis (Pritchard *et al.*, 2000) followed by Yamasaki *et al.* (2020). We also used the ones from the upper reaches above large waterfall(s), where *R. brunneus* is not or rarely found sympatrically. Note that no artificial dam lake is present in Iriomote-jima Island.

The specimens examined in this study are deposited in the following institutions: Kanagawa Prefectural Museum of Natural History, Odawara (KPM); National Museum of Nature and Science (NSMT); Osaka Museum of Natural History, Osaka (OMNH); Museum of Natural and Environmental History, Shizuoka (SPMN). The type series and comparative materials were collected at a time when no permission for the collection was required, or with permission from Okinawa or Kagoshima Prefectures.

All specimen lengths given are standard lengths (SL). Measurements were made point-to-point with calipers (in the case of the lengths more than 10 mm), or micrometer (10 mm or less) attached to the microscope to the nearest 0.1 mm. The methods for measurements followed those of Suzuki *et al.* (2020). The methods for counting followed Prince Akihito *et al.* (1984), except for scales between the origin of a dorsal fin and dorsal insertion of a pectoral fin (counting scales in an oblique row from the dorsalmost point of pectoral-fin base to the origin of the first dorsal fin). The information about squamation is based on specimens stained with Alizarin Red S. The count of scales was examined based on specimens temporary stained with cyanine blue or stained with Alizarin Red S. The observation of cephalic sensory system was examined based on specimens temporary stained with cyanine blue. The number of the first branches of the fifth segmented pelvic-fin ray was counted based on specimens stained with Alizarin Red S at the position where the proximal most segment of each branch alignes transversely (e.g., Fig. 3). Osteological features were observed from radiographs. The method of Akihito *et al.* (2013) is used in describing the pattern of the interdigitation of the dorsal-fin pterygiophores and neural spines ("P-V") with exceptions given below: "9&10" behind the second slash shows that anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of the 9th and 10th vertebrae. The counts of vertebrae follow Akihito *et al.* (2013). Notations of cephalic sensory-canal pores and sensory-papillae rows followed Prince Akihito *et al.* (1984) and Suzuki *et al.* (2017), respectively. In the description, if there are differences between the holotype and paratypes data, data from the holotype are indicated

Table 1. Measurements for *Rhingobius aonumai aonumai*

Cat. No.	OMNH-P 40256	OMNH-P 40254	OMNH-P 40257	KPM-NI 59988	SPMN-PI 46248	OMNH-P 40040	OMNH-P 40043	OMNH-P 43796
Type status	Holotype	Paratypes						
Locality	Hinai-gawa River				Urauchi-gawa River			
Sex	Male	Male	Female	Female	Male	Male	Female	Female
SL (mm)	65.9	66.0	61.5	56.5	48.0	46.3	46.0	47.5
As % in SL								
Head length	34.9	34.1	30.1	31.2	33.3	33.5	32.6	33.7
Predorsal length	41.0	41.7	38.4	38.4	40.2	40.4	39.1	40.6
Length of snout to D2 origin	60.7	62.1	61.3	61.2	60.4	60.5	60.9	61.1
Length of snout to anus	59.2	59.5	57.7	58.4	59.8	57.2	60.0	58.9
Length of snout to A origin	63.1	63.6	62.8	62.1	66.5	63.9	65.2	63.2
Prepelvic length	29.6	28.8	25.4	26.2	31.3	30.2	30.2	29.5
Caudal peduncle length	25.2	23.5	24.4	26.5	24.0	23.8	23.9	22.9
Caudal peduncle depth	12.3	12.1	12.2	12.6	13.1	13.4	13.0	13.5
Length of D1 base	15.9	14.4	16.1	14.5	17.1	15.3	17.2	16.8
Length of longest D1 spine*	15.0 (3rd)	broken	13.0 (2nd)	12.7 (2nd)	21.7 (2nd)	19.2 (2nd)	14.8 (2nd)	15.6 (3rd)
Length of D2 base	17.0	16.7	17.1	16.5	17.1	17.5	17.8	17.7
Length of longest D2 ray*	12.9 (7th)	14.2 (7th)	13.7 (6th)	14.0 (4th)	17.7 (7th)	17.5 (8th)	14.1 (4th)	14.3 (2nd)
Length of last D2 ray	12.9	12.3	11.2	10.3	16.3	15.8	10.7	12.6
Length of A base	13.5	14.8	13.5	13.3	14.6	15.1	13.5	15.4
Length of longest A ray*	13.7 (7th)	12.7 (5th)	12.5 (6th)	13.3 (6th)	15.2 (5th)	14.9 (6th)	13.7 (4th)	13.1 (6th)
Caudal-fin length	22.8	21.2	22.0	20.4	22.9	23.3	23.5	21.7
Pectoral-fin length	23.8	24.8	21.6	23.9	26.0	25.5	23.9	22.1
Pelvic-fin length	15.9	16.4	16.3	15.9	17.9	17.3	18.9	16.4
Body depth of A origin	15.3	14.7	15.9	15.6	15.8	16.6	16.5	17.5
Body width of A origin	12.3	11.4	12.8	12.4	13.5	11.4	12.4	12.0
Length of P2 origin to anus	30.3	32.7	32.8	35.2	29.2	31.5	32.2	31.8
As % in HL								
Snout length	35.7	35.6	32.4	31.3	37.5	34.8	32.7	31.3
Eye diameter	19.1	20.0	21.1	19.3	20.0	20.6	22.0	19.4
Postorbital length	49.1	48.9	48.1	46.0	45.6	45.8	49.3	50.0
Cheek depth	28.3	28.4	27.6	28.4	31.3	29.7	26.7	26.9
Head width in maximum	65.2	62.2	70.8	68.2	61.3	58.1	65.3	51.9
Head depth in maximum	51.7	48.9	56.8	51.1	54.4	52.3	54.0	59.4
Bony interorbital width	4.3	3.6	4.9	4.5	5.0	5.2	4.7	5.0
Upper jaw length	40.9	44.0	37.8	35.8	45.6	41.9	38.0	38.8
As % in Caudal peduncle length								
Caudal peduncle depth	48.8	51.6	50.0	47.3	54.8	56.4	54.5	58.7

Abbreviations: SL: standard length; D1: first dorsal-fin; D2: second dorsal-fin, A: anal fin, P2: pelvic fin. *Longest ray is indicated in parentheses.

by asterisks. In the description of the counts, the frequency of each count is given in parentheses following the relevant count. Description of the coloration was based on digital images photographed on a white background. The names of colors follow those of the Japan Color Research Institute (1995). In "Variations between rivers", proportional characters were measured horizontally on the holotype and 21 paratypes images including all river populations in Iriomote-jima Island.

In the description of *R. aonuma aonumai*, proportional measurements on the holotype and seven paratypes are given in Table 1; The following observations of characteristics are based on the material in parentheses: proportion and fins (holotype and seven paratypes), the first dorsal fin and length of pelvic fin (holotype and 23 paratypes), branching of the pectoral-fin rays, first branches of fifth segmented pelvic-fin ray, and sensory canal pores (holotype and 27 paratypes), squamation (20 paratypes), sensory-papillae rows and coloration when preserved in alcohol (holotype and a paratype), coloration when freshly collected (holotype and 19 paratypes), and coloration when alive (four underwater photographs). In the description of *R. aonumai ishigakiensis,* proportional measurements on the holotype and six paratypes are given in Table 2; the following observations of characteristics are based on the material in parentheses:

Table 2. Measurements for *Rhingobius aonumai ishigakiensis*

Cat. No.	SPMN-PI 49269	KPM-NI 65588	SPMN-PI 49270	OMNH-P 40912	OMNH-P 40914	OMNH-P 40911	OMNH-P 40913
Type status	Holotype	Paratypes					
Locality	Sakuta-gawa River			Miyara-gawa River		Sokobaru-gawa River	
Sex	Male	Male	Female	Male	Female	Male	Female
SL (mm)	51.5	55.9	45.0	38.0	38.4	39.9	33.3
As % in SL							
Head length	32.4	34.0	31.8	31.8	33.9	33.6	31.5
Predorsal length	41.7	42.9	40.0	41.3	40.4	41.4	42.0
Length of snout to D2 origin	60.4	61.7	61.1	62.1	59.9	60.7	60.1
Length of snout to anus	57.1	60.6	59.8	61.1	59.9	59.1	58.6
Length of snout to A origin	61.7	64.4	65.6	64.2	63.5	62.9	61.9
Prepelvic length	30.3	32.2	29.1	29.2	26.0	28.8	24.9
Caudal peduncle length	26.2	25.4	26.2	25.0	23.4	24.8	26.4
Caudal peduncle depth	12.6	12.9	13.3	13.9	14.3	14.3	13.5
Length of D1 base	15.9	14.0	15.8	15.5	14.8	15.0	15.0
Length of longest D1 spine*	25.6 (3rd)	20.8 (2nd)	17.8 (2nd)	31.1 (2nd)	15.6 (2nd)	24.3 (2nd)	14.1 (3rd)
Length of D2 base	16.5	15.4	16.4	17.4	17.2	16.8	15.0
Length of longest D2 ray*	16.1 (8th)	19.7 (7th)	17.1 (2th)	21.1 (7th)	18.0 (3rd)	20.6 (7th)	broken
Length of last D2 ray	14.0	18.2	10.2	19.2	12.2	19.3	8.7
Length of A base	14.2	12.0	12.0	16.3	16.4	15.8	13.5
Length of longest A ray*	15.0 (7th)	17.2 (6th)	16.0 (6th)	17.6 (7th)	16.7 (6th)	17.5 (7th)	14.7 (4th)
Caudal-fin length	24.3	23.3	23.3	27.4	26.0	26.3	24.0
Pectoral-fin length	25.0	23.3	24.4	27.4	24.2	23.8	23.1
Pelvic-fin length	15.5	15.7	18.0	18.2	16.9	16.8	16.2
Body depth of A origin	15.9	16.8	17.3	16.6	17.4	16.8	18.0
Body width of A origin	11.7	10.7	12.4	11.6	10.7	12.0	11.4
Length of P2 origin to anus	29.1	30.1	30.7	31.8	33.9	30.1	34.5
As % in HL							
Snout length	34.1	36.8	35.0	37.2	30.0	35.8	28.6
Eye diameter	18.0	18.9	21.0	22.3	21.5	23.9	24.8
Postorbital length	50.3	46.8	48.3	54.5	46.2	47.8	48.6
Cheek depth	26.3	28.9	25.9	29.8	23.1	28.4	22.9
Head width in maximum	62.3	58.4	66.4	59.5	54.6	63.4	52.4
Head depth in maximum	51.5	52.1	55.9	60.3	53.8	53.0	58.1
Bony interorbital width	4.8	3.2	5.6	4.1	3.8	4.5	4.8
Upper jaw length	41.3	44.7	37.1	46.3	33.1	43.3	37.1
As % in Caudal peduncle length							
Caudal peduncle depth	48.1	50.7	50.8	55.8	61.1	57.6	51.1

Abbreviations: SL: standard length; D1: first dorsal-fin; D2: second dorsal-fin, A: anal fin, P2: pelvic fin. *Longest ray is indicated in parentheses.

proportion and fins (holotype and six paratypes), the first dorsal fin, branching of the pectoral-fin rays, length of the pelvic fin, first branches of fifth segmented pelvic-fin ray and sensory canal pores (holotype and 11 paratypes), squamation (five paratypes), sensory-papillae rows and coloration when preserved in alcohol (holotype and a paratype), coloration when freshly collected (holotype and six paratypes), and coloration when alive (two underwater photographs).

The heights of waterfalls in Iriomote-jima Island refer to Kano *et al.* (2012), except for the Kanpire Fall of Urauchi-gawa River. The heights of Kanpire Fall and waterfalls of Sakuta-gawa River in Ishigaki-jima Island was calculated using the GSI Maps (Geospatial Information Authority of Japan, 2021).

Comparative materials were treated as supplementary materials.

Results

Rhinogobius aonumai sp. nov.

(New Standard Japanese name: Painu-kibara-yoshinobori)

Holotype. OMNH-P 40256, male, 65.9 mm SL, Hinai-gawa River, Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan, upper reaches above Pinaisara Fall (total vertical height 58.7 m), 24°22' 54.99"N 123°49' 13.29"E, 104 m above sea level, 19 Aug. 2012, Fig. 1.

Paratypes. Total 28 specimens (15 males and 13 females, 35.9–70.5 mm SL), collected from Iriomote-jima Island. Aira-gawa River: OMNH-P 40849 (Fig. 6B) and 40852 (Fig. 6A), female and male, 42.3 and 47.5 mm SL, stained with Alizarin Red S., 24°20'54"N 123°54'1"E, 54 m above sea level, 7 May 2014; Geta-gawa River: OMNH-P 40832 (Fig. 6C) and 40836 (Fig. 6D), male and female, 58.0 and 40.5 mm SL, stained with Alizarin Red S., upper reaches above Geta Fall (total vertical height 32.7 m), 24°23'18.59"N 123°51' 36.09"E, 100 m above sea level, 6 May 2014; Hinai-gawa River: KPM-NI 59987 (formerly OMNH-P 40254), and 59988 (formerly OMNH-P 40255) (Fig. 2), male and female, 66.0 and 56.5 mm SL, collected with the holotype; OMNH-P 40257, female, 61.5 mm SL, collected with the holotype; OMNH-P 43694, male, 70.5 mm SL, stained with Alizarin Red S., same locality with the holotype, 13 Aug. 1993; NSMT-P 138485 (Fig. 6E) and 138486 (Fig. 6F), male and female, 56.0 and 55.5 mm SL, stained with Alizarin Red S., collected with the holotype; Kuira-gawa River: KPM-NI 59984 (Fig. 6H), 59985 (Fig. 6G) and 59986, female, male and female, 38.5, 35.9 and 39.0 mm SL, two females are stained with Alizarin Red S., 24°17'23.18"N 123°45'4.25"E, 30 m above sea level, 23 July 2003; Kura-gawa River: OMNH-P 43162 (Fig. 6I) and 43165 (Fig. 6J), male and female, 42.0 and 36.0 mm SL, stained with Alizarin Red S., upper reaches above Kura Fall (total vertical height 7.6 m), 24°23'53.07"N 123°50' 47.73"E, 15 m above sea level, 28 July 2015; Nakara-gawa River: OMNH-P 43153 (Fig. 7A) and 43156 (Fig. 7B), male and female, 47.3 and 37.0 mm SL, stained with Alizarin Red S., upper reaches above Nakara Fall (total vertical height 27.9 m), 24°19'30.16"N 123°47' 9.93"E, 52 m above sea level, 31 July 2015; Nishida-gawa River: OMNH-P 40533 (Fig. 7C) and 40537 (Fig. 7D), male and female, 58.3 and 50.3 mm SL, stained with Alizarin Red S., upper reaches above Sangara Fall (total vertical height 7.2 m), 24°23'1.37"N 123°49' 58.81"E, 30 m above sea level, 1 Aug 2013; Urauchi-gawa River: OMNH-P 40040 (Fig. 7E), male, 46.3 mm SL, upper reaches above Kampire Fall (total vertical height 13.0 m), 24°21'17.18"N 123°48' 27.00"E, 65 m above sea level, 28 March 2012; SPMN-PI 46248 (formerly OMNH-P 40042) and SPMN-PI 46249 (formerly OMNH-P40043) (Fig. 7F), male and female, 48.0 and 46.0 mm SL, collected with OMNH-P 40040; OMNH-P 43689, 43690, 43691 and 43796, three males and female, 51.3, 47.5, 51.1 and 47.5 mm SL, three males are stained with Alizarin Red S., upper reaches above Mariudo Fall (total vertical height 19.4 m), 24°21'25.49"N 123°48' 12.27"E, 34 m above sea level, 10 Aug 1993; Yuchin-gawa River: OMNH-P 40323 (Fig. 7G) and 40327 (Fig. 7H), male and female, 63.0 and 49.2 mm SL, stained with Alizarin Red S., upper reaches above Yuchin Right Falls (total vertical height 44.2 m), 24°21'58.82"N 123°53' 2.39"E, 253 m above sea level, 27 March 2013.

Diagnosis. *Rhinogobius aonumai* is distinguished from all congeneric species by having the following combination of features: 9–15 predorsal scales; 32–38 longitudinal scales; 26–28 vertebrae, mode 27; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 4–14 and 3–10 vertical rows of dark spots or forming dark zigzag bands in males and females, respectively; a pair of short, vertically aligned, rod-shaped dark mark on caudal-fin base in females.

Rhinogobius aonumai aonumai subsp. nov.
(New Standard Japanese name: Iriomote-painu-kibara-yoshinobori)
(Figs. 1–8 & 17A; Tables 1 & 3)

Rhinogobius brunneus (not of Temminck & Schlegel): Hayashi, 1984: 259 (in part: Medium Egg Type, Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

Rhinogobius sp. YB: Iwata, 1989: 598 (in part: Iriomote-jima Island); Akihito *et al.*, 1993: 1082 (in part: Iriomote-jima Island); Akihito et al., 2000: 1255 (in part: Iriomote-jima Island); Akihito *et al.*, 2002: 1255 (in part: Iriomote-jima Island); Suzuki et al., 2004: 460 (in part: Iriomote-jima Island); Akihito et al., 2013: 1461 (in part: Iriomote-jima Island) ; Suzuki *et al.*, 2021: 466 (in part: Iriomote-jima Island).

Holotype. As for *Rhinogobius aonumai* sp. nov.

Paratypes. As for *Rhinogobius aonumai* sp. nov.

Photograph Records from Image Database of Fishes. Arabara-gawa River: KPM-NR 211716–211721, 3 males and 3 females, 28.0–43.0 mm SL, 24°22'14.43"N 123°45' 47.16"E, 118 m above sea level, 18 February 2013, Yo Y. Yamasaki; Hora-gawa River: KPM-NR 211722– 211733, 4 males and 8 females, about 40–55 mm SL, upper reaches above Hora Fall (total vertical height 23.3 m), 24°22'10.71"N 123°54' 3.61"E, 174 m above sea

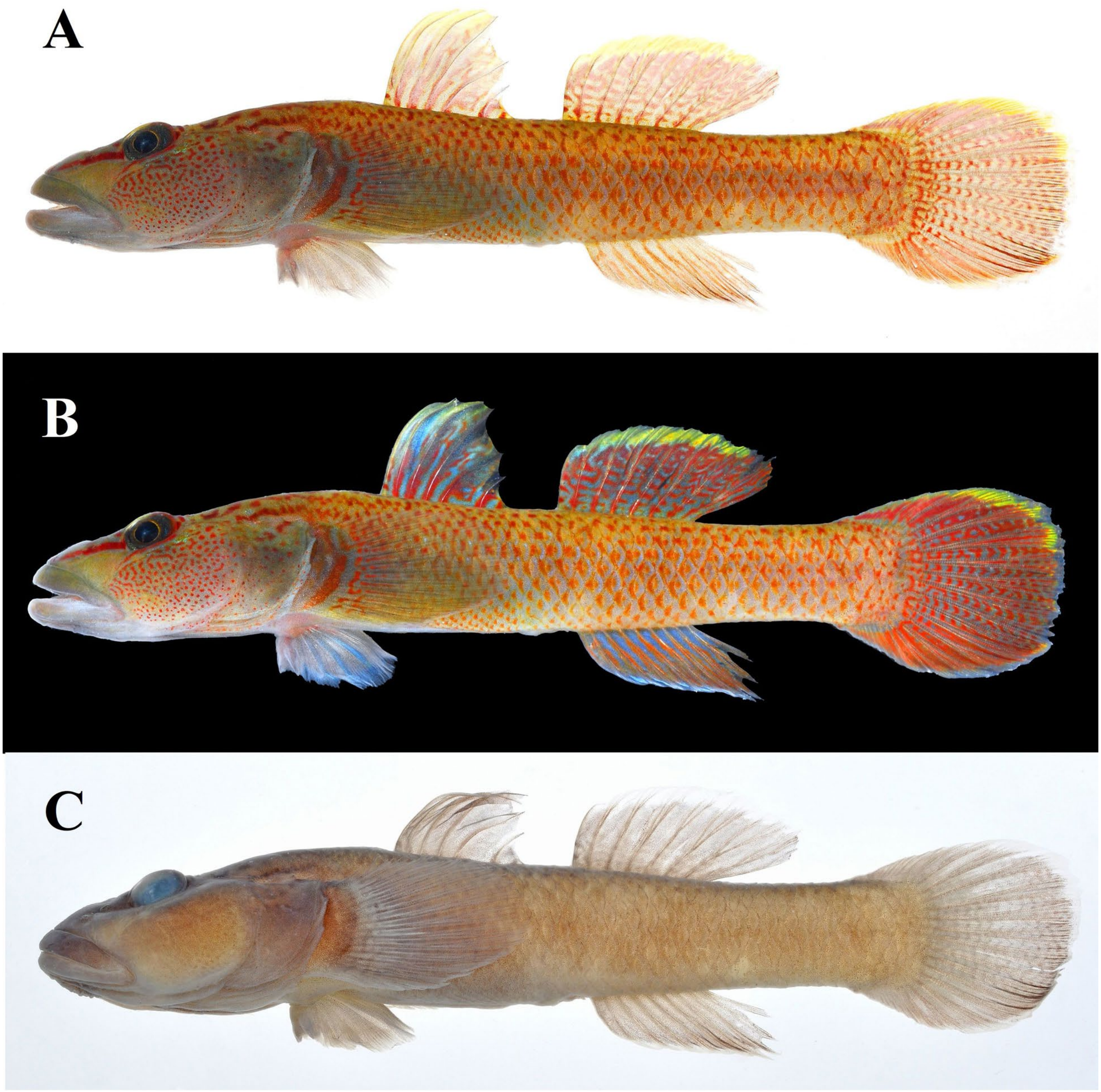

Fig. 1. Holotype of *Rhinogobius aonumai aonumai* (OMNH-P 40256, male, 65.9 mm SL) collected from Hinai-gawa River, Iriomote-jima Island, the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

level, 12 June 2011, Yuichi Kano; Namure-gawa River, a tributary of the Nakama-gawa River System: KPM-NR 211734–211738, 3 males and 2 females, about 40 mm SL, 24°17'50.7"N 123°49' 43.4"E, 15 m above sea level, 15 March 2020, Yoshiyuki Akatuka; Nishifunatsuki-gawa River, a tributary of the Nakama-gawa River System: KPM-NR 211739 and 211740, male and female, about 40 mm SL, 24°18'33.9"N 123°51' 31.0"E, 87 m above sea level, 27 June 2020, Yoshiyuki Akatuka.

Diagnosis. *Rhinogobius aonumai aonumai* is distinguished from all congeneric species-group taxa (species and subspecies) by having the following combination of features: 9–15 predorsal scales; 32–37 longitudinal scales; 11+15–17=26–28 vertebrae, mode 27; anteriormost two pterygiophores (proximal radials) of second dorsal fin mounted over neural spine of tenth (sometimes nineth and tenth) vertebra; fifth segmented pelvic-fin ray divided into 3–4 (usually four) branches at the position where proximal most segment of each branch alignes transversely; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 4–14 and 3–10 vertical rows of dark spots or forming dark zigzag bands in males and females, respectively; a pair of short, vertically aligned,

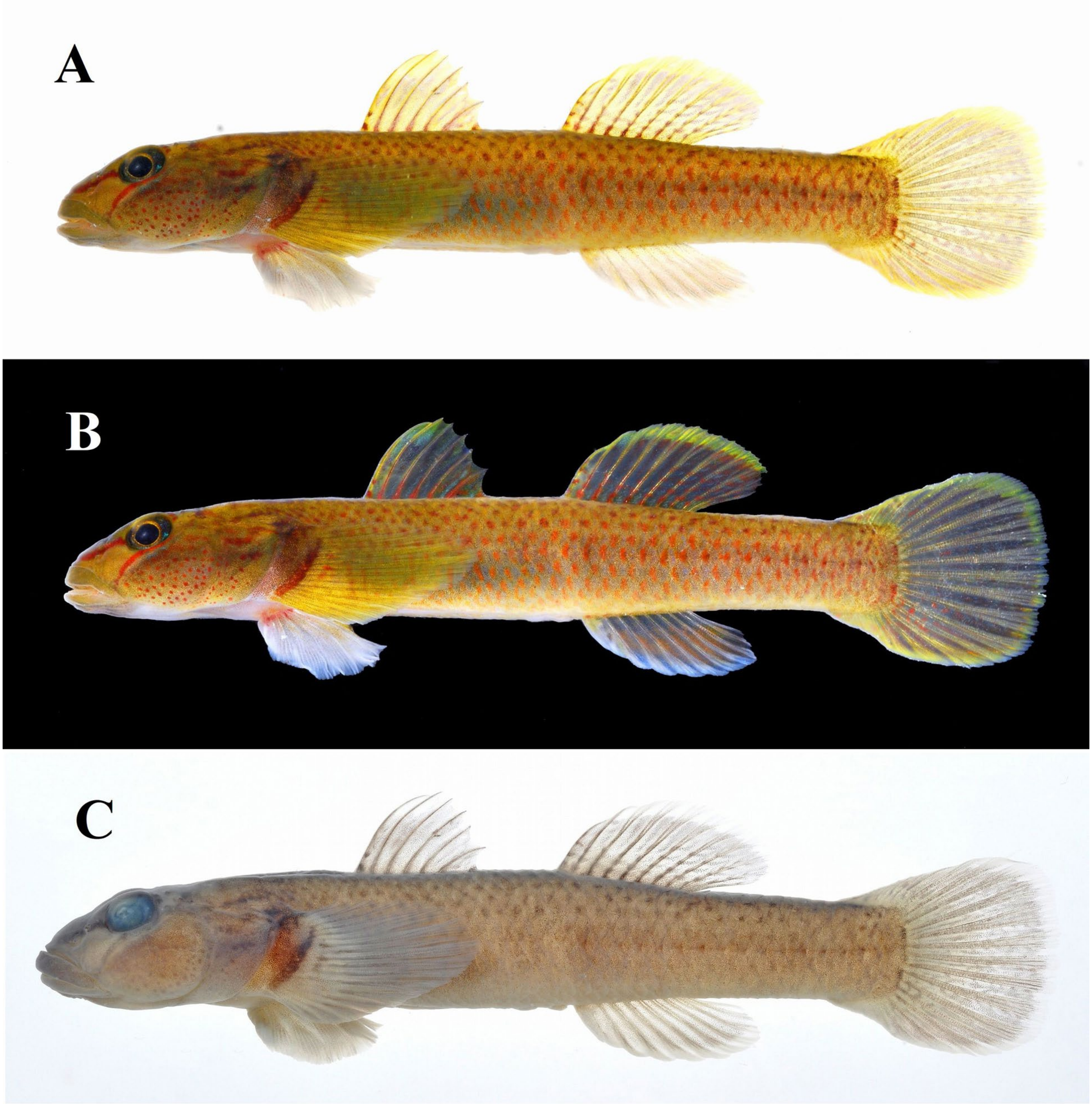

Fig. 2. Female paratype of *Rhinogobius aonumai aonumai* (KPM-NI 59988, 56.5 mm SL) collected from Hinai-gawa River, Iriomote-jima Island, the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

rod-shaped dark mark on caudal-fin base in females.

Description. First dorsal-fin rays V(2), VI*(25) or VII (2); second dorsal-fin rays I, 7* (1), I, 8 (21) or I, 9 (7); anal-fin rays I, 8* (21) or I, 9 (8); pectoral-fin rays (left/right) 18/19 (2), 19/18 (1), 19/19 (16), 20/19 (2) or 20/20* (7); pelvic-fin rays I, 5 (28); segmented caudal-fin rays (upper part + lower part) 8+8 (1) or 9+8* (27); branched caudal-fin rays (upper part + lower part) 6+6 (1), 7+7* (15), 7+8 (2), 8+7 (5), 8+8 (4) or 9+8 (1); longitudinal scales 32 (1), 33 (6), 34 (7), 35 (6), 36* (7) or 37 (1); transverse scales 9 (4), 10 (12), 11 (8) or 12* (3); scales between origin of dorsal fin and dorsal insertion of pectoral fin 7 (8), 8 (9), 9* (9), or 10 (2); predorsal scales 9 (2), 10 (3), 11* (9), 12 (5), 13(5), 14(3) or 15 (1); number of the first branches of fifth segmented pelvic-fin ray (left/right) at the position where proximal most segment of each branch alignes transversely (Fig. 3A) 3/3 (7), 3/4 (1), 4/3 (2) or 4/4* (18); P-V 3/122000/10 (1), 3/122100/10 (1), 3/122110/10 (1), 3/122111/10(1), 3/21210/9&10 (3), 3/212100/10 (1), 3/212101/10 (1), 3/212110/10 (1), 3/221010/10 (1), 3/22110/9&10* (3) or 3/221100/10 (8); vertebrae (abdominal + caudal = total vertebrae) 11+15=26 (1), 11+16=27* (18) or 11+17=28 (2).

Head slightly large and slightly depressed; body slightly slender, slightly compressed anteriorly, and compressed posteriorly (see "Variations between rivers"). Snout nearly pointed, long and longer than eye diameter; snout length of males greater than that of females. Eye large, dorsolateral

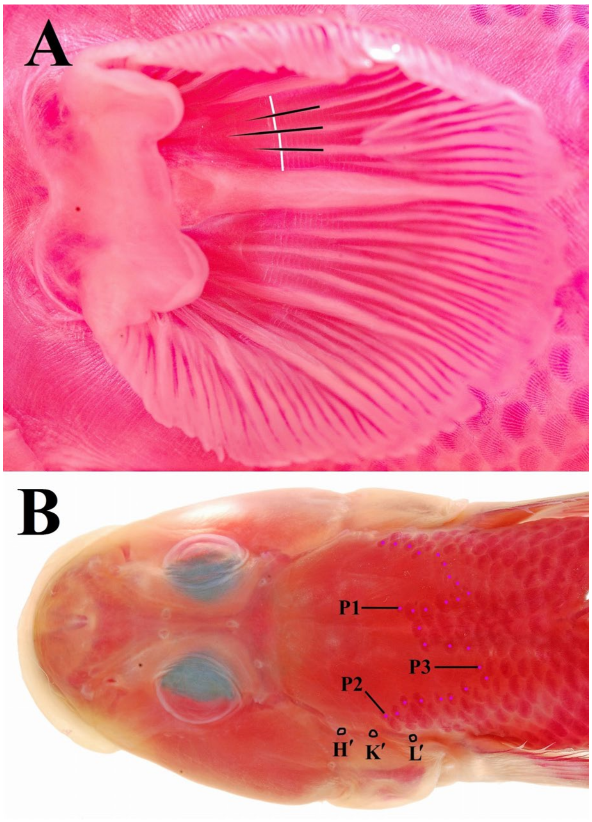

Fig. 3. Ventral view of pelvic fin (A) and dorsal view of head (B), in *Rhinogobius aonumai aonumai* stained with Alizarin Red S. OMNH-P 43694, paratype, male, 70.5 mm SL. White lines indicate position where proximal most segment of each branch alignes transversely with the fifth segmented ray. Black wedge indicates slits between branches. Black circles with black letters H′, K′, and L′ indicate sensory-canal pores. P1, P2, and P3 indicate anteriormost point of anterior extension of scaly area along predorsal midline, anteriormost point of anterior extensions of scaly area on temporal region, and greatest concaved point of scaly area between P1 and P2, respectively. Photographed and annotated by T. Suzuki.

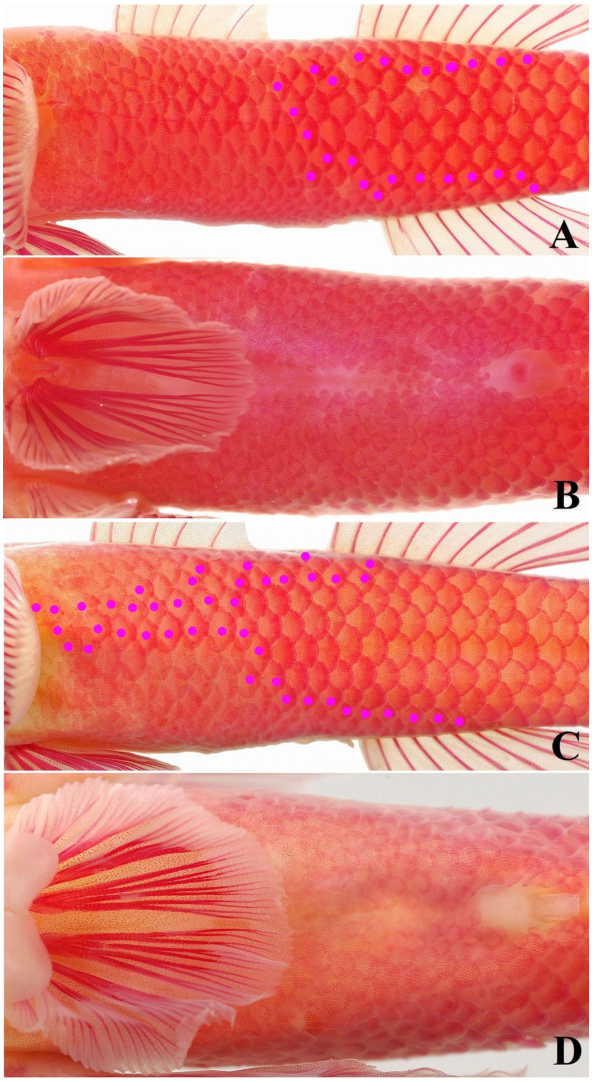

Fig. 4. Lateral view of body (A, C) and ventral view of belly (B, D) in *Rhinogobius aonumai aonumai* stained with Alizarin Red S. A and B: NSMT-P 138485, paratype, male, 56.0 mm SL, Hinai-gawa River; C and D: OMNH-P 40852, paratype, male, 47.5 mm SL, Aira-gawa River. Pink spots indicate the lateral anterior margin of ctenoid scale area. Photographed and annotated by T. Suzuki.

on head, and located slightly behind a vertical through midpoint between snout tip and posterior margin of preopercle. Cheek somewhat bulbous and fleshy. Lips thick and fleshy; anterior tips of both lips even or usually lower lip slightly protruding anteriorly*; gape slightly oblique; posterior margin of lower jaw extending posteriorly to a vertical through anterior margin of eye in females and slightly beyond it* in males. Anterior naris a short tube without skin flap at its tip, and its base located slightly behind the midpoint between snout tip and anterior margin of eye. Posterior naris a round pore with low rim and located at the midpoint between base of anterior naris and anterior margin of eye. Gill opening extending anteriorly to a vertical through posterior margin of preopercle. Gill membranes broadly attached to isthmus. No fleshy papillae or finger-like projections on lateral margin of shoulder girdle. Tongue free from floor of mouth, with rounded anterior margin. Genital papillae cone-shaped* in males and oval in females.

Origin of first dorsal fin about an eye diameter behind a vertical through dorsal insertion of pectoral-fin; first dorsal fin in males near falcate shape, pentagon (becoming slightly narrower distally) shape or semioval*, and higher than second dorsal fin, whereas, in females, semicircular or semioval, and slightly higher than, or slightly lower than second dorsal fin in height; usually second, or third* spine longest; all dorsal-fin spines slender and flexible; usually not filamentous*, or distal tip of second or third spine slightly filamentous; posterior tip (= distal tip of second, usually third, or fourth* spines) of first dorsal fin

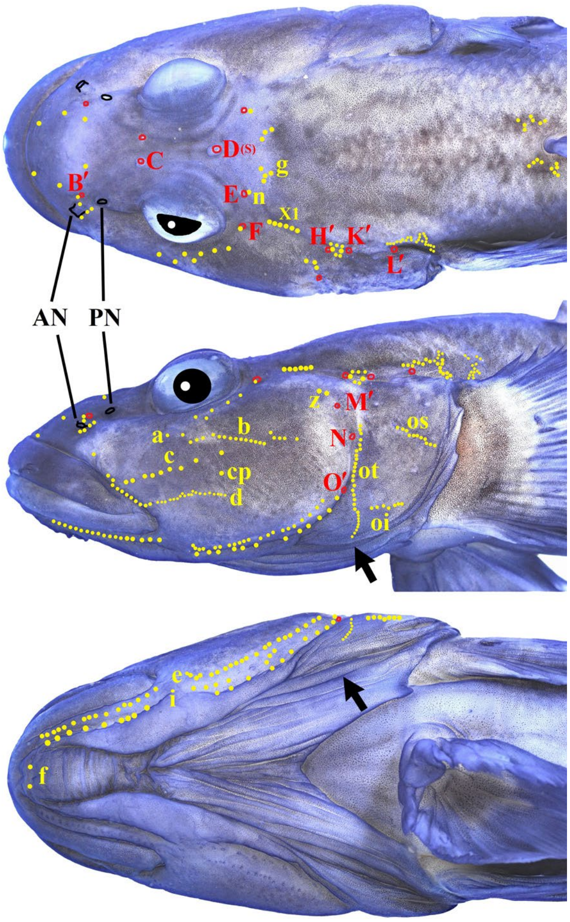

Fig. 5. Dorsal (top), lateral (middle), and ventral (bottom) views of head of *Rhinogobius aonumai aonumai* stained with cyanine blue showing cephalic sensory pores and papillae. OMNH-P 40256, holotype, male, 65.9 mm SL Red circles with red letters indicate sensory canal pores (letters with prime marks indicate terminal openings of sensory canals); rows of yellow spots indicated by yellow letters represent sensory papillae rows; black arrows show ventralmost positions of gill opening. Abbreviations: AN, anterior narial pore; PN, posterior narial pore. Photographed and annotated by T. Suzuki.

in males extending between front of origin* and base of third segmented ray of second dorsal fin when adpressed, whereas, in females, posterior tip (= distal tip of third, fourth, fifth or usually sixth spines) not extending to origin of second dorsal fin when adpressed. First and second dorsal fins not connected by membrane (see “Variations between rivers”); all segmented rays of second dorsal fin branched; seventh* or eighth, and second, fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last* or penultimate segmented ray) of second dorsal fin usually not extending* to caudal fin, or to it when adpressed; posterior end of second dorsal-fin above posterior end of anal-fin base. Origin of anal fin below between bases of first and second segmented rays of second dorsal fin; anal fin equal to*, or usually slightly lower than second dorsal fin in height; all segmented anal-fin rays branched; fifth, sixth or seventh* and fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate* segmented ray) of anal fin not extending to caudal fin when adpressed. Pectoral fin oval, extending posteriorly to a vertical between base of sixth spine and posterior end of base* of first dorsal fin; all pectoral-fin rays branched, except for dorsalmost ray usually not branched (left side*), or branched once (right side*); ventralmost ray not branched (both side*), or branched once. Pelvic fins fused medially by well-developed frenum (between spines) and connecting membrane (between innermost rays), forming a circular cup-like disc; pelvic fins extending posteriorly to a vertical between bases of second and fifth spines of first dorsal fin (base of third spine*) (see “Variations between rivers”); pelvic fins not reaching to anus; pelvic-fin spine with a rounded membranous lobe at its tip; all segmented rays of pelvic fin branched. Caudal fin elliptical or fan-shaped*.

Scales on body largely ctenoid, becoming smaller anteriorly; anterior edge of scaled areas with ctenoid scales on lateral, dorsal and ventral sides of body reaching respectively to a vertical between a little behind pectoral-fin axilla and origin of second dorsal fin, to second dorsal-fin base, and to anal-fin base (see “Variations between rivers”; Figs. 4A, 4C). Small cycloid scales on anterodorsal part of body before the area with ctenoid scales. Predorsal squamation with trifurcate anterior edge; its mid-anterior extension (Fig. 3B: P1) extending anteriorly to between transverse lines through sensory-canal pores H′ and L′; anterior extensions of lateral sides (Fig. 3B: P2) extending anteriorly to posterior oculoscapular canal; greatest concaved point of anterior margin of scaled area of predorsal region between P1 and P2 (Fig. 3B: P3) extending posteriorly to above between sensory-canal pore H′ and upper end of pectoral-fin axilla. Pectoral-fin base usually naked, or with one or two small cycloid scales. Prepelvic area usually naked, or with some small cycloid scales. Scaled area on belly with small cycloid scales usually extending anteriorly to, or not, side of prepelvic area; belly above pelvic fin naked, and its following anterior part of ventral midline of belly usually naked, or with some small cycloid scales (see “Variations between rivers”; Figs. 4B, 4D).

Cephalic sensory systems are illustrated in Fig. 5. Nasal extension of anterior oculoscapular canal with terminal pore B′ located above both nares. Anterior interorbital sections of the anterior oculoscapular canal separated

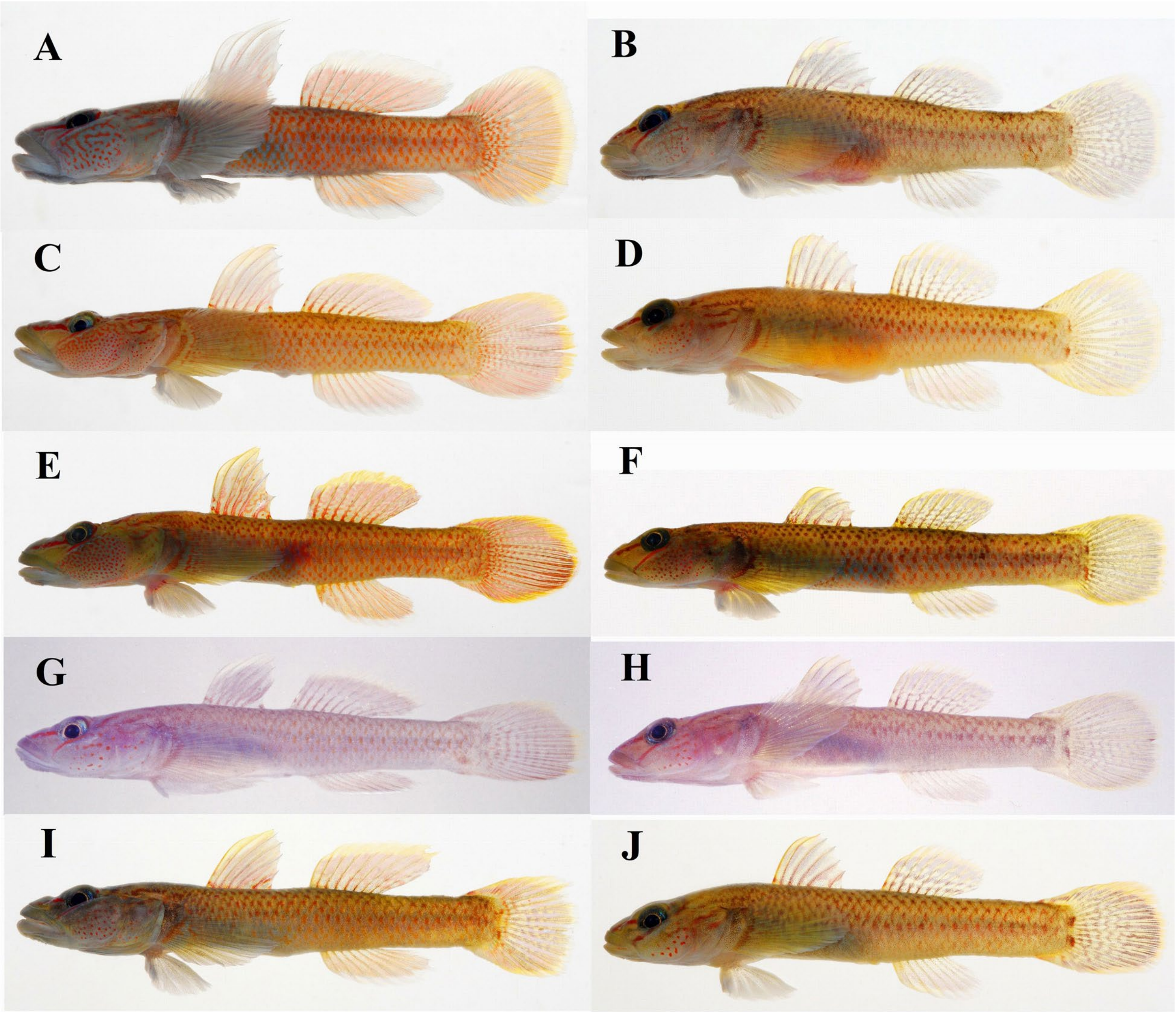

Fig. 6. Freshly-collected paratypes of *Rhinogobius aonumai aonumai* from Iriomote-jima Island, the Ryukyu Islands, Japan. Aira-gawa River: A (OMNH-P 40852, male, 47.5 mm SL) and B (OMNH-P 40849, female, 42.3 mm SL); Geta-gawa River: C (OMNH-P 40832, male, 58.0 mm SL) and D (OMNH-P 40836, female, 40.5 mm SL); Hinai-gawa River: E (NSMT-P 138485, male, 56.0 mm SL) and F (NSMT-P 138486, female, 55.5 mm SL); Kuira-gawa River G: (KPM-NI 59985, male, 35.9 mm SL) and H (KPM-NI 59984, female, 38.5 mm SL); Kura-gawa River: I (OMNH-P 43162, male, 42.0 mm SL) and J (OMNH-P 43165, female, 36.0 mm SL). Photographed by T. Suzuki.

bilaterally, with paired pore C and a single pore D. Pore E present just behind posterior edge of eye. Lateral section of anterior oculoscapular canal with anterior pore F and terminal pore H′. Posterior oculoscapular canal with two terminal pores K′ and L′. A gap between anterior and posterior oculoscapular canals much narrower than the length of the posterior oculoscapular canal. Preopercular canal present, with three pores M′, N, and O′. In the three paratypes, there is an accessory pore on the canal between pores C and D, pores D and E, and pores M′ and N. Sensory-papillae row “a” oblique and uniserial, composed of loosely-arranged papillae, and extending anteriorly to a vertical through midpoint between anterior margin of eye and anterior margin of pupil. Row “b” longitudinal, composed of densely-arranged papillae, extending anteriorly to a vertical through the center of pupil, and longer than eye diameter in length. Row “c” composed of loosely-arranged papillae, extending posteriorly to a vertical through posterior margin of the eye. Row “d” composed of densely-arranged papillae, extending posteriorly to a vertical through posterior margin of the pupil. Row “cp” comprising a single papilla. Row “f” comprising paired papillae. Anterior end of row “oi” a little separated from a vertical row “ot”.

Coloration of male when freshly collected (Figs. 1A, 1B, 6A, 6C, 6E, 6G, 6I, 7A, 7C, 7E, 7G). Ground color of head and body usually reddish yellow. Belly pale yellow, and its side greenish. Head grayish; cheek, operculum and gill membrane usually with densely-arranged, many small reddish orange dots (see “Variations between rivers”). Snout with a broad oblique reddish orange stripe between anterior margin of eye and anteriormost part

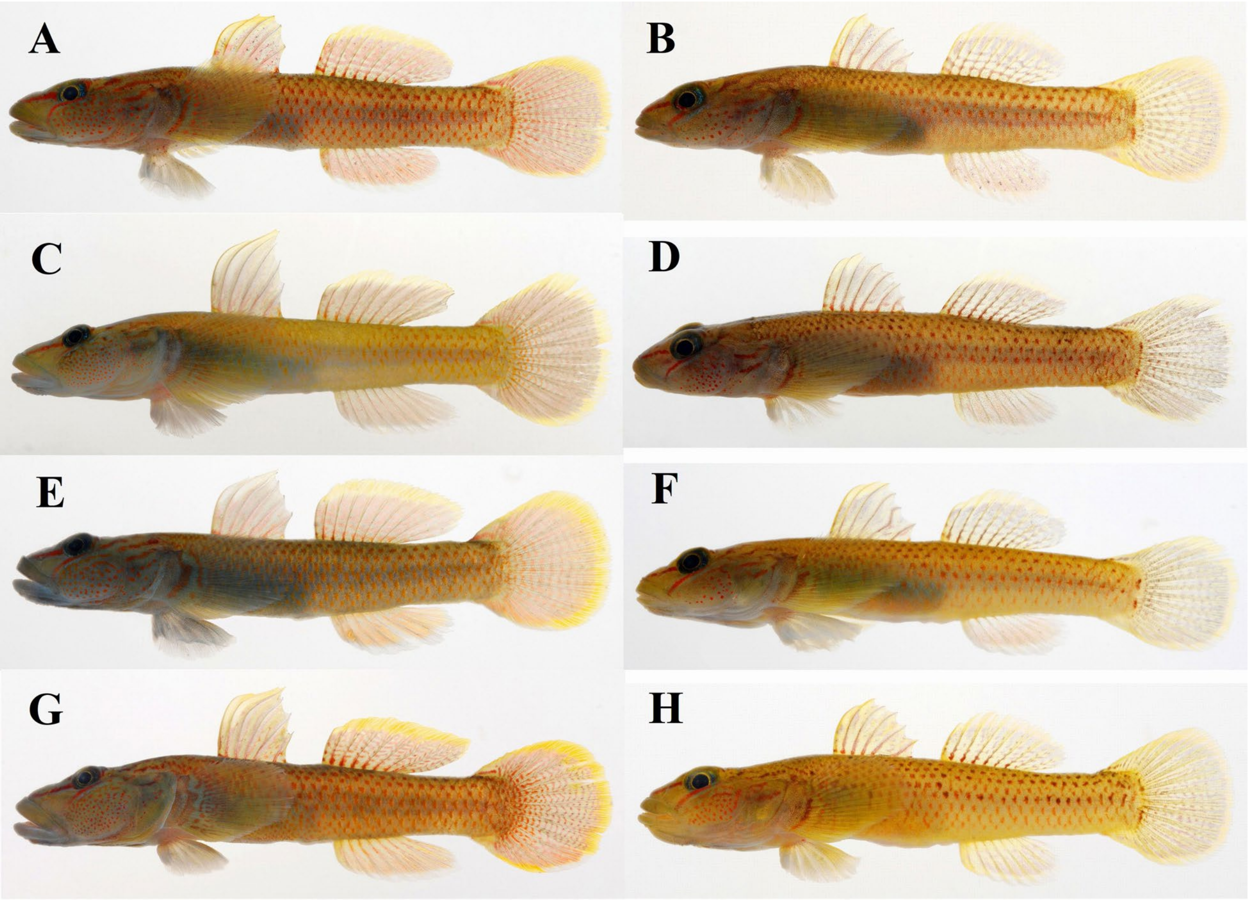

Fig. 7. Freshly-collected paratypes of *Rhinogobius aonumai aonumai* from Iriomote-jima Island, the Ryukyu Islands, Japan. Nakara-gawa River: A (OMNH-P 43153, male, 47.3mm SL) and B (OMNH-P 43156, female, 37.0 mm SL); Nishida-gawa River: C (OMNH-P 40533, male, 58.3 mm SL) and D (OMNH-P 40537, female, 50.3 mm SL); Urauchi-gawa River: E (OMNH-P 40040, male, 43.6 mm SL) and F (SPMN-PI 46249, female, 46.0 mm SL); Yuchin-gawa River: G (OMNH-P 40323, male, 63.0 mm SL) and H (OMNH-P 40327, female, 49.2 mm SL). Photographed by T. Suzuki.

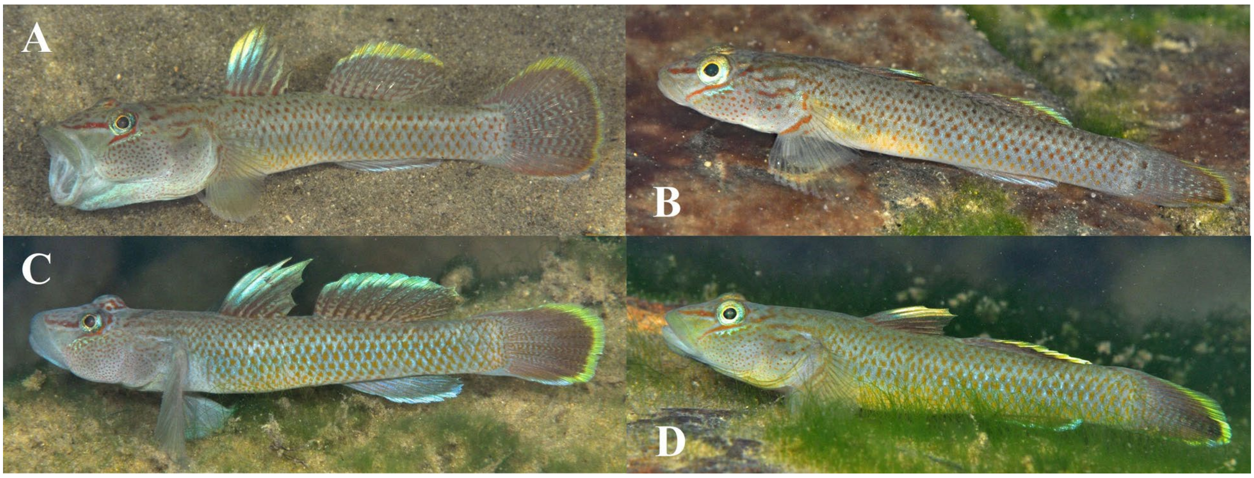

Fig. 8. Underwater photographs of *Rhinogobius aonumai aonumai* taken at Iriomote-jima Island, the Ryukyu Islands, Japan. Hinai-gawa River: A (male, about 60 mm SL) and B (female, about 45 mm SL); Urauchi-gawa River: C (male, about 50 mm SL) and D (female, about 40 mm SL). Photographed by M. Suzuki.

of snout. Several irregular-shaped, short reddish orange stripes and minute spots on dorsal surface of snout and interorbital space, occipital region and nape; anterodorsal margin of cheek with a narrow reddish orange line edged ventrally by a bluish green line; temporal region above operculum with usually a short reddish orange stripe*, or dashed line; upper part of operculum with usually an oblique reddish orange stripe, or dashed line*. Almost all scale pockets on body with reddish orange spots. Ground color of fin membranes pale yellow, partially with bluish sheen. Spines and segmented rays gray. Anterodorsal part of first dorsal fin and distal margin of second dorsal bright yellow; distal margin of anal fin white; distal margin of caudal fin usually bright yellow, or ventral margin white*; membranes around spines and segmented rays of first dorsal and anal fins, and upper and lower parts of caudal fin reddish orange. Second dorsal fin with usually 1–6 (5*) longitudinal rows of reddish orange spots* or forming zigzag stripes; indistinct pale orange mottles above these spots or stripes. Central part of caudal fin with usually 4–14 (13*) vertical rows of reddish orange* or pale orange spots, or forming zigzag bands. Pectoral-fin base with a distinct reddish orange bow-shaped mark, and one or two* vertical rows of reddish orange spots behind it.

Coloration of female when freshly collected (Figs. 2A, 2B, 6B, 6D, 6F, 6H, 6J, 7B, 7D, 7F, 7H). Resembles that of male, except as follows. Orange marks of head and body darker. Orange dots on cheek fewer in number; on operculum fewer in number or absent; gill membranes with no orange dots. Ventral side of body usually with no orange dots. Spines and segmented rays of fins except for those of first dorsal and pelvic fins yellowish. Second dorsal fin with 3–5 longitudinal rows of reddish orange spots or forming zigzag stripes; membrane above these spots or stripes pale pink. Caudal-fin base with a pair of short, vertically aligned, rod-shaped deep reddish orange mark; central part of caudal fin with 3–10 vertical rows of pale pink spots or forming zigzag bands, membrane behind these spots or bands usually pale pink, or forming indistinct mottles. Pectoral-fin base with a deep reddish orange band or a vertical row of or reddish orange spots behind a bow-shaped mark.

Coloration when alive based on underwater photographs (Fig. 8). Coloration when alive in the populations of Hinai-gawa and Urauchi-gawa rivers in Iriomote-jima Island resembles that of freshly-collected coloration, except as follows. In the population of Hinai-gawa River, ground color of head and body light grayish green; belly bright yellow. In the population of Urauchi-gawa River, head and body greenish; belly bright yellow; orange and pink color darker; anterodorsal part of first dorsal fin and distal margin of second dorsal fin bright greenish in males.

Coloration when preserved in alcohol (Figs. 1C, 2C). Ground color of head and body turned to yellowish gray; blue, green, pink, yellow and white color faded; orange and red color turns to brown or gray. Upper half of body with 1–2, and 3–4 longitudinal row(s) of brown spots in males and females, respectively.

Variations between rivers. In females of the population of Hinai-gawa River, head length is shorter [28.1–28.9 % in SL (average 28.6 %) vs 29.0–31.6 % (average 30.8 %) than those of the other populations]; head, body, and caudal peduncle are lower in depth [head depth 14.2–14.8 % (average 14.5 %) vs 16.4–18.7 % (average 17.5 %), body depth at origin of pelvic-fin 14.2–14.8% (average 14.5 %) vs 16.4–18.7 % (average 17.6 %), caudal peduncle depth 11.0–11.4 % (average 11.2 %) vs 10.7–12.8 % (average 12.2 %)]; interspace between dorsal fins longer [8.2–8.6 % (average 8.5 %) vs 4.9–8.1 % (average 6.5 %)] than females of the other populations.

In the population of Hinai-gawa River, pelvic fins are shorter, extending usually posteriorly to a vertical between bases of the second and third spines of the first dorsal fin, whereas the fins extend posteriorly to a vertical between bases of the third and fifth spines of the first dorsal fin in the other populations. In the populations of Kuira-gawa and Aira-gawa rivers, anterior margin of the ctenoid scale area extends to a little behind pectoral-fin axilla (Fig 4A), whereas the area extends to a vertical between origins of first and second dorsal fins in the other population (Fig. 4C); ventral midline of belly largely covered with small cycloid scales (Fig. 4D), although naked except posterior part in other populations (Fig. 4B).

Furthermore, in the population of Kuira-gawa River, some small reddish orange dots on the cheek, the lower half of operculum, and gill membrane in males (Fig. 6G), but many densely-arranged dots are present in those of the other populations; females lack orange spots on the lower half of body, whereas many spots are seen in those of the other populations (Fig. 6H).

Distribution and habitat. *Rhinogobius aonumai aonumai* is known only from Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. In this island, it is seen in the upper reaches of freshwater streams above waterfalls in montane areas of Geta-gawa, Hinai-gawa, Kura-gawa, Nakara-gawa, Nishida-gawa, Urauchi-gawa and Yuchin-gawa rivers, and the upper reaches of swift freshwater streams without waterfalls of Aira-gawa and Kuira-gawa rivers. In addition, we identified the gobies in the photographs taken from Arabara-gawa, Hora-gawa, and Nakama-gawa rivers registered in the Image Database of Fishes, as *R. a. aonumai.* The subspecies is usually found in small pools of streams, called “Fuchi” in Japanese.

Etymology. The specific/subspecific name, *aonumai*, refers to Dr. Yoshimasa Aonuma, who was the pioneer in the taxonomic study of the genus *Rhinogobius* in the Ryukyu Islands. In his master's thesis, he was the first to suggest that the number of vertebrae in *Rhinogobius* sp.

Table 3. Comparisons of 55 species-group taxa (species and subuspecies) of *Rhinogobius* with high or unknown vertebral counts

Species	Vertebrae	Longitudinal scales	Dark spot of D1	Bands or vertical rows of dark spots on C.	Reference
R. albimaculatus	28	29–31	present	present	Chen *et al*., 1999a
R. aonumai aonumai	26–28	32–37	absent	present	This study
R. a. ishigakiensis	26–28	33–38	absent	present	This study
R. boa	27	30–33	present	present	Chen & Kottelat, 2005
R. bucculentus	unknown	40–44	absent	absent	Herre, 1927
R. carpenteri	28–29	34–40	absent	absent	Endruweit, 2017; Seale, 1910
R. changtinensis	27	28–30	present	present	Huang & Chen, 2007
R. cheni	27	34–36	present	present	Chen *et al*., 2008; Koumans, 1940; Nichols, 1931
R. chiengmaiensis	28	26–30	present	present	Chen *et al*., 2008; Fowler, 1934; Koumans, 1940
R. cliffordpopei	unknown	28–29	present	present	Nichols, 1925; Wu & Chen, 2008
R. coccinella	28	27–30	present	present	Endruweit, 2018
R. davidi	28	30–31	present	absent in male; present in female	Chen & Miller, 1998; Sauvage & Dabry de Thiersant, 1874
R. duospilus	27	29–31	present	present	Herre, 1935; Huang & Chen, 2007
R. filamentosus	27	30–33	present	present	Chen *et al*., 2008; Wu, 1939
R. flumineus	27–28	30–36	absent	absent or present	Akihito *et al*., 2013 & Errata; Mizuno, 1960b; This study
R. fukushimai	unknown	30–31	absent	present	Mori, 1934; Wu & Chen, 2008
R. genanematus	27	27–29	absent	present	Chen & Fang, 2006; Zhong & Tzeng, 1998
R. henryi	28	30–32	present	present	Chen *et al*., 2008; Herre, 1938
R. honghensis	28	32–34	present	present	Chen *et al*., 1999c
R. houheensis	30	37–40	present	absent	Wanghe *et al.*, 2020
R. imfasciocaudatus	27–28	30–31	present	absent	Endruweit, 2018; Nguyen & Vo, 2005
R. immaculatus	27–28	29–31	absent	present	Li *et al*., 2018
R. lentiginis	27	30–32	present	absent in male; present in female	Chen & Miller, 1998; Wu & Zheng, 1985; Wu & Chen, 2008
R. lindbergi	27–28	30–32	present	present	Berg,1933; Sakai *et al*., 2000
R. lineatus	28	29–32	present	present	Chen *et al*., 1999a
R. linshuiensis	27–28	27–31	present	present	Chen & Miller, 2014; Chen *et al*., 2002
R. liui	29	35–39	present	present	Chen & Wu, 2008
R. longyanensis	27	30–32	present	absent	Chen *et al*., 2008
R. lungwoensis	28	31–34	present	present	Huang & Chen, 2007
R. maculagenys	27	32–34	present	present	Wu *et al*., 2018
R. maculicervix	28	29–32	present	present	Chen & Kottelat, 2000
R. maxillivirgatus	27	28–30	present	present	Xia *et al*., 2018
R. mekongianus	28	29–31	present	present	Chen *et al*., 1999a; Pellegrin & Fang 1940
R. milleri	27	28–31	present	present	Chen & Kottelat, 2003
R. multimaculatus	29	33–37	present	present	Chen *et al*., 1999b; Wu & Zheng, 1985
R. nammaensis	28	30–32	present	present	Chen & Kottelat, 2003
R. nanophyllum	28	29–32	present	present	Endruweit, 2018
R. ngutinhoceps	29	30–32	present	present	Endruweit, 2018
R. niger	27–28	35–37	present	absent in male; present in female	Huang *et al*., 2016
R. parvus	27	28–30	absent	absent in male; present in female	Huang *et al*., 2016; Luo, 1989
R. philippinus	unknown	36–40	absent	absent	Herre, 1927
R. phuongae	28–29	28–32	present	absent in male; present in female	Endruweit, 2018
R. ponkouensis	28	32–34	present	present	Huang & Chen, 2007
R. rubromaculatus	27–28	29–32	present	present	Lee & Chang, 1996; Wu & Chen, 2008
R. shennongensis	unknown	31–33	absent	present	Wu & Chen, 2008; Yang & Xie, 1983
R. sowerbyi	unknown	35–36	absent	absent	Ginsburg, 1917
R. sulcatus	27–28	26–29	present	present	Chen & Kottelat, 2005
R. szechuanensis	27	30–34	absent	present	Chen *et al.,* 2008; Tchang, 1939; Wu & Chen, 2008
R. taenigena	27	29	present	present	Chen *et al*., 1999a
R. vermiculatus	28	32–34	present	present	Chen & Kottelat, 2003
R. wangchuangensis	27	28–30	present	absent in male; present in female	Chen & Miller, 2014; Chen *et al*., 2002
R. wangi	27	26–27	present	present	Chen & Fang, 2006
R. wuyanlingensis	27	30–32	present	present	Yang *et al*., 2008
R. xianshuiensis	27	29–32	present	present	Chen *et al*., 1999b; Wu & Chen, 2008
R. yaoshanensis	28	29–31	present	present	Chen *et al*., 2008; Luo 1989

Abbreviations: D1, first dorsal fin; C, caudal fin

YB from the Yaeyama Group of the Ryukyu Islands was higher than those from other islands of the Ryukyu Islands (Aonuma, 1992).

Rhinogobius aonumai ishigakiensis **subsp. nov.**

(New Standard Japanese name: Ishigaki-painu-kibara-yoshinobori)
(Figs 9–15 & 17B; Tables 2 & 3)

Rhinogobius brunneus (not of Temminck & Schlegel): Hayashi, 1984: 259 (in part: Medium Egg Type, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

Rhinogobius sp. YB: Iwata, 1989: 598 (in part: Ishigaki-jima Island); Akihito *et al.*, 1993: 1082 (in part: Ishigaki-jima Island); Akihito *et al.*, 2000: 1255 (in part: Ishigaki-jima Island); Akihito et al., 2002: 1255 (in part: Ishigaki-jima Island); Suzuki *et al.*, 2004: 460 (in part: Ishigaki-jima Island); Akihito *et al.*, 2013: 1461 (in part: Ishigaki-jima Island); Suzuki *et al.*, 2021: 466 (in part: Ishigaki-jima Island).

Holotype. SPMN-PI 49269, male, 51.5 mm SL, Sakuta-

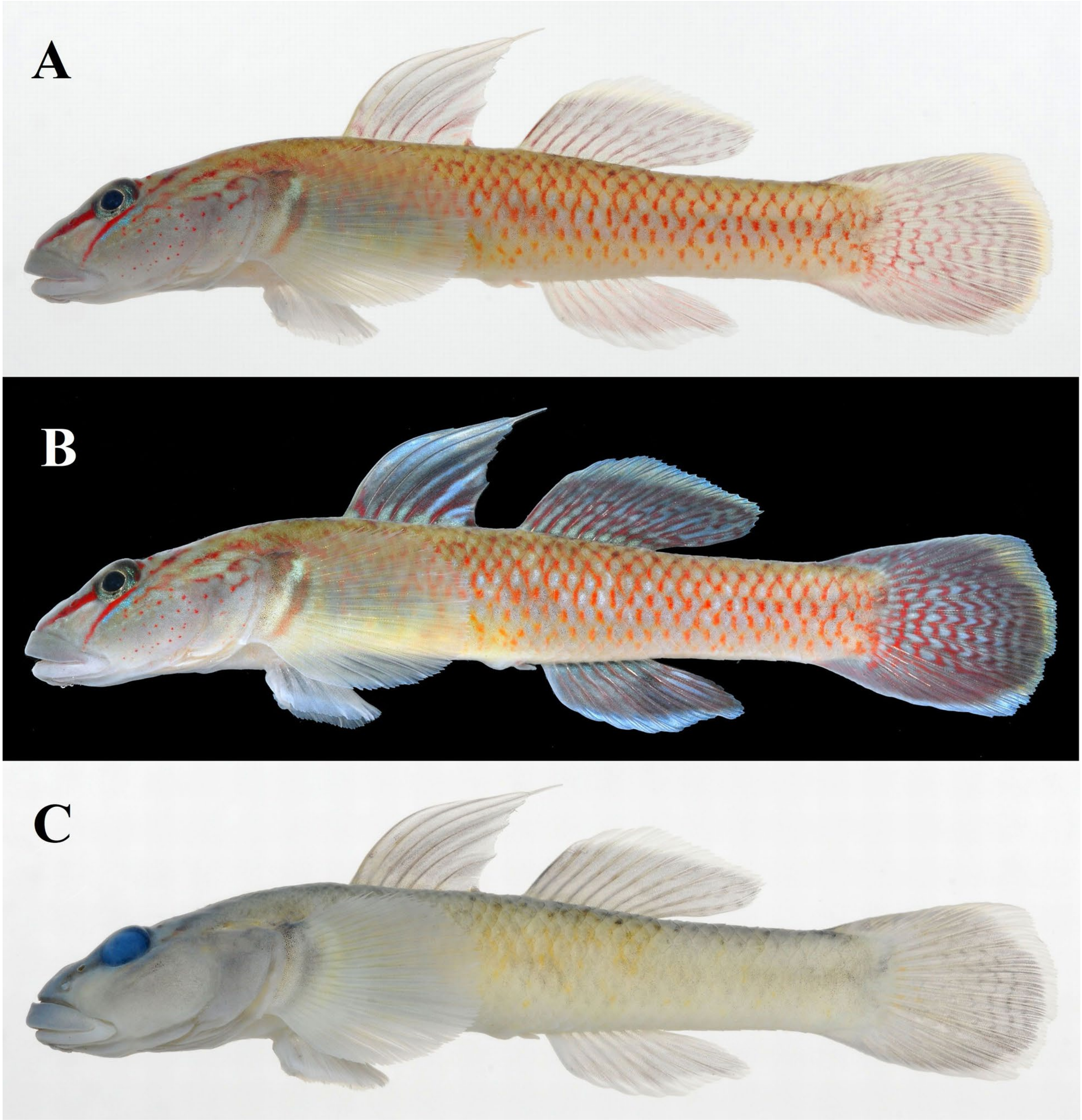

Fig. 9. Holotype of *Rhinogobius aonumai ishigakiensis* (SPMN-PI 49269, male, 51.5 mm SL) collected from Sakuta-gawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

gawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan, upper reaches above some falls (total vertical height about 100 m), 165 m above sea level, 26 Oct. 2020, Fig. 9.

Paratypes. Total 11 specimens (6 males and 5 females, 33.3–56.5 mm SL), collected from Ishigaki-jima Island. Miyara-gawa River: OMNH-P 40912 (Fig. 14A) and 40914 (Fig. 14B), male and female, 38.0 and 38.4 mm SL, upper stream above Maezato Dam, 93 m above sea level, 19 June 2014; Sakuta-gawa River: OMNH-P 48920, 48922, 48923 and 48924, three males and female, 38.0, 37.2, 35.5 and 35.5 mm SL, stained with Alizarin Red S., the same locality as holotype, 18 Sep 2020, KPM-NI 65589 and 65588 (Fig. 14C), female and male, 56.5 and 55.9 mm SL, female is stained with Alizarin Red S., the same locality as holotype, 19 Oct. 2020, SPMN-PI 49270 (Figs. 10, 14D), female, 45.0 mm SL, collected with the holotype; Sokobaru-gawa River, a tributary of the Miyara-gawa River System: OMNH-P 40911 (Fig. 14E) and 40913 (Fig. 14F), male and female, 39.9 and 33.3 mm SL, upper stream above Sokobaru Dam, 159.6 m above sea level, 22 June 2014.

Photograph Records from Image Database of Fishes. Ayamashi-gawa River, a tributary of the Miyara-gawa River System: KPM-NR 217906 , male, about 40 mm SL, 24°23′47.59″N 124°11′07.28″E, 34 m above sea

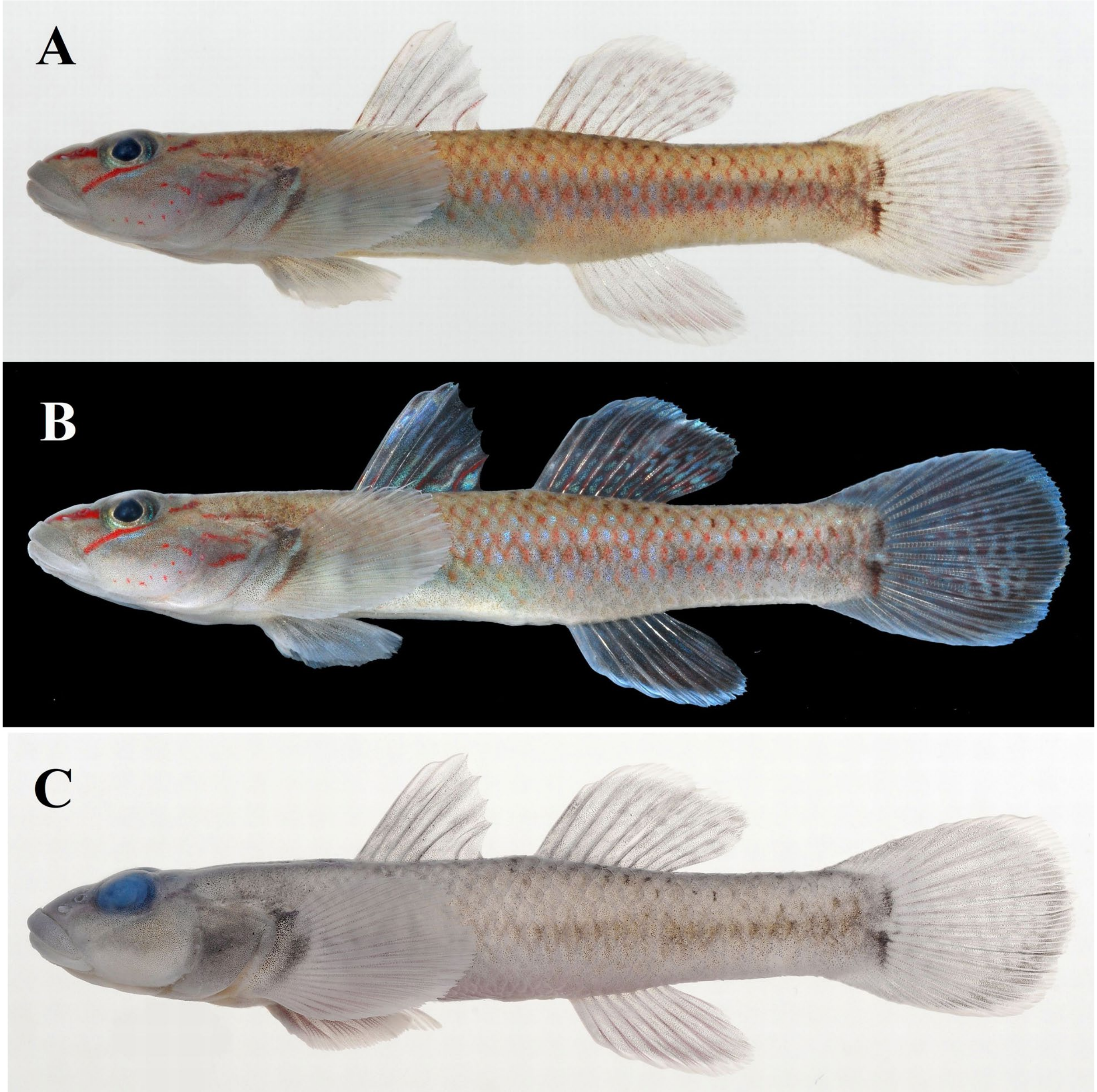

Fig. 10. Female paratype of *Rhinogobius aonumai ishigakiensis* (SPMN-PI 49270, female, 45.0 mm SL) collected from Sakuta-gawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

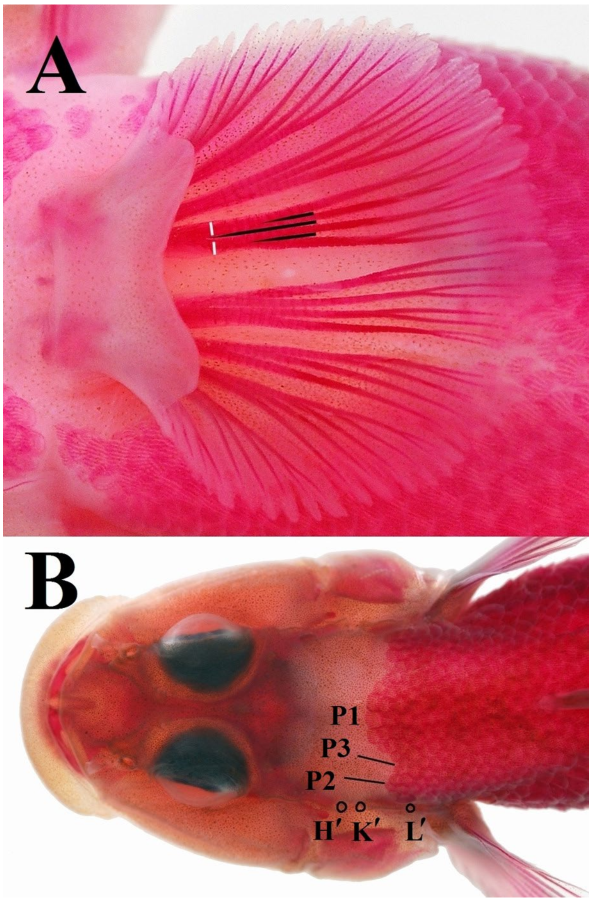

Fig. 11. Ventral view of pelvic fin (A) and dorsal view of head (B), in *Rhinogobius aonumai ishigakiensis* stained with Alizarin Red S. OMNH-P 48923, paratype, male, 35.5 mm SL, Sakuta-gawa River. White lines indicate position where proximal most segment of each branch alignes transversely with the fifth segmented ray. Black wedge indicates slits between branches. Black circles with black letters H′, K′, and L′ indicate sensory-canal pores. P1, P2, and P3 indicate anteriormost point of anterior extension of scaly area along predorsal midline, anteriormost point of anterior extensions of scaly area on temporal region, and greatest concaved point of scaly area between P1 and P2, respectively. Photographed and annotated by T. Suzuki.

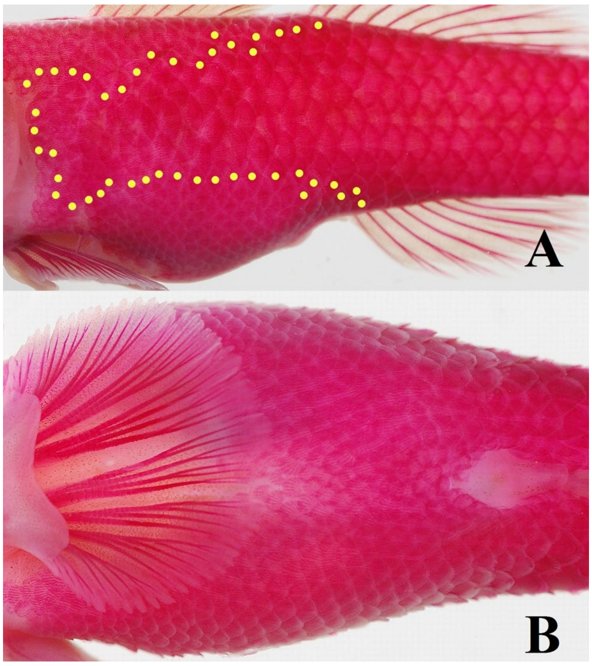

Fig. 12. Lateral view of body (A) and ventral view of belly (B) in *Rhinogobius aonumai ishigakiensis* stained with Alizarin Red S. OMNH-P 48923, paratype, male, 35.5 mm SL, Sakuta-gawa River. Yellow spots indicate the lateral anterior margin of ctenoid scale area. Photographed and annotated by T. Suzuki.

level, 17 September 2021, Naoharu Oseko; Isobe-gawa River: KPM-NR 217904 and 217905, male and female, about 35 mm SL, upper stream above Ishigaki Dam, 24°22′28.39″N 124°09′59.25″E, 113 m above sea level, 17 September 2021, Naoharu Oseko; Pensann-kara River, a tributary of the Uratabaru-gawa River System: KPM-NR 217910 and 217911, male and female, about 40 and 35 mm SL, 24°22′37.00″N 124°09′39.45″E, 90 m above sea level, 7 March 2018, Naoharu Oseko; Todoroki-gawa River: KPM-NR 217907 and 217908, male and female, about 35 mm SL, 24°23′52.60″N 124°13′36.59″E, 25 m above sea level, 10 March 2021, Naoharu Oseko; A tributary of the Todoroki-gawa River: KPM-NR 217909, male, about 35 mm SL, 24°23′37.23″N 124°13′04.84″E, 25 m above sea level, 10 March 2021, Naoharu Oseko.

Diagnosis. *Rhinogobius aonumai ishigakiensis* is distinguished from all congeneric species-group taxa by having the following combination of features: 10–14 predorsal scales; 33–38 longitudinal scales; 10+16–18=26–28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of second dorsal fin mounted over neural spine of nineth vertebra; fifth segmented pelvic-fin ray divided into 2–3 (usually two) branches at the position where proximal most segment of each branch alignes transversely; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 7–9 and 3–6 dark zigzag bands in males and females, respectively; a pair of short, vertically aligned, rod-shaped dark mark on caudal-fin base in females.

Description. First dorsal-fin rays V (1) or VI* (11); second dorsal-fin rays I, 8* (9) or I, 9 (3); anal-fin rays I, 7 (2), I, 8* (2) or I, 9 (8); pectoral-fin rays (left/right) 18/18 (1), 18/19 (1), 19/18 (1), 19/19 (4), 19/20 (3), 20/19* (1) or 20/20 (1); pelvic-fin rays I, 5 (12); segmented caudal-fin rays (upper part + lower part) 9+8* (10) or 9+9 (2); branched caudal-fin rays (upper part + lower part) 7+7 (6), 8+7* (5) or 9+7 (1); longitudinal scales 33 (2), 34 (1), 35 (6), 36 (1), 37* (1) or 38 (1); transverse scales 9 (7) or 10*(5); scales between origin of dorsal fin and dorsal insertion of pectoral fin 8 (5) or 9* (7); predorsal scales 10 (1), 11 (4), 12 (2) or 14* (5); number of the first branches of fifth segmented pelvic-fin ray (left/right) at the position where proximal most segment of each branch alignes transversely (Fig. 11A) 2/2* (10), 2/3 (1) or 3/2 (1); P-V 3/21210/9 (1), 3/22100/9 (1), 3/22110/9 (9) or 3/22200/9* (1); vertebrae (abdominal + caudal = total vertebrae) 10+16=26 (3), 10+17=27 (7) or 10+18=28* (2).

Head slightly large and slightly depressed; body slightly slender, slightly compressed anteriorly, and compressed posteriorly. Snout nearly pointed, long, and longer than eye diameter; snout length of males greater than that of females. Eye large, dorsolateral on head, and located slightly behind a vertical through midpoint between snout tip and posterior margin of preopercle. Cheek somewhat bulbous and fleshy. Lips thick and fleshy; anterior tips of both lips even* or lower lip slightly protruding anteriorly; gape slightly oblique; posterior margin of lower jaw extending posteriorly to a vertical through anterior margin of eye in females and slightly beyond it in males. Anterior naris a short tube without skin flap at its tip, and its base located slightly behind the midpoint between snout tip and anterior margin of eye. Posterior naris a round pore with low rim and located the midpoint between base of anterior naris and anterior margin of eye. Gill opening usually extending anteriorly to center of operculum*, or to a vertical through posterior margin of preopercle. Gill membranes broadly attached to isthmus. No fleshy papillae or finger-like projections on lateral margin of shoulder girdle. Tongue free from floor of mouth, with rounded anterior margin. Genital papillae cone-shaped in males and oval in females.

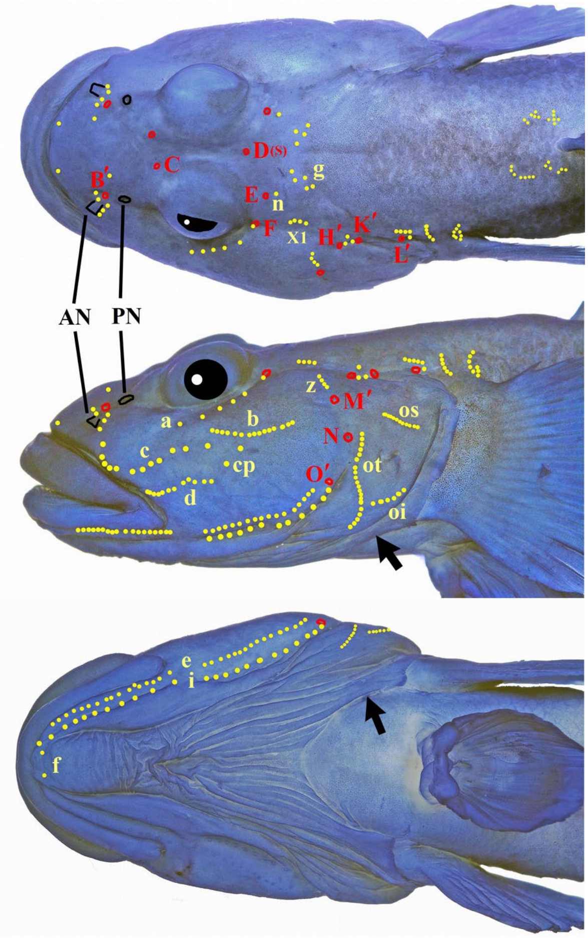

Fig. 13. Dorsal (top), lateral (middle), and ventral (bottom) views of head of *Rhinogobius aonumai ishigakiensis* stained with cyanine blue showing cephalic sensory pores and papillae. KPM-NI 65588, paratype, male, 55.9 mm SL. Red circles with red letters indicate sensory canal pores (letters with prime marks indicate terminal openings of sensory canals); rows of yellow spots indicated by yellow letters represent sensory papillae rows; black arrows show ventralmost positions of gill opening. Abbreviations: AN, anterior narial pore; PN, posterior narial pore. Photographed and annotated by T. Suzuki.

Origin of first dorsal fin about an eye diameter behind a vertical through dorsal insertion of pectoral-fin; first dorsal fin in males usually near falcate shape, and higher than second dorsal fin in height, whereas, in females, semicircular or semioval, and usually slightly higher than, or slightly lower than second dorsal fin in height; usually second, or third* spine longest; all dorsal-fin spines slender and flexible; distal tip of first dorsal fin in males usually second or third* spine filamentous, or with non-filamentous spines, whereas, in females with non-filamentous spines; posterior tip (= distal tip of second or third* spines) of first dorsal fin in males extending between bases of first and fifth segmented ray of second dorsal fin (forth ray*) when adpressed, whereas, in females, posterior tip (= distal tip of third, fourth or sixth spine) usually not, or extending to origin of second dorsal fin, when adpressed. First and second dorsal fins not connected by membrane; all segmented rays of second dorsal fin branched; seventh or eighth* and second or third segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate* segmented ray) of second dorsal fin not extending to caudal fin when adpressed; posterior end of second dorsal-fin base above posterior end of anal-fin base. Origin of anal fin below between origin and base of second segmented ray of second dorsal fin (between bases of first and second segmented rays*); anal fin slightly lower than second dorsal fin in height; all segmented anal-fin rays branched; sixth or seventh*, and fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate* segmented ray) of anal fin not extending to caudal fin when adpressed. Pectoral fin oval, extending posteriorly to a vertical between base of sixth spine of first dorsal fin and origin of

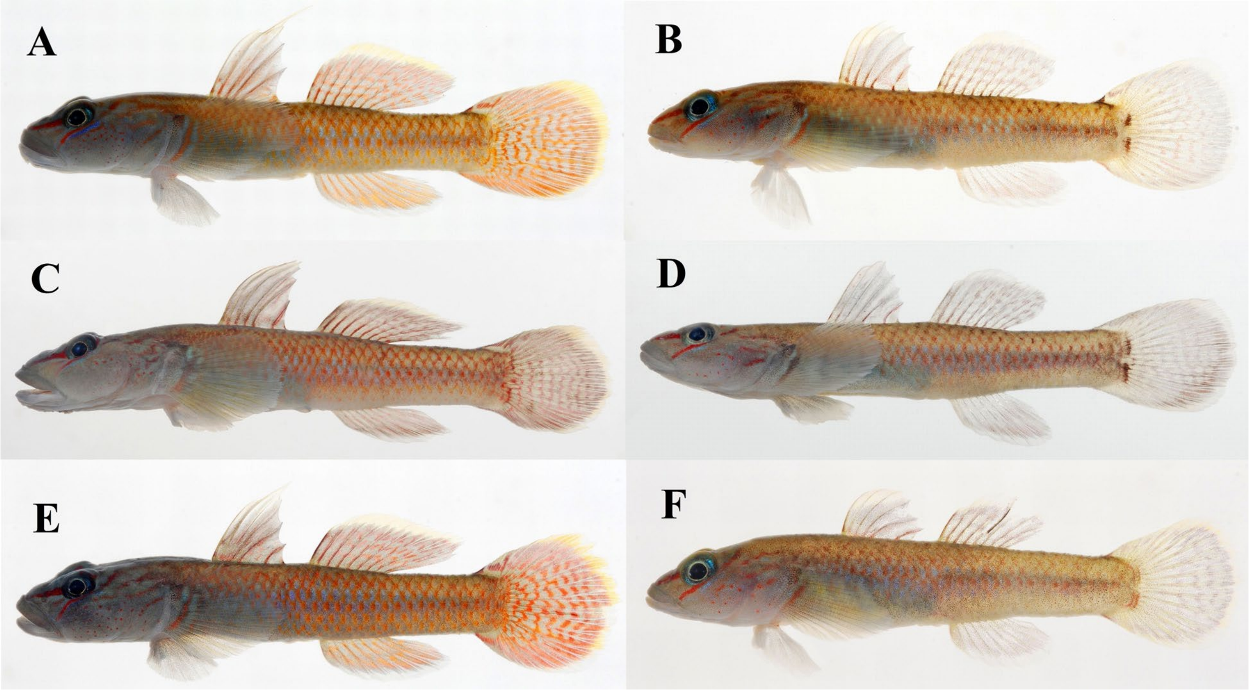

Fig. 14. Freshly-collected paratypes of *Rhinogobius aonumai ishigakiensis* from Ishigaki-jima Island, the Ryukyu Islands, Japan. Miyara-gawa River: A (OMNH-P 40912, male, 38.0 mm SL) and B (OMNH-P 40914, female, 38.4 mm SL); Sakuta-gawa River: C (KPM-NI 65588, male, 55.9 mm SL) and D (SPMN-PI 49270, female, 40.5 mm SL); Sokobaru-gawa River: E (OMNH-P 40911, male, 39.9 mm SL) and F (OMNH-P 40913, female, 33.3 mm SL). Photographed by T. Suzuki.

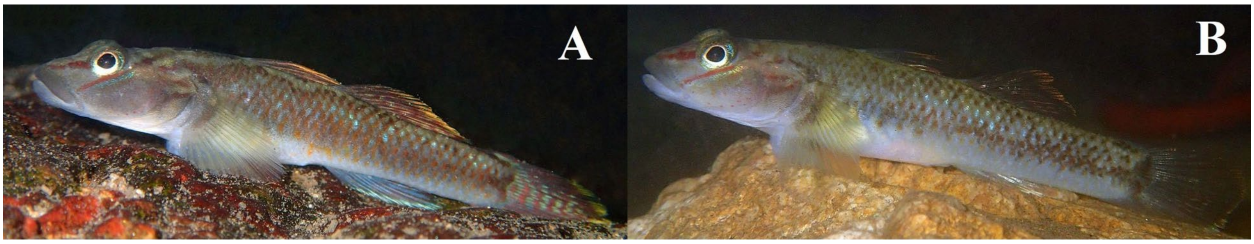

Fig. 15. Underwater photographs of *Rhinogobius aonumai ishigakiensis* taken at Ishigaki-jima Island, the Ryukyu Islands, Japan. Sakuta-gawa River: A (male, about 40 mm SL) and B (female, about 40 mm SL). Photographed by N. Oseko.

second dorsal fin (posterior end of first dorsal-fin base*); pectoral-fin rays branched, except for dorsalmost and ventralmost rays unbranched. Pelvic fins fused medially by well-developed frenum (between spines) and connecting membrane (between innermost rays), forming a circular cup-like disc; pelvic fins extending posteriorly to a vertical between bases of second and fifth* spines of first dorsal fin, not reaching to anus; pelvic-fin spine with a rounded membranous lobe at its tip; all segmented rays of pelvic fin branched. Caudal fin elliptical or fan-shaped*.

Scales on body largely ctenoid, becoming smaller anteriorly; anterior edge of scaled areas with ctenoid scales on lateral, dorsal and ventral sides of body reaching respectively to a little behind pectoral-fin axilla, to between end of first dorsal fin and end of second dorsal fin, and to anal-fin base (Fig. 12A). Small cycloid scales on anterodorsal part of body before the area with ctenoid scales. Predorsal squamation with trifurcate anterior edge; its mid-anterior extension (Fig. 11B: P1) extending anteriorly to between transverse lines through sensory-canal pore H′ and K′; anterior extensions of lateral sides (Fig. 11B: P2) extending anteriorly to posterior oculoscapular canal; greatest concaved point of anterior margin of scaled area of predorsal region between P1 and P2 (Fig. 11B: P3) extending posteriorly to above between sensory-canal pore K′ and upper end of pectoral-fin axilla. Pectoral-fin base usually naked, or with one small cycloid scale. Prepelvic area usually naked, or with some small cycloid scales. Scaled area on belly with small cycloid scales usually extending anteriorly to, or not side of prepelvic area; belly above pelvic fin naked, and its following ventral midline of belly usually with small cycloid scale, or its anterior part naked (Fig. 12B).

Cephalic sensory systems are illustrated in Fig. 13. Nasal

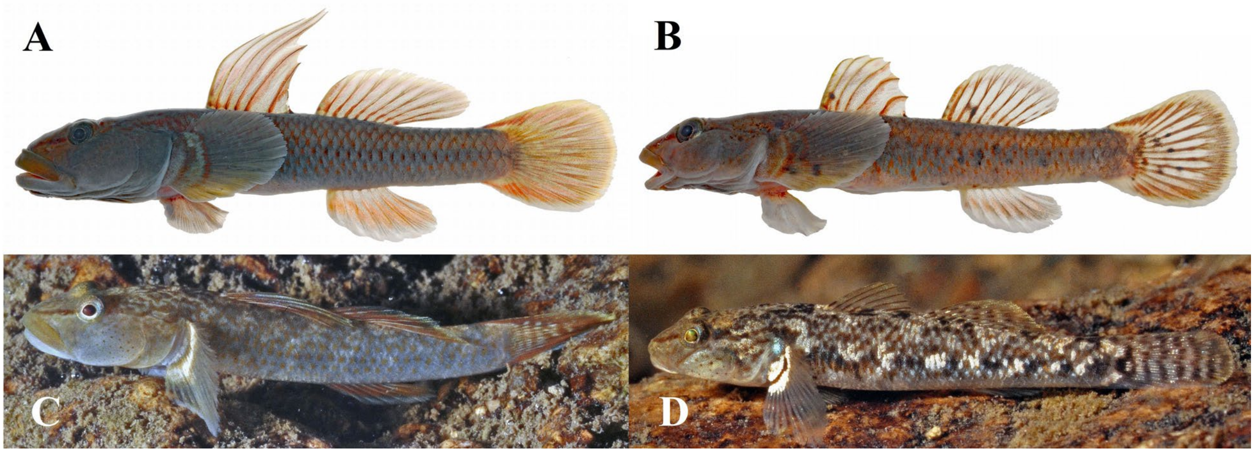

Fig. 16. *Rhinogobius flumineus* at Ina-gawa River, Ina-gawa, Hyogo Prefecture, Japan. Freshly-collected: A (OMNH-P 43228, 52.3 mm SL, male) and B (OMNH-P 43229, 50.6 mm SL, female), photographed by T. Suzuki.; underwater photographs: C (male, about 40 mm SL) and D (female, about 40 mm SL), photographed by M. Suzuki.

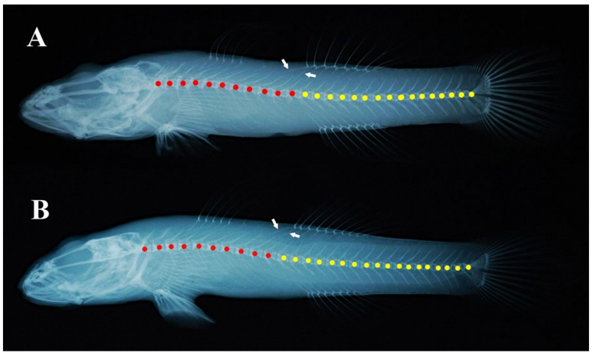

Fig. 17. Soft X-ray negatives of holotypes of two subspecies of *Rhinogobius aonumai*. A: *Rhinogobius aonumai aonumai*, B: *Rhinogobius aonumai ishigakiensis*. Red and yellow spots indicate abdominal and caudal vertebrae, respectively; white arrows show anteriormost two pterygiophores (proximal radials) of second dorsal fin. Photographed and annotated by T. Suzuki.

extension of anterior oculoscapular canal with terminal pore B′ located above between both nares. Anterior interorbital sections of anterior oculoscapular canal separated bilaterally, with paired pore C and a single pore D. Pore E present just behind posterior edge of eye. Lateral section of anterior oculoscapular canal with anterior pore F and terminal pore H′. Posterior oculoscapular canal with two terminal pores K′ and L′. Gap between anterior and posterior oculoscapular canals much narrower than length of posterior oculoscapular canal. Preopercular canal present, with three pores M′, N, and O′. As unusual variations, there is an accessory pore on the canal between pores F and H′ in a paratype, and pore N is absent in two paratypes. Sensory-papillae row “a” oblique and uniserial, composed of loosely-arranged papillae, and extending anteriorly to a vertical through midpoint between anterior margin of eye and anterior margin of pupil. Row “b” longitudinal, composed of densely-arranged papillae, extending anteriorly to a vertical through center of pupil, and equal to eye diameter in length. Row “c” composed of loosely-arranged papillae, extending posteriorly to a vertical through posterior margin of eye. Row “d” composed of densely-arranged papillae, extending posteriorly to a vertical through midpoint of pupil. Row “cp” comprising a single papilla. Row “f” comprising paired papillae. Anterior end of row “oi” a little separated from a vertical row “ot”.

Coloration of male when freshly collected (Figs. 9A, 9B, 14A, 14C, 14E, 14G). Ground color of head and body usually reddish yellow. Belly pale yellow and its side greenish. Head grayish; cheek, lower half of operculum and gill membrane with sparsely-arranged, some small purplish red dots. Snout with a broad oblique purplish red stripe between anterior margin of the eye and anteriormost part of snout. Several irregular-shaped, short purplish red stripes and minute spots on dorsal surface of snout and interorbital space, occipital region and nape; anterodorsal margin of cheek with a narrow purplish red line edged ventrally by a bright blue line; temporal region above operculum with a short purplish red stripe; upper part of operculum with two oblique purplish red stripes. Almost all scale pockets on body with reddish orange spots. Lateral side of body below dorsal-fin base with two longitudinal rows of blue spots. Ground color of fin membranes yellowish gray, partially with bluish sheen. Spines and segmented rays gray. Anterodorsal part of first dorsal fin and distal margin of second dorsal fin pale yellow; distal margin of anal fin white; dorsal and middle margins of caudal fin pale yellow and ventral margin white; membranes around spines and segmented rays of first dorsal and anal fins, and upper and lower parts of caudal fin purplish red. Second dorsal fin with three longitudinal rows of reddish orange* or purplish red spots, or usually forming zigzag stripes; a reddish orange or purplish red* mottles above these spots or stripes. Central part of caudal fin with 7–9 (8*) reddish orange or purplish red* zigzag bands. Upper end of pectoral-fin base with a black triangle mark; pectoral-fin base with a reddish orange* or purplish red bow-shaped mark, and in some specimens (except holotype), a vertical row of spots behind it.

Coloration of female when freshly collected (Figs. 10A, 10B, 14B, 14D, 14F). Resembles that of male, except as follows. Red or orange marks of head and body darker. Purplish red dots on cheek and operculum fewer in number, usually absent on operculum; gill membrane with no orange dots. Ventral side of body usually with no reddish orange spots. Second dorsal fin with 0–4 longitudinal rows of purplish orange spots or forming zigzag stripes. Caudal-fin base with a pair of short, vertically aligned, rod-shaped grayish brown mark; central part of caudal fin with 3–6 indistinct purplish red zigzag bands. Pectoral-fin base with no vertical rows of spots behind a bow-shaped mark.

Coloration when alive based on underwater photographs (Fig. 15). Coloration when alive in the populations of Sakuta-gawa River, in Ishigaki-jima Island resembles that of freshly-collected coloration, except as follows. Ground color of head and body grayish; upper half of body with some pale blue spots; caudal-fin base with a Y-shaped, reddish gray mark in male.

Coloration when preserved in alcohol (Figs. 9C, 10C). Ground color of head and body turned to light gray; blue, green, yellow and white color faded; orange and red color turns to brown or gray. Dorsal side of body with 1–2 longitudinal row(s) of dark gray spots; mid lateral side of trunk with a brownish gray broad stripe in female.

Distribution and habitat. *Rhinogobius aonumai ishigakiensis* is known only from Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. In this island, it is seen in the upper reaches of freshwater streams above waterfalls in montane areas of Sakuta-gawa River, and the upper streams above Maezato Dam of Miyara-gawa River and Sokobaru Dam of Sokobaru-gawa River of Miyara-gawa River System. In addition, we identified the gobies in the photographs taken from Isobe-gawa, Todoroki-gawa and Uratabaru-gawa rivers registered in the Image Database of Fishes, as *R. a. ishigakiensis*. The subspecies is usually found in small pools of streams, called "Fuchi" in Japanese.

Etymology. The subspecific name, *ishigakiensis*, refers to the type locality (Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

Discussion

Subgroups of *Rhinogobius* sp. YB. *Rhinogobius* includes both amphidromous and freshwater resident species (Huang & Chen, 2007; Mizuno, 1960a). The latter is divided further into fluvial, lake-river migrating and lentic species (Takahashi & Okazaki, 2002).

Nishijima (1968) named a fluvial species of *Rhinogobius* collected from Okinawa-jima Island of Okinawa Group of the Ryukyu Islands as the Medium-egg type ("Chuuran-gata" in Japanese) with a note that it was characterized by its intermediate size and number of eggs between the small eggs of amphidromous species of Japanese *Rhinogobius* and the large eggs of *Rhinogobius flumineus*, and that it can spend its whole life in freshwater stream. Nakayama (1975) reported that there were two morphotypes of the Medium-egg type with yellow or blue bellies from Okinawa-jima Island. Hayashi (1984) reported that the Medium-egg type with a yellow belly was distributed from Amami-oshima Island (Amami Group of the Ryukyu Islands) to Taiwan. Iwata (1989) assumed this as a distinct unnamed species, tentatively named it as "*Rhinogobius* sp. YB" with a new Japanese vernacular name "Kibara-yoshinobori"; he stated that it was distributed in the Ryukyu Islands, and that its distribution in the area outside of Japan was unknown. Akihito *et al.* (1993, 2000, 2002, 2013) reported *Rhinogobius* sp. YB occurred in the Ryukyu Islands. Suzuki *et al.* (2004) reported *Rhinogobius* sp. YB was distributed Amami-oshima, Kakeroma-jima, Tokuno-shima and Okinoerabu-jima islands of Amami Group, Okinawa-jima and Kume-jima islands of Okinawa Group, and Ishigaki-jima and Iriomote-jima islands, the Yaeyama Group of the Ryukyu Islands, Japan. Suzuki *et al.* (2021), an updated and revised version of Suzuki *et al.* (2004), added Tokashiki-jima Island of Okinawa Group to its distribution areas.

Nishida (1994) hypothesized that *Rhinogobius* sp. YB speciated multiple times from *Rhinogobius brunneus* as an ancestor species in the Ryukyu Islands, Japan. Kano *et al.* (2012) collected *Rhinogobius* sp. YB and *R. brunneus* from multiple rivers in Iriomote-jima Island. They showed that mtDNA divergence between *Rhinogobius* sp. YB populations in respective rivers was larger than between *Rhinogobius* sp. YB and *R. brunneus* populations. They estimated that *Rhinogobius* sp. YB populations in respective rivers independently speciated from a common migratory ancestor in the rivers where they inhabited. However, this hypothesis was rejected by a subsequent analysis using multiple nuclear gene markers (Yamasaki *et al.*, 2020). Yamasaki *et al.* (2020) revealed that the speciation of *Rhinogobius* sp. YB from its migratory ancestor, *R. brunneus*, occurred in parallel across five islands or islands groups (Amami-oshima Island, Tokuno-shima + Okinoerabu-jima islands, Okinawa-jima Island, Kume-jima Island, and Ishigaki-jima + Iriomote-jima islands) using population genetic analysis.

Our investigation further revealed that the population of *Rhinogobius* sp. YB of the Yaeyama Group can be morphologically differentiated from the congeners, including the population of *Rhinogobius* sp. YB from the other island groups of the Ryukyu Islands, and that the population of *Rhinogobius* sp. YB of Ishigaki-jima and Iriomote-jima islands can be morphologically distinguished. Considering together with the result of the molecular studies made by Yamasaki *et al.* (2020), therefore, we here recognize the population of *Rhinogobius* sp. YB of the Yaeyama Group as a distinct species (*R. aonumai*), comprising two subspecies (*R. a. aonumai* from Iriomote-jima Island and *R. a. ishigakiensis* from Ishigaki-jima Island).

Comparisons with the congeners. *Rhinogobius* is currently known as the most specious freshwater gobiid genus, comprising 88 valid species-group taxa (Suzuki *et al.*, 2020; Wanghe *et al.*, 2020; present study). As indicated by Chen & Shao (1996) and Suzuki *et al.* (2015), the genus is divided into two distinct groups; one comprises only a single species *R. similis*, whereas the other includes all the remaining species. *Rhinogobius similis* differs from the other congeners by having large ctenoid scales on the nape (vs. nape naked or with cycloid scales in the others) and several short transverse rows of sensory papillae on the cheek (vs. no distinct transverse rows of sensory papillae on the cheek). Suzuki *et al.* (2020) assigned all species of the genus but *R. similis* to the "*Rhinogobius brunneus* complex", following Chen & Shao (1996). *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis* described here also belong to the *R. brunneus* complex.

Furthermore, Suzuki *et al.* (2020) attempted to divide the *R. brunneus* complex into two subgroups: one almost always has 27 or more vertebrae (named as "Group I"), whereas the others have lower counts of vertebrae (25–27, almost always 26) ("Group II"). The groups I and II, both of which appear to be phylogenetic grades merely assembled by the vertebral counts (Suzuki *et al.*, 2020), hitherto comprise at least 47 and 32 described species, respectively. Unfortunately, Suzuki *et al.* (2020) failed to assign the remaining six species to these subgroups due to the lack of information on their vertebral counts. *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis*, having 26–28 vertebrae (usually 27), belong to Group I, making the total number of species-group taxa in the group 49.

Species of Group I and the assemblage with no information about vertebral counts including 49+6=55 species-group taxa are compared in Table 3. Within 55 species-group taxa, *R. a. aonumai* and *R. a. ishigakiensis* are most similar to *Rhinogobius flumineus* (Mizuno, 1960) and *R. szechuanensis* (Tchang, 1939), by having the following combination of characters: the number of longitudinal scales (32–37 in *R. a. aonumai*; 33–38 in *R. a. ishigakiensis*; 30–36 in *R. flumineus*; 30–34 in *R. szechuanensis*); no dark spot on first dorsal fin; vertical rows of dark spots or dark bands on caudal fin (absent or vertical rows of dark spots in *R. flumineus*). *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis* are, however, distinguished from *R. flumineus* and *R. szechuanensis* by having more than 9 predorsal scales (9–15 in *R. a. aonumai*; 10–14 in *R. a. ishigakiensis* vs. 2–9 in *R. flumineus*; 0 in *R. szechuanensis*). Furthermore, *R. aonumai aonumai* and *R. a. ishigakiensis* are distinguished from *R. flumineus* by having a yellow-colored body in freshly-collected (vs. gray in *R. flumineus*), and a pair of short, vertically aligned, rod-shaped mark on the caudal-fin base in females (vs. single rod-shaped mark in both sexes) (Fig. 16). The previously-recognized "*Rhinogobius* sp. YB" (including *R. a. aonumai* and *R. a. ishigakiensis*) and *Rhinogobius flumineus* are distinguishable by genetic features, and the genetic distance between them is as large as that between the species of *Rhinogobius* (Yamasaki *et al.*, 2015). *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis* are distinguished from *R. szechuanensis* by having sensory canals on the head (vs. absent in *R. szechuanensis*). In the above comparisons, data on *R. flumineus* follow Akihito *et al.* (2013) and its errata (Nakabo & Tokai University Press, 2014), Mizuno (1960), as well as the comparative materials examined here (see below), and data on *R. szechuanensis* follow Chen *et al.* (2008), Tchang (1939) and Wu & Chen (2008).

Rhinogobius aonumai aonumai and *R. a. ishigakiensis* are distinguished from the populations of *Rhinogobius* sp. YB from the other islands by having usually 27 vertebrae (vs. 26 in *Rhinogobius* sp. YB from the other islands). Furthermore, *Rhinogobius* sp. YB populations showed a large divergence between the Middle Ryukyu and South Ryukyu (including the Yaeyama Group), and *Rhinogobius* sp. YB has speciated in five islands in the Ryukyu Islands (Yamasaki *et al.*, 2020). The Yaeyama Group is one of them.

Rhinogobius aonumai aonumai is distinguished from *R. a. ishigakiensis* by having 11 abdominal vertebrae (vs. 10 in *R. aonumai ishigakiensis*), the anteriormost two pterygiophores (proximal radials) of second dorsal fin mounted over the neural spine of the tenth vertebra (vs. ninth vertebra) (Fig. 17), fifth segmented pelvic-fin ray usually divided into four branches (vs. usually two branches) at the position where proximal most segment of each branch alignes transversely, no filamentous spine of first dorsal fin (vs. usually second spine filamentous in males), around the midline of belly usually naked except posterior part (vs. usually scaly), cheek, the lower half of operculum and gill membrane usually with many small orange spots densely (vs. some red spots sparsely), and the lower margin of caudal fin usually bright yellow (vs. white).

Current statuses of the populations. *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis* are the endemic subspecies of Iriomote-jima Island and Ishigaki-jima Island, respectively, and are the second and third known species-group taxa of the genus *Rhinogobius* from Japan, with a high vertebral count. In the Red List 2020 of the Ministry of the Environment of Japan, *Rhinogobius* sp. YB (including *R. a. aonumai* and *R. a. ishigakiensis*) is ranked as EN "Endangered" (Ministry of the Environment Government of Japan, 2020). Both *R. a. aonumai* and *R. a. ishigakiensis* are the noteworthy species-group taxa (species and subspecies) with the smallest distribution area and population size among the congeners hitherto known from Japan. Gobies of the previously-recognized "*Rhinogobius* sp. YB" has diversified independently in five islands groups of the Ryukyu Islands (Yamasaki *et al.*, 2020), but the taxonomic assessment of all but the populations from the Yaemyama Group (herein described as *R. a. aonumai* and *R. a. ishigakiensis*) has not yet made properly. Resolving the taxonomic statuses and proper naming of the populations of the "*Rhinogobius* sp. YB" are urgently needed for assessing respective population statuses and developing their conservation measure adequately, and this paper is the first step for the task.

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References

Akihito, A. Iwata, K. Sakamoto & Y. Ikeda, 1993. Gobioidei. In Nakabo, T. (ed.), Fishes of Japan with pictorial keys to the species (1st ed.), pp. 997–1116, 1355–1366. Tokai University Press, Tokyo. (In Japanese).

Akihito, K. Sakamoto, Y. Ikeda & M. Aizawa, 2013. Gobioidei. In Nakabo, T. (ed.), Fishes of Japan with pictorial keys to the species (3rd ed.), pp. 1347–1608, 2109–2211. Tokai University Press, Kanagawa. (In Japanese).

Akihito, K. Sakamoto, Y. Ikeda & A. Iwata, 2000. Gobioidei. In Nakabo, T. (ed.), Fishes of Japan with pictorial keys to the species (2nd ed.), pp. 1139–1310, 1606–1628. Tokai University Press, Tokyo. (In Japanese).

Akihito, K. Sakamoto, Y. Ikeda & K. Sugiyama, 2002. Gobioidei. In Nakabo, T. (ed.), Fishes of Japan with pictorial keys to the species (English ed.), pp. 1139–1310, 1596–1619. Tokai University Press, Tokyo.

Aonuma, Y., 1992. Review of genus *Rhinogobius* (Pisces: Gobiidae) in Taiwan. Master of Science Thesis of Ryukyu University.

Berg, L. S., 1933. Les poissons des eaux douces de l'U.R.S.S. et des pays limitrophes. 3-e édition, revue et augmentée. Leningrad. Les poissons des eaux douces de l'U.R.S.S., Part 2: 544–903. (In Russian).

Chen, I-S., Y.-H. Cheng & K.-T. Shao, 2008. A new species of *Rhinogobius* (Teleostei: Gobiidae) from the Julongjiang basin in Fujian Province, China. Ichthyological Research, 55: 335–343.

Chen, I-S. & L.-S. Fang, 2006. A new species of *Rhinogobius* (Teleostei: Gobiidae) from the Hanjiang Basin in Guangdong Province, China. Ichthyological Research, 53: 247–253.

Chen, I-S. & M. Kottelat, 2000. *Rhinogobius maculicervix*, a new species of goby from the Mekong basin in northern Laos (Teleostei: Gobiidae). Ichthyological Exploration of Freshwaters, 11: 81–87.

Chen, I-S. & M. Kottelat, 2003. Three new freshwater gobies of the genus *Rhinogobius* (Teleostei: Gobiidae) from northeastern Laos. Raffles Bulletin of Zoology, 51: 87–95.

Chen, I-S. & M. Kottelat, 2005. Four new freshwater gobies of the genus *Rhinogobius* (Teleostei: Gobiidae) from northern Vietnam. Journal of Natural History, 39: 1407–1429.

Chen, I-S., M. Kottelat & P. J. Miller, 1999a. Freshwater gobies of the genus *Rhinogobius* from the Mekong Basin in Thailand and Laos, with descriptions of three new species. Zoological Studies, 38: 19–32.

Chen, I-S. & P. J. Miller, 1998. Redescription of a Chinese freshwater goby, *Gobius davidi* (Gobiidae), and comparison with *Rhinogobius lentiginis*. Cybium, 22: 211–221.

Chen, I-S. & P. J. Miller, 2014. A new freshwater goby of *Rhinogobius* (Teleostei: Gobiidae) from Hainan Island, southern China. Journal of Marine Science and Technology, 21, Supplement: 124–129.

Chen, I-S., P. J. Miller, H.-L. Wu & L.-S. Fang, 2002. Taxonomy and mitochondrial sequence evolution in non-diadromous species of *Rhinogobius* (Teleostei: Gobiidae) of Hainan Island, southern China. Marine and Freshwater Research, 53: 259–273.

Chen, I-S. & K.-T. Shao, 1996. A taxonomic review of the gobiid fish genus *Rhinogobius* Gill, 1859, from Taiwan, with description of three new species. Zoological Studies, 35: 200–214.

Chen, I-S. & H.-L. Wu, 2008. *Rhinogobius liui* Chen et Wu nom. nov. In Wu, H.-L. & Zhong, J.-S. (eds.), Fauna Sinica, Osteichthyes, Perciformes (V), Gobioidei, p.612. Science Press, Beijing. (In Chinese).

Chen, I-S., H.-L. Wu & K.-T. Shao, 1999b. A new species of *Rhinogobius* (Teleostei: Gobiidae) from Fujian Province, China. Ichthyological Research, 46:171–178.

Chen, I-S., J.-X. Yang & Y.-R. Chen, 1999c. A new goby of the genus *Rhinogobius* (Teleostei: Gobiidae) from the Honghe Basin, Yunnan Province, China. Acta Zoologica Taiwanica, 10: 45–52.

Endruweit, M., 2017. Neotype designation for *Rhinogobius carpenteri* Seale 1910, and its placement in *Tukugobius* Herre 1927 (Teleostei: Gobiidae). Zootaxa, 4277(4): 549–560.

Endruweit, M., 2018. Description of four new species of freshwater gobies from the Black River drainage in China and Vietnam (Teleostei: Gobiidae). Zootaxa, 4486(3): 284–310.

Fowler, H. W., 1934. Zoological results of the third De Schauensee Siamese Expedition, Part I, Fishes. Proceedings of the Academy of Natural Sciences of Philadelphia, 86: 67–163, pl.12.

Geospatial Information Authority of Japan, 2021. GSI Maps. Online. Available from internet: https://maps. gsi.go.jp. (last modified on 2021-1-6 by the author).

Gill, T. N., 1859. Notes on a collection of Japanese fishes, made by Dr. J. Morrow. Proceedings of the Academy of Natural Sciences of Philadelphia, 11: 144–150.

Ginsburg, I., 1917. On two species of fishes from the Yalu River, China. Proceedings of the United States National Museum, 54: 99–101.

Hayashi, M., 1984. Genus *Rhinogobius*. In Masuda, H., K. Amaoka, C. Araga, T. Uyeno. & T. Yoshino (eds.), The fishes of the Japanese Archipelago (1st ed.), pp. 269–270, pls.248–249. Tokai University Press, Tokyo.

Herre, A. W. C. T., 1927. Gobies of the Philippines and the China Sea. Monographs, Bureau of Science Manila, 23: 1–352.

Herre, A. W. C. T., 1935. Notes on fishes in the Zoological Museum of Stanford University. VI. New and rare Hong Kong fishes obtained in 1934. Hong Kong Naturalist, 6: 285–293.

Herre, A. W. C. T., 1938. Notes on a small collection of fishes from Kwangtung Province including Hainan, China. Lingnan Science Journal, Canton, 17: 425–437.

Huang, S.-P. & I-S. Chen, 2007. Three new species of *Rhinogobius* Gill, 1859 (Teleostei: Gobiidae) from the Hanjiang basin, Southern China. Raffles Bulletin of Zoology, 14, 101–110.

Huang, S.-P., I-S. Chen & K.-T. Shao, 2016. A new species of *Rhinogobius* (Teleostei: Gobiidae) from Zhejiang Province, China. Ichthyological Research, https://doi.org/10.1007/s10228-016-0516-9.

Iwata, A. 1989. *Rhinogobius* sp. YB. In Kawanabe, H. & N. Mizuno (eds.), Freshwater fishes of Japan, pp. 598. Yama-Kei Publishing Company Ltd, Tokyo. (In Japanese).

Japan Color Research Institute (ed.), 1995. Concise manual of color names, revised edition. 90 pp. Japan Color Enterprise Co. Ltd, Tokyo. (In Japanese).

Jordan, D. S. & A. Seale, 1906. Descriptions of six new species of fishes from Japan. Proceedings of The United States National Museum, 30: 143–148.

Kano Y., S. Nishida & J. Nakajima. 2012. Waterfalls drive parallel evolution in a freshwater goby. Ecology and Evolution, 2(8): 1805–1817.

Koumans, F. P. 1940. Results of a reexamination of types and specimens of gobioid fishes, with notes on the fishfauna of the surroundings of Batavia. Zoologische Mededelingen, Leiden, 22: 121–210.

Lee, S.-C. & J.-T. Chang, 1996. A new goby, *Rhinogobius rubromaculatus* (Teleostei: Gobiidae), from Taiwan. Zoological Studies, 35: 30–35.

Li, F., S. Li & J.-K. Chen, 2018. *Rhinogobius immaculatus*, a new species of freshwater goby (Teleostei: Gobiidae) from the Qiantang River, China. Zoological Research, 39 (6): 1–10.

Luo, Y. L., 1989. *Ctenogobius parvus*. In Zheng C.-Y. (ed.), Fishes of the Zhujiang River, pp. 354–355. Sceience Press, Beijing.

Ministry of the Environment Government of Japan, 2020. The Red List 2020. Online. Available from internet: https://www.env.go.jp/press/files/jp/114457 (In Japanese) (downloaded on 2021-8-13).

Mizuno, N., 1960a. Study on a freshwater goby, *Rhinogobius similis* Gill, with a proposition on the relationships between land-locking and speciation of some freshwater gobies in Japan. Memoirs of the College of Science, University of Kyoto, Series B, 27: 97–115.

Mizuno, N., 1960b. Description of a new freshwater goby from Japan. Memoirs of the College of Science, Kyoto University, Series B, 27: 117–119.

Mori, T., 1934. The fresh water fishes of Jehol. Report of the first scientific expedition to Manchoukuo, Tokyo, Section 5, Zoology, Part 1: 1–28 + 1–61, pls.1–21,

Nakabo, T. & Tokai University Press, 2014. Fishes of Japan with pictorial keys to the species (3rd ed.), errata. Online. Available from internet: https://www.press.tokai.ac.jp/seigohyo/gyoruikensakuseigo.pdf (In Japanese) (downloaded on 2021-8-13)

Nakayama, H., 1975. On the Yoshinobori inhabiting the rivers of Okinawa. Freshwater fishes, (1): 113–115. (In Japanese).

Nguyen, V. H. & V. B. Vo, 2005. Gobiidae, Perciformes. In Nguyen, V. H. (ed.), The freshwater fishes of Vietnam, Vol. 3, pp. 635–640. MARD Informatic Center Agriculture Publisher, Hanoi. (In Vietnamese).

Nichols, J. T., 1925. Some Chinese Fresh-water fishes. XII. A small goby from the central Yangtze. American Museum Novitates, (185): 5.

Nichols, J. T., 1931. Some Chinese fresh-water fishes. XXIX, A new goby from Hokou, Kiangsi. XXX, Six type specimens figured. American Museum Novitates, (499): 1–3.

Nishida, M., 1994. Life-history variation and speciation in the *Rhinogobius* species-complex (In Japanese). In Goto A, Tsukamoto K, & Maekawa K (eds.) , Freshwater fishes migrating between rivers and the sea, pp. 154–169, Tokai University Press, Tokyo.

Nishijima, S., 1968. Two forms of the gobioid fish *Rhinogobius brunneus* from Okinawa Islands. Zoological magazine, 77:397–398. (In Japanese).

Pellegrin, J. & P.-W. Fang, 1940. Poissons du Laos recueillis par Mm. Delacour, Greenway, Ed. Blanc. Description d'un genre, de cinq espèces et d'une variété. Bulletin de la Société Zoologique de France, 65: 111–123.

Prince Akihito, M. Hayashi, T. Yoshino, K. Shimada, H. Senou & T. Yamamoto, 1984. Suborder Gobioidei. In Masuda, H., K. Amaoka, C. Araga, T. Uyeno & T. Yoshino (eds.), The fishes of the Japanese Archipelago (1st ed.), pp. 236–289, pls.235–258, 353–355. Tokai University Press, Tokyo. (In Japanese).

Pritchard, J. K., Stephen, M., & Donnelly, P. (2000). Inference of population genetic structure using multilocus genotype data. Genetics, 155, 945–959.

Sakai, H., K. Ikoma, S. V. Frolov, Y. Yamazaki, H. Takahashi & H. Ida, 2000. Morphological features of a Russian freshwater goby, *Rhinogobius lindbergi* (Pisces: Gobiidae), and its genetic relationships to Japanese species. Biogeography, 2: 51–61.

Sauvage, H.-E. & P. Dabry de Thiersant, 1874. Notes sur les poissons des eaux douces de Chine. Annales des Sciences Naturelles, Paris, Zoologie et Paléontologie, Sér. 6, 1 (art. 5): 1–18.

Seale, A., 1910. New species of Philippine fishes. The Philippine Journal of Science, Section A, 4: 491–543, pls.1–13.

Suzuki, T., I-S. Chen & H. Senou, 2012. A new species of *Rhinogobius* Gill, 1859 (Teleostei: Gobiidae) from the Bonin Islands, Japan. Journal of Marine Science and Technology, 19: 693–701.

Suzuki, T., S. Kimura & K. Shibukawa, 2019. Two new lentic, dwarf species of *Rhinogobius* Gill, 1859 (Gobiidae) from Japan. Bulletin of Kanagawa Prefectural Museum, (Natural Science), (48): 21–36.

Suzuki, T., N. Oseko, S. Kimura & K. Shibukawa, 2020. Two new species of torrential gobies of the genus *Rhinogobius* from the Ryukyu Islands, Japan. Bulletin of Kanagawa Prefectural Museum, (Natural Science), (49): 7–28.

Suzuki, T., K. Shibukawa & M. Aizawa, 2017. *Rhinogobius mizunoi*, a new species of freshwater goby (Teleostei: Gobiidae) from Japan. Bulltin of Kanagawa Prefectural Museum, (Natural Science), 46: 79–95.

Suzuki, T., K. Shibukawa, H. Senou & I-S. Chen, 2015. Redescription of *Rhinogobius similis* Gill 1859 (Gobiidae: Gobionellinae), the type species of the genus *Rhinogobius* Gill 1859, with designation of the neotype. Ichthyological Research.

Suzuki, T., K. Yano & T. Yonezawa, 2004. Kibara Yoshinobori *Rhinogobius* sp. YB. In Senou, H. (ed.), A Photographic Guide to the Gobioid Fishes of Japan, pp. 460–461. Heibonsha, Tokyo. (In Japanese).

Suzuki, T., K. Yano & T. Yonezawa, 2021. Kibara Yoshinobori Rhinogobius sp. YB. In Senou, H. (ed.), A Photographic Guide to the Gobioid Fishes of Japan. Revised edition, pp. 466–467. Heibonsha, Tokyo. (In Japanese).

Takahashi, S. & T. Okazaki, 2002. A new lentic form of the Yoshinobori species complex, *Rhinogobius* spp. from Lake Biwa, Japan, compared with lake-river migrating *Rhinogobius* sp. OR. Ichthyological Research, 49: 333–339.

Takahashi, S. & T. Okazaki, 2017. *Rhinogobius biwaensis*, a new gobiid fish of the "yoshinobori" species complex, *Rhinogobius* spp., endemic to Lake Biwa, Japan. Ichthyological Research, https://doi.org/1 0.1007/s10228-017-0577-4.

Tanaka, S., 1908. Descriptions of eight new species of fishes from Japan. Annotationes Zoologicae Japonenses, 7(1): 27–47.

Tanaka, S., 1925. Figures and descriptions of the fishes of Japan including Riukiu Islands, Bonin Islands, Formosa, Kurile Islands, Korea and southern Sakhalin. Figure and description of the fishes of Japan, 34: 629–644, pls.151–153.

Tchang, T.-L., 1939. Studies on Chinese *Glossogobius*. Bulletin of the Fan Memorial Institute of Biology, Peiping (Zoology Series), 9: 67–70.

Temminck, C. J. & H. Schlegel, 1845. Pisces. In von Siebold, P. F., Fauna Japonica, Parts VIII, pp. 133–152. Lugduni Batavorum.

Wanghe, K.-Y., F.-X. Hu, M.-H. Chen and X.-F. Luan, 2020. *Rhinogobius houheensis*, a new species of freshwater goby (Teleostei: Gobiidae) from the Houhe National Nature Reserve, Hubei province, China. Zootaxa, 4820(2): 351-365.

Wu, H.-L. & I-S. Chen, 2008. *Rhinogobius* Gill, 1859. In Wu, H.-L. & Zhong, J.-S. et al. (eds.), Fauna Sinica, Osteichthyes, Perciformes (V), Gobioidei, pp. 568–635. Science Press, Beijing. (In Chinese).

Wu, Q., X. Deng, Y. Wang & Y. Liu, 2018. *Rhinogobius maculagenys*, A new species of freshwater goby (Teleostei: Gobiidae) from Hunan, China. Zootaxa, 4476 (1): 118–129.

Wu, H.-L. & M.-L. Zheng, 1985. *Ctenogobius multimaculatus, Ctenogobius lentiginis*. In Zheng, M.-L. & H.-L. Wu. A study on the freshwater gobioid fishes of Zhejiang Province, China, with descriptions of two new species (Perciformes: Gobiidae), pp. 328–331. Acta Zootaxonomica Sinica, 10. (In Chinese).

Wu, H.-W., 1939. On the fishes of Li-Kiang. Sinensia, 10: 92–142, pls.1–3.

Xia, J.-H., H.-L. Wu, C.-H. Li, Y.-Q. Wu & S.-H. Liu, 2018. A new species of *Rhinogobius* (Pisces: Gobiidae), with analyses of its DNA barcode. Zootaxa, 4407(4): 553–562.

Yamasaki, Y. Y., M. Nishida, T. Suzuki, T. Mukai, & K.

Watanabe, 2015. Phylogeny, hybridization, and life history evolution of *Rhinogobius* gobies in Japan, inferred from multiple nuclear gene sequences. Molecular Phylogenetics and Evolution, 90: 20–33.

Yamasaki, Y. Y., H. Takeshima, Y. Kano, N. Oseko, T. Suzuki, M. Nishida & K. Watanabe, 2020. Ecosystem size predicts the probability of speciation in migratory freshwater fish. Molecular Ecology, https://do i.org/10.1111/mec.15415.

Yang, G.-Y. & C.-X. Xie, 1983. A new species of fishes from Mount Shennong. Zoological Research, 4: 71–74. (In Chinese).

Yang, J.-Q., H.-L. Wu & I-S. Chen, 2008. A new species of *Rhinogobius* (Teleostei: Gobiidae) from the Feiyunjiang Basin in Zhejiang Province, China. Ichthyological Research, 55: 379–385

Zhong, J.-S. & C.-S. Tzeng, 1998. A new species of *Rhinogobius* from China (Perciformes: Gobioidae). Zoological Research, 19: 237–241. (In Chinese).

Supplementary materials

Supplementary Material #1 is shown in the last page of the article.

摘　要

鈴木寿之・大迫尚晴・山﨑　曜・木村清志・渋川浩一, 2022. 琉球列島八重山諸島から得られたハゼ科ヨシノボリ属魚類の2新亜種を含む1新種. 神奈川県立博物館研究報告（自然科学）, (51): 9–34. [Suzuki, T., N. Oseko, Yo Y. Yamasaki, S. Kimura & K. Shibukawa, 2022. A New Species with Two New Subspecies of *Rhinogobius* (Teleostei: Gobiidae) from Yaeyama Group, the Ryukyu Islands, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 9–34.]

琉球列島八重山諸島の河川渓流域に生息するハゼ科ヨシノボリ属魚類の2新亜種（*Rhinogobius aonumai aonumai* と *Rhinogobius aonumai ishigakiensis*）をふくむ1新種 *Rhinogobius aonumai*（新標準和名パイヌキバラヨシノボリ）を記載した。*Rhinogobius aonumai aonumai*（新標準和名イリオモテパイヌキバラヨシノボリ）は西表島のみに分布し、背鰭前方鱗数9–15、縦列鱗数32–37、脊椎骨数11+15–17=26–28（モードは27）、第2背鰭前端の2個の担鰭骨は第10脊椎骨の神経棘をまたぐ、腹鰭第5軟条は最初に3または4分岐（ふつう4分岐）する、頬の孔器列は縦列する、生鮮時の体の地色は黄色系である、第1背鰭に暗色斑はない、尾鰭に暗色の横点列かジグザグ横線が並ぶ、雌の尾鰭基底に垂直に並ぶ1対の暗色の短い棒状斑があるなどの特徴で同属の他種階級タクソン（種及び亜種）から区別できる。*Rhinogobius aonumai ishigakiensis*（新標準和名イシガキパイヌキバラヨシノボリ）は石垣島のみに分布し、背鰭前方鱗数10–14、縦列鱗数33–38、脊椎骨数10+16–18==26–28（モードは27）、第2背鰭前端の2個の担鰭骨は第9脊椎骨の神経棘をまたぐ、腹鰭第5軟条は最初に2または3分岐（ふつう2分岐）する、頬の孔器列は縦列する、生鮮時の体の地色は黄色系である、第1背鰭に暗色斑はない、尾鰭に暗色のジグザグ横線が並ぶ、雌の尾鰭基底に垂直に並ぶ1対の暗色の短い棒状斑があるなどの特徴で同属の他種階級タクソン（種及び亜種）から区別できる。

Supplementary Material 1. Comparative materials

Rhinogobius flumineus: 9 specimens (41.0–53.5 mm SL): OMNH-P 43227–43230, 2 males and 2 females, 47.5–53.5 mm SL, Ina-gawa River, Inagawa, Hyogo Prefecture, Japan, 34°54'42.93"N 135°22'8.05"E, 10 October 2015; OMNH-P 42905, male, 48.1 mm SL, stained with Alizarin Red S., Ina-gawa River, 34°55'0.54"N 135°21'18.62"E, 29 August 2014; OMNH-P 48836 and 48838, male and female, 41.0 and 46.5 mm SL, stained with Alizarin Red S., Kuma-gawa River, Kumakogen, Ehime Prefecture, Japan, 33°36'55.65"N 132°57'16.91"E, 20 February 2020; OMNH-P 48840 and 48841, male and female, 42.0 and 44.5 mm SL, stained with Alizarin Red S., Shimanto-gawa River, Nakatosa, Kochi Prefecture, Japan, 33°21'52.10"N 133°8'4.99"E, 20 February 2020.

***Rhinogobius* sp. YB**: 38 specimens: (27.0–61.0 mm SL). Amami-oshima Island (Amami Group of the Ryukyu Islands, Japan): OMNH-P 43030–43033, three males and a female, 38.3–42.3 mm SL, Kawauchi-gawa River, 28°20'0.88"N 129°25'13.9"E, 27 July 2014; OMNH-P 42870–42872, two males and a female, 37.0–42.8 mm SL, Akina-gawa River, 28°24'52.3"N 129°33'36.5"E, 28 July 2014. Tokuno-shima Island (Amami Group): OMNH-P 43021 and 43023, male and female, 41.0–50.8 mm SL, Agon-gawa River, 27°42'34.93"N 128°55'6.28"E, 30 January 2015; OMNH-P 43024 and 43026, male and female, 41.5 and 46.5 mm SL, Uwanaru-gawa River, 27°44'12.25"N 128°55'37.15"E, 27 January 2015; OMNH-P 43016 and 43017, male and female, 48.0 and 53.6 mm SL, Akigiyan-gawa River, 27°46'43.33"N 128°57'28.61"E, 28 January 2015; OMNH-P 43027, male, 38.0 mm SL, Oose-gawa River, 27°43'53.78"N 128°59'38.25"E, 30 January 2015. Okinoerabu-jima Island (Amami Group): OMNH-P 43011–43014, two males and two females, 38.0–53.5 mm SL, Amata-gawa River, 27°21'46.1"N 128°36'40.9"E, 22 or 24 January 2014. Sampling in Kagoshima Prefecture was approved by Kagoshima Prefecture. Okinawa-jima Island (Okinawa Group of the Ryukyu Islands, Japan): OMNH-P 38191–39194, two males and two females, 44.5–61.0 mm SL, Sukuta-gawa River, 26°33'41.6"N 127°59'18"E, 11 December 2011; OMNH-P 43048 and 43050, male and female, 40.0 and 45.5 mm SL, Okukubi-gawa River, 26°28'59.24"N 127°54'20.25"E, 2 March 2015; OMNH-P 43051–43054, two males and two females, 39.3–49.1 mm SL, Yofuke-gawa River, 26°33'31.1"N 128°00'23.3"E, 2 March 2015. Tokashiki-jima Island (Okinawa Group): OMNH-P 48916–48919, three males and a female, 36.5–38.0 mm SL, a small stream with an unknown name, 26°10'07.8"N 127°21'18.1"E, 27 February 2018. Kume-jima Island (Okinawa Group): OMNH-P 42910–42912, two males and female, 27.0–32.0 mm SL, Suhara-gawa River, 26°18'57.87"N 126°47'27.41"E, 5 October 2014; OMNH-P 42913–42915, two males and female, 28.0–38.4 mm SL, Shirase-gawa River, 26°21'31.04"N 126°46'22.62"E, 6 or 8 October 2014.

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Original article

Molecular Phylogenetic and Morphological Problems of the Aki Salamander *Hynobius akiensis*: Description of Two New Species from Chugoku, Japan

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Abstract. Two new species of the genus *Hynobius* are described from the southern part of Hiroshima Prefecture, Japan. *Hynobius akiensis* was divided into three groups (Hiroshima-Ehime group, Higashihiroshima group, Northern Hiroshima group) based on morphological and molecular analyses; thus, the Hiroshima-Ehime and Higashihiroshima groups were described as *Hynobius geiyoensis* sp. nov. and *Hynobius sumidai* sp. nov., respectively. According to morphological comparisons, males of *H. geiyoensis* sp. nov. have significantly larger snout–vent length relative to those of males of the other two species, whereas members of *H. sumidai* sp. nov., unlike *H. akinesis*, have a distinct brownish–yellow stripe on the dorsal side of their tail. Following this description, the distribution area of *H. akiensis* has changed substantially; thus, the habitat status of this species or two new species should be reassessed for its conservation.

Key words: cytochrome b, fragmented habitat, Hiroshima Prefecture, natural monument, Seto Inland Sea

Introduction

The Aki salamander, *Hynobius akiensis*, was described from Mirasakacho Haizuka, Miyoshi-shi, Hiroshima Prefecture, but it is distributed in Hiroshima and Ehime Prefectures across the Seto Inland Sea (Setonaikai) (Matsui *et al.*, 2019). Based on mitochondrial DNA analyses, this species has three genetically distinct groups, namely the Northern Hiroshima, Higashihiroshima, and Hiroshima-Ehime groups; monophyly is strongly supported in the latter two groups, whereas the Northern Hiroshima group is not part of the monophyletic group with the Higashihiroshima, and Southern Hiroshima groups (Matsui *et al.*, 2019). However, these results were obtained from analysis of only 14 samples; thus, the monophyly of the three groups should be reassessed using many populations across the entire distribution range of *H. akiensis*. In addition, the morphological uniformity of the three *H. akiensis* groups is unreliable because Matsui *et al.* (2019) did not make comparisons among them; therefore, such comparisons are still required.

In the present study, we evaluated the species validity of the three aforementioned groups using the morphological, phylogenetic, and evolutionary species concepts, similar to Sugawara *et al.* (2018). To resolve the taxonomic issues related to *H. akiensis*, we performed statistical analyses of morphological characteristics to compare the three groups. We also used additional DNA sequence data to reconstruct the phylogeny of *H. akiensis* collected from the entire distribution range of this species. Finally, we revealed the new distribution ranges of the three *H. akiensis* groups in detail.

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Materials and methods

Molecular analysis

For phylogenetic analysis, we collected DNA samples from personal property or fields from February 2007 to April 2021 (Table 1; Fig. 1). We obtained a single tailbud embryo from each paired egg sac or tissue samples from larvae when tissue sampling from fields; these tissues were preserved in 99.5 % ethanol. The DNA extraction was performed using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For constructing phylogenetic trees, we sequenced a 630-bp mitochondrial cytochrome b gene (630 bp) using the two primers: L14010 (5′-TAHGGWGAHGGATTWGAWGCMACWGC-3′) and H14778 (5′-AARTAYGGGTGRAADGRRAYTTTRTCT-3′) (Matsui *et al.*, 2007). We deposited the acquired sequences into the DNA Data Bank of Japan (Table 1). DNA sequences were aligned using MEGA X (Kumar *et al.*, 2018) and then phylogenetic analyses of the aligned sequences were conducted using Bayesian inference (BI) and maximum likelihood (ML) estimations with several species of *Hynobius* and *Salamandrella keyserlingii* as the outgroup (Table 1). We estimated the best-fit nucleotide substitution model based on the Bayesian information criterion (Schwarz, 1978) and corrected Akaike's information criterion (AICc) (Sugiura, 1978) via jModelTest 2 (Darriba *et al.*, 2012). We selected the Hasegawa-Kishino-Yano model (gamma distribution) for BI and Tamura-Nei model (invariant sites) for ML and constructed Bayesian and maximum likelihood trees using MrBayes 3.2 (Ronquist *et al.*, 2012) and MEGA X (Kumar *et al.*, 2018), respectively. For Bayesian analyses, we performed two independent MCMC runs for 2,000,000 generations with a sampling frequency of 100; we examined the stationarity of the likelihood scores of sampled trees using Tracer version 1.7 (http://tree.bio.ed.ac.uk/software/tracer/) with the first 25 % of generations discarded as burn-in. Finally, we assessed monophyly using the values of posterior probability (PP) and bootstrap (BS) based on the criteria of Huelsenbeck and Rannala (2004) and Hillis & Bull (1993): monophyletic group = PP ≥ 0.95 and BP ≥ 70.

Morphological analysis

We sampled 54 individuals of *H. akiensis* from February 2017 to April 2021: 19 individuals of the Hiroshima-Ehime group from 2 populations (Pops. 1, 2), 7 individuals of the Higashihiroshima group from 1 population (Pop. 8), and 28 individuals of the Northern Hiroshima group from 8 populations (Pops. 14, 15, 20, 29, 30, 34, 39, 54) (Table 1; Fig. 1). In this study, we used only male individuals for morphological analyses, as did Matsui *et al.* (2019), because we could not collect enough females for statistical analyses. The collected individuals were measured under anesthesia using ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich®, St. Louis, MO, USA) diluted 1,000-fold with water (Bennett, 1991). For conservation, measured individuals were subsequently returned to their site of capture except for the candidate individuals of type specimens. Before individuals were returned, photographs were taken of their dorsal, ventral, and lateral sides with a black background, and tissue samples (preserved to 99.5 % ethanol) were collected from the tail tips of all individuals as evidence of collection. All examined individuals were measured using a vernier caliper with the following 22 measurements: snout–vent length (SVL), trunk length

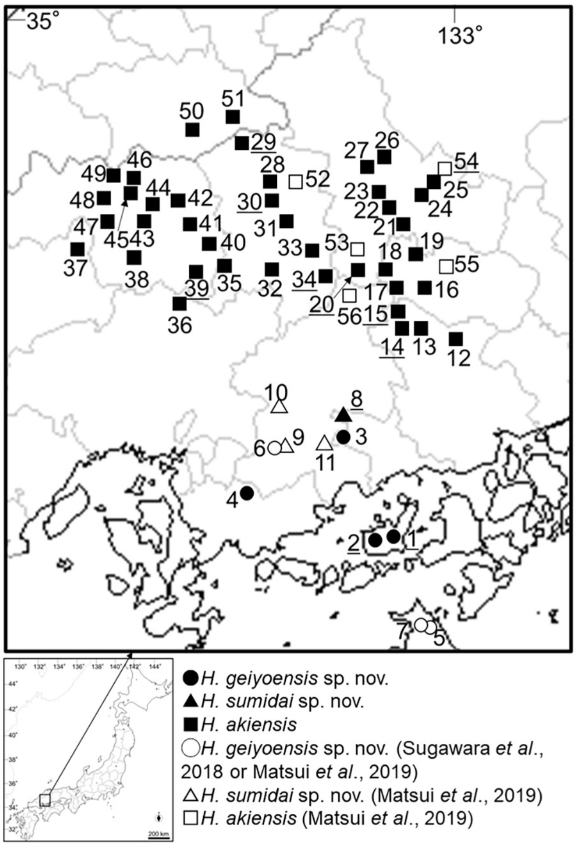

Fig. 1. Sampling map of the three *Hynobius* species used in this study. The enlarged area includes the central to the western part of Hiroshima Prefecture and the northernmost part of Ehime Prefecture. Closed symbols correspond to each of the three species sequenced in the current study. Open symbols correspond to each of the three species sequenced by other studies. The underlined localities show the sampling points of individuals for morphological comparisons: Pops. 1 (18 males) and 2 (1 male) for *H. geiyoensis* sp. nov.; Pop. 8 (7 males) for *H. sumidai* sp. nov.; Pops. 14 (3 males), 15 (7 males), 20 (6 males), 29 (3 males), 30 (1 male), 34 (6 males), 39 (1 male), 54 (1 male) for *H. akiensis*.

Table 1. List of samples used for molecular analyses

Population	Species	Sampling licality	Accession number / Label in Fig. 2
1	*Hynobius geiyoensis* sp. nov.	Nakano, Osakikamijima-cho, Hiroshima*	LC647835 / GEI01
2	*Hynobius geiyoensis* sp. nov.	Harada, Osakikamijima-cho, Hiroshima	LC647836 / GEI02
3	*Hynobius geiyoensis* sp. nov.	Nikacho, Takehara-shi, Hiroshima	LC647837 / GEI03
4	*Hynobius geiyoensis* sp. nov.	Goharacho, Kure-shi, Hiroshima	LC647838 / GEI04
5	*Hynobius geiyoensis* sp. nov.	Chikamicho, Imabari-shi, Ehime	LC279198 / EIC2018
6	*Hynobius geiyoensis* sp. nov.	Saijocho Goso, Higashihiroshima-shi, Hiroshima	LC436389 / H24
7	*Hynobius geiyoensis* sp. nov.	Namikatacho Hinokuchi, Imabari-shi, Ehime	LC436387 / H22
8	*Hynobius sumidai* sp. nov.	Tamaricho, Takehara-shi, Hiroshima*	LC647839 / SUM01
9	*Hynobius sumidai* sp. nov.	Saijocho Goso, Higashihiroshima-shi, Hiroshima	LC436390 / H25
10	*Hynobius sumidai* sp. nov.	Hachihonmatsucho Yoshikawa, Higashihiroshima-shi, Hiroshima	LC436392 / H27
11	*Hynobius sumidai* sp. nov.	Akitsucho Mitsu, Higashihiroshima-shi, Hiroshima	LC436394 / H29
12	*Hynobius akiensis*	Kuicho Hagura, Mihara-shi, Hiroshima	LC647840 / AKI01
13	*Hynobius akiensis*	Daiwacho Shimotokura, Mihara-shi, Hiroshima	LC647841 / AKI02
14	*Hynobius akiensis*	Daiwacho Ogu, Mihara-shi, Hiroshima	LC647842 / AKI03
15	*Hynobius akiensis*	Daiwacho Kuramune, Mihara-shi, Hiroshima	LC647843 / AKI04
16	*Hynobius akiensis*	Tsukuchi, Sera-cho, Hiroshima	LC647844 / AKI05
17	*Hynobius akiensis*	Yoshiwara, Sera-cho, Hiroshima	LC647845 / AKI06
18	*Hynobius akiensis*	Naka, Sera-cho, Hiroshima	LC647846 / AKI07
19	*Hynobius akiensis*	Nagata, Sera-cho, Hiroshima	LC647847 / AKI08
20	*Hynobius akiensis*	Miwacho Kamiichi, Miyoshi-shi, Hiroshima	LC647848 / AKI09
21	*Hynobius akiensis*	Uedamachi, Miyoshi-shi, Hiroshima	LC647849 / AKI10
22	*Hynobius akiensis*	Miwakamachi, Miyoshi-shi, Hiroshima	LC647850 / AKI11
23	*Hynobius akiensis*	Megurikamimachi, Miyoshi-shi, Hiroshima	LC647851 / AKI12
24	*Hynobius akiensis*	Kisacho Shikiji, Miyoshi-shi, Hiroshima	LC647852 / AKI13
25	*Hynobius akiensis*	Mirasakacho Mirasaka, Miyoshi-shi, Hiroshima	LC647853 / AKI14
26	*Hynobius akiensis*	Minamihatajikimachi, Miyoshi-shi, Hiroshima	LC647854 / AKI15
27	*Hynobius akiensis*	Nishisakeyamachi, Miyoshi-shi, Hiroshima	LC647855 / AKI16
28	*Hynobius akiensis*	Takamiyacho Kurumegi, Akitakata-shi, Hiroshima	LC647856 / AKI17
29	*Hynobius akiensis*	Midoricho Ikeda, Akitakata-shi, Hiroshima	LC647857 / AKI18
30	*Hynobius akiensis*	Midoricho Yokota, Akitakata-shi, Hiroshima	LC647858 / AKI19
31	*Hynobius akiensis*	Yoshidacho Aio, Akitakata-shi, Hiroshima	LC647859 / AKI20
32	*Hynobius akiensis*	Yoshidacho Oyama, Akitakata-shi, Hiroshima	LC647860 / AKI21
33	*Hynobius akiensis*	Kodacho Kamiobara, Akitakata-shi, Hiroshima	LC647861 / AKI22
34	*Hynobius akiensis*	Mukaiharacho Saka, Akitakata-shi, Hiroshima	LC647862 / AKI23
35	*Hynobius akiensis*	Yachiyocho Sasai, Akitakata-shi, Hiroshima	LC647863 / AKI24
36	*Hynobius akiensis*	Kabecho Nabara, Asakita-ku, Hiroshima-shi, Hiroshima	LC647864 / AKI25
37	*Hynobius akiensis*	Inoshiyama, Akiota-cho, Hiroshima	LC647865 / AKI26
38	*Hynobius akiensis*	Tsushimi, Kitahiroshima-cho, Hiroshima	LC647866 / AKI27
39	*Hynobius akiensis*	Minamigata, Kitahiroshima-cho, Hiroshima	LC647867 / AKI28
40	*Hynobius akiensis*	Yorohoyobara, Kitahiroshima-cho, Hiroshima	LC647868 / AKI29
41	*Hynobius akiensis*	Haruki, Kitahiroshima-cho, Hiroshima	LC647869 / AKI30
42	*Hynobius akiensis*	Kawado, Kitahiroshima-cho, Hiroshima	LC647870 / AKI31
43	*Hynobius akiensis*	Kamiishi, Kitahiroshima-cho, Hiroshima	LC647871 / AKI32
44	*Hynobius akiensis*	Motsuna, Kitahiroshima-cho, Hiroshima	LC647872 / AKI33
45	*Hynobius akiensis*	Ikadazu, Kitahiroshima-cho, Hiroshima	LC647873 / AKI34
46	*Hynobius akiensis*	Otuka, Kitahiroshima-cho, Hiroshima	LC647874 / AKI35
47	*Hynobius akiensis*	Mizoguchi, Kitahiroshima-cho, Hiroshima	LC647875 / AKI36
48	*Hynobius akiensis*	Utsunohara, Kitahiroshima-cho, Hiroshima	LC647876 / AKI37
49	*Hynobius akiensis*	Takano, Kitahiroshima-cho, Hiroshima	LC647877 / AKI38
50	*Hynobius akiensis*	Iwaya, Onan-cho, Shimane	LC647878 / AKI39
51	*Hynobius akiensis*	Asuna, Onan-cho, Shimane	LC647879 / AKI40
52	*Hynobius akiensis*	Takamiyacho Bogo, Akitakata-shi, Hiroshima	LC436381 / H16
53	*Hynobius akiensis*	Miwacho Kamiitaki, Miyoshi-shi, Hiroshima	LC436383 / H18
54	*Hynobius akiensis*	Mirasakacho Haizuka, Miyoshi-shi, Hiroshima*	LC436384 / H19
55	*Hynobius akiensis*	Kurobuchi, Sera-cho, Hiroshima	LC436385 / H20
56	*Hynobius akiensis*	Toyosakacho Kiyotake, Higashihiroshima-shi, Hiroshima	LC436386 / H21
	Hynobius abuensis		LC436395 / H. abuensis
	Hynobius hidamontanus		LC225434 / H. hidamontanus
	Hynobius tosashimizuensis		LC436447 / H. tosashimizuensis
	Hynobius utsunomiyaorum		LC436376 / H. utsunomiyaorum
	Salamandrella keyserlingii		NC_026032 / S. keyserlingii

The number of males indicates the individuals used in morphological analyses. Asterisks with sampling localities indicate the type locality of three species.

(TRL), axilla-groin distance (AGD), head length (HL), tail length (TAL), median tail width (MTAW), median tail height (MTAH), vomerine teeth length (VTL), and vomerine teeth wide (VTW), head width (HW), forelimb length (FLL), hindlimb length (HLL), second finger length (2FL), third finger length (3FL), third toe length (3TL), five toe length (5TL), internarial distance (IND), interorbital distance (IOD), upper eyelid length (UEL), snout length (SL), upper eyelid width (UEW), and lower jaw length (LJL). On each individual, we also checked for the presence of the following markings: distinct black dots on the dorsal side of the body (DBDD), distinct white dots on the ventral side of the body (DWDV), distinct white dots on the lateral sides of the body (DWDL), distinct brownish–yellow lines on the dorsal (DTBYLD), and ventral (DTBYLV) sides of the tail, and distinct gular mottling (DGM). The number of costal folds between the adpressed limbs (CFBALN) and the number of costal grooves (CGN) were counted, with the counting method of Matsui *et al.* (2019) used to count CGN. Prior to performing morphological comparisons among the three groups, we tested the data for normality using a Shapiro–Wilk test; when the data followed a normal distribution, we tested for homoscedasticity using Bartlett's test. When the variances among populations were equal, we performed Tukey–Kramer tests; when variances were not equal, we performed Games–Howel tests. When data did not follow a normal distribution and variances among populations were not equal, we performed Steel–Dwass tests. To examine the overall morphological variation among the three groups, we performed canonical discriminant analysis using SVL and standardized values for the 21 measurements: RTRL, RAGD, RHL, RTAL, RMTAW, RMTAH, RVTL, RVTW, RHW, RFLL, RHLL, R2FL, R3FL, R3TL, R5TL, RIND, RIOD, RUEW, RSL, RUEL, RLJL. Statistical analyses were conducted using R with $\alpha = 0.05$ (Ihaka & Gentleman, 1996).

From type specimens, we took 43 measurements as follows: SVL, TRL, AGD, HL, TAL, MTAW, MTAH, basal tail width (BTAW), basal tail height (BTAH), VTL, VTW, HW, left forelimb length (LFLL), left hindlimb length (LHLL), right forelimb length (RFLL), right hindlimb length (RHLL), left first finger length (L1FL), left second finger length (L2FL), left third finger length (L3FL), left fourth finger length (L4FL), right first finger length (R1FL), right second finger length (R2FL), right third finger length (R3FL), right fourth finger length (R4FL), left first toe length (L1TL), left second toe length (L2TL), left third toe length (L3TL), left fourth toe length (L4TL), left fifth toe length (L5TL), right first toe length (R1TL), right second toe length (R2TL), right third toe length (R3TL), right fourth toe length (R4TL), right fifth toe length (R5TL), IND, IOD, left upper eyelid length (LUEL), right upper eyelid length (RUEL), SL, left upper eyelid width (LUEW), right upper eyelid width (RUEW), and LJL. Measurements were taken after specimens were fixed in 10 % formalin and transferred to 70 % ethanol.

Two holotype specimens designated in this study are stored in the Shobara Municipal Hiwa Museum for Natural History: 1119-1, Hiwacho Hiwa, Shobra-shi, Hiroshima Prefecture, 727-0301, Japan. All paratypes of the two new species and a single topotype of *H. akiensis* are stored in the Kanagawa Prefectural Museum: 499 Iryuda, Odawara-shi, Kanagawa Prefecture, 250-0031, Japan. Further details are available only by contacting the corresponding author or the museums for avoiding overcollection of the species.

Results

The monophyly of *H. akiensis* was not supported by PP and BS values (Fig. 2); indeed, the Hiroshima-Ehime, Higashihiroshima, and Northern Hiroshima groups were genetically distinguishable. In contrast, the monophyly of *H. akiensis*, *Hynobius utsunomiyaorum*, and *Hynobius hidamontanus* was strongly supported by both BI and ML (Fig. 2). The transition type of *H. akiensis* was polyphyletic, included in the clade of the Northern Hiroshima group of *H. akiensis* (Fig. 2).

Morphological measurements of the three groups and significant values of all measurements among these groups are shown in Table 2 and Table 3, respectively. Males of the Hiroshima-Ehime and Higashihiroshima groups differed significantly in terms of nine morphological characteristics; males of the Hiroshima-Ehime and Northern Hiroshima groups differed significantly in terms of ten morphological characteristics; males of the Higashihiroshima and Northern Hiroshima groups differed significantly in terms of three morphological characteristics (Table 3). The canonical discriminant analysis also indicated that the three groups were different and that the distribution area of scores did not overlap (Fig. 3).

Morphological observations are given in Table 4. The Hiroshima-Ehime group always had DWDV (19/19 = 100 %), almost always had DTBYLD (17/19 = 89.5 %), 12 CGN (17/19 = 89.5 %), and −2–0 CFBALN (18/19 = 94.7 %), usually had DWDL (16/19 = 84.2 %), and almost always had no DGM (18/19 = 94.7 %), and never had DTBYLV (19/19 = 100 %). The Higashihiroshima group always had DTBYLD (7/7 = 100 %), usually had −1–0 CFBALN (6/7 = 85.7 %), and never had DTBYLV (7/7 =

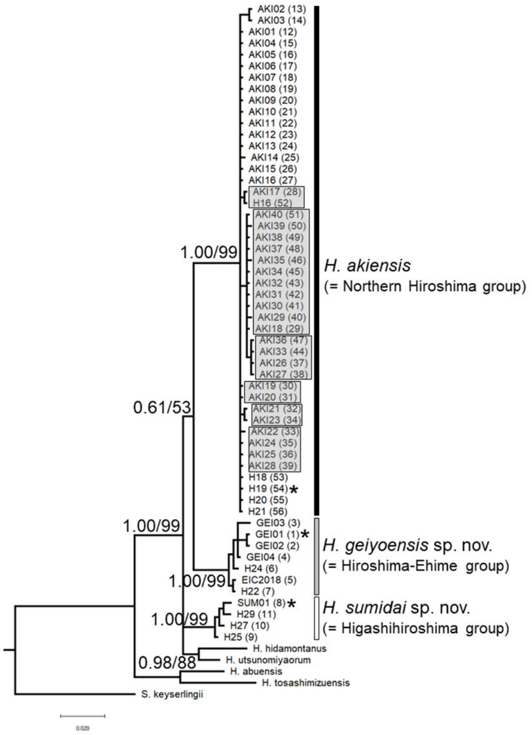

Fig. 2. Phylogenetic tree produced by Bayesian inference using 630-bp cytochrome b genes. *Salamandrella keyserlingii* was used as an outgroup. Scale bar shows genetic distance (expected changes per site). Numbers located near the nodes are posterior probabilities (PP) for Bayesian inference and bootstrap (BS) values for maximum likelihood estimation. Values appearing in parentheses after the haplotype names correspond to population localities as indicated in Table 1 and Fig. 1. Asterisks after the parentheses (Pops. 1, 8, and 54) indicate the type locality of the three species. The labels covered by shaded boxes indicate the transition type of *Hynobius akiensis*.

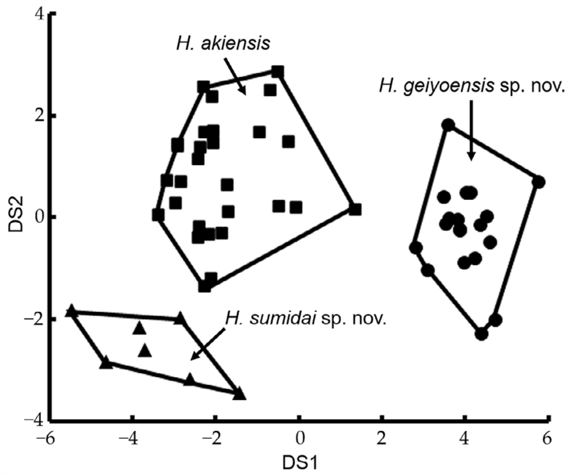

Fig. 3. Two-dimensional plots of canonical discriminant analysis in males. The x and y axes show discriminant score 1 (DS1) and discriminant score 2 (DS2), respectively. The contribution ratios of DS1 and DS2 were 88.03 % and 11.97 %, respectively.

100 %) and DGM (7/7 = 100 %). The northern Hiroshima group usually lacked the DTBYLD (25/28 = 89.3 %), usually had DWDV (23/28 = 82.1 %), 12 CGN (24/28 = 85.7 %), and −1.5–0 CFBALN (89.3 %), but never had DTBYLV (7/7 = 100 %).

According to the results from molecular and morphological analyses, the Hiroshima-Ehime and Higashihiroshima groups of *H. akiensis* should be distinct species based on the three species concepts. Thus, we described them as new species below.

Taxonomy

Hynobius geiyoensis **sp. nov.**

(New standard Japanese name: *Geiyo-sanshouo*)

(Figs. 4–5)

Hynobius nebulosus: Okawa *et al.*, 1990: 50, in part.

Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Etymology. The specific name is derived from "*Geiyo*." In ancient times, the combined area of Aki (= central to the western part of Hiroshima Prefecture) and Iyo (= Ehime Prefecture), where the new species occurred, was called *Geiyo*.

Holotype. An adult male (specimen number: HMNH-AM-101) from Nakano, Osakikamijima-cho, Hiroshima Prefecture, Chugoku, Japan [34° 13′ N, 132° 53′ E; elevation = 150 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on February 23, 2019.

Paratype. An adult female (specimen number: KPM-NFA 943) from Harada, Osakikamijima-cho, Hiroshima Prefecture, Chugoku, Japan [34° 14′ N, 132° 52′ E; elevation = 30 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on 23 February 2019. An adult male (specimen number: KPM-NFA 944) from the same locality as the holotype, collected by Jun-ichi Naito on February 23, 2019.

Diagnosis. A comparatively large species (with a mean snout–vent length of 60.1 mm in males) within the Japanese lentic *Hynobius*: snout–vent length usually more than 50 mm; distinct white dots on the ventral side of the body always present; distinct brownish–yellow line on the dorsal edge of the tail almost always present; distinct white dots on the lateral side of the body usually present; distinct yellow stripe on the ventral edge of the tail always absent; distinct gular mottling almost always absent in males; fifth toe of hindlimb always present; V-shaped vomerine teeth

Table 2. Measurements (mm) of SVL and character ratios (R = %SVL) of TRL to LJL (ranges are shown in parentheses)

	H. geiyoensis sp. nov.		*H. sumidai* sp. nov.		*H. akiensis*	
Trait	Holotype	*n* = 19	Holotype	*n* = 7	Topotype	*n* = 28
SVL	58.4	60.1±3.23	48.2	47.2±2.23	55.3	51.2±4.07
		(50.5-64.5)		(44.9-49.7)		(42.6-60.5)
RTRL	78.3	77.2±0.80	75.9	76.9±1.26	77.8	77.0±1.14
		(75.5-78.4)		(75.3-78.3)		(74.6-78.9)
RAGD	54.1	52.5±1.32	53.9	53.5±1.78	50.3	52.9±1.41
		(49.8-54.4)		(51.0-56.8)		(49.7-55.3)
RHL	21.4	23.1±0.86	23.4	24.3±1.54	23.1	24.2±0.82
		(21.3-24.9)		(22.6-27.4)		(23.1-26.0)
RTAL	85.6	75.7±6.46	71.8	68.9±7.47	58.2	68.2±5.91
		(60.5-85.7)		(55.5-76.2)		(57.2-78.4)
RMTAW	6.8	6.0±0.78	5.0	5.9±0.53	4.9	6.5±0.86
		(4.7-7.5)		(5.0-6.7)		(4.2-8.1)
RMTAH	14.0	11.7±1.00	9.8	10.7±1.19	10.1	10.4±1.18
		(9.5-14.0)		(9.3-12.6)		(7.7-12.7)
RVTL	5.4	4.9±0.48	5.4	5.2±0.49	4.7	4.7±0.38
		(3.9-5.9)		(4.3-5.6)		(3.3-5.3)
RVTW	4.8	5.3±0.26	5.2	5.4±0.24	5.6	5.5±0.35
		(4.8-5.7)		(5.1-5.7)		(4.9-6.1)
RHW	17.5	16.4±0.60	16.6	16.6±0.58	17.7	16.7±0.61
		(15.3-17.8)		(15.7-17.4)		(15.4-17.8)
RFLL	23.6	23.0±1.29	24.5	25.1±1.42	22.8	25.1±1.22
		(20.6-25.0)		(23.9-28.1)		(22.7-27.5)
RHLL	30.5	30.6±1.03	29.3	30.1±1.80	31.8	30.6±1.29
		(28.4-32.7)		(27.6-32.7)		(27.2-32.9)
R2FL	5.5	5.0±0.54	4.4	4.8±0.27	5.8	4.9±0.58
		(4.2-6.1)		(4.4-5.1)		(3.8-5.8)
R3FL	5.3	4.5±0.64	3.9	3.8±0.21	5.2	4.2±0.43
		(3.0-5.3)		(3.6-4.2)		(3.4-5.3)
R3TL	9.1	8.2±0.49	6.8	7.5±0.72	8.9	7.9±0.69
		(7.4-9.3)		(6.7-8.7)		(6.3-9.5)
R5TL	2.7	2.4±0.44	1.7	1.3±0.37	2.2	1.3±0.79
		(1.7-3.4)		(0.8-1.8)		(0.0-2.6)
RIND	5.0	5.4±0.43	4.8	5.2±0.35	5.2	5.3±0.47
		(4.7-6.1)		(4.7-5.7)		(4.1-6.2)
RIOD	6.5	5.8±0.31	6.2	6.4±0.62	6.0	6.3±0.33
		(5.1-6.6)		(5.6-7.6)		(5.7-7.0)
RUEW	2.7	2.9±0.20	3.3	3.4±0.19	2.7	3.4±0.35
		(2.6-3.4)		(3.2-3.8)		(2.6-4.0)
RSL	6.8	6.3±0.36	7.1	6.2±0.47	7.2	6.5±0.47
		(5.7-6.8)		(5.7-7.1)		(5.4-7.3)
RUEL	4.3	3.9±0.21	5.0	4.7±0.35	4.2	4.7±0.31
		(3.6-4.4)		(4.4-5.1)		(3.8-5.1)
RLJL	12.5	13.4±0.61	14.3	13.9±0.62	13.6	14.3±0.73
		(12.5-14.4)		(13.0-14.9)		(13.1-15.8)

See Materials and Methods for definitions of morphological characteristics.

series; 12 (rarely 11) costal grooves; the number of costal folds between adpressed limbs always −2 to 0.5 in males.

Description of holotype. A moderately large individual: HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; clearly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on both sides; five toes on each hindlimb, order of length III > IV > II > I > V on both sides; V-shaped vomerine teeth; skin smooth and shiny; DBDD present; DWDV and DWDL present (it became unclear after preservation); DTBYLD present; DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL =58.4, TRL = 45.7, AGD = 31.6, HL = 12.5, TAL = 50.0, MTAW = 4.0, MTAH = 8.2, BTAW = 8.0, BTAH = 7.3, VTL = 3.2,

Table 3. Significant values of the 22 morphological traits in males among three species

	Male		
Trait	GEI vs. SUM	GEI vs. AKI	SUM vs. AKI
SVL	**P < 0.001**	**P < 0.0001**	P < 0.05
RTRL	NS	NS	NS
RAGD	NS	NS	NS
RHL	P < 0.05	P < 0.01	NS
RTAL	P < 0.05	**P < 0.001**	NS
RMTAW	NS	NS	NS
RMTAH	NS	**P < 0.001**	NS
RVTL	NS	NS	P < 0.05
RVTW	NS	NS	NS
RHW	NS	NS	NS
RFLL	P < 0.01	**P < 0.0001**	NS
RHLL	NS	NS	NS
R2FL	NS	NS	NS
R3FL	P < 0.01	NS	P < 0.01
R3TL	P < 0.05	NS	NS
R5TL	**P < 0.0001**	**P < 0.0001**	NS
RIND	NS	NS	NS
RIOD	NS	**P < 0.0001**	NS
RUEW	**P < 0.001**	**P < 0.0001**	NS
RSL	NS	NS	NS
RUEL	**P < 0.001**	**P < 0.0001**	NS
RLJL	NS	**P < 0.001**	NS
P < 0.05	3	0	2
P < 0.01	2	1	1
P < 0.001	3	3	0
P < 0.0001	1	6	0
Total	9	10	3

GEI, SUM, and AKI are abbreviations for *Hynobius geiyoensis* sp. nov., *H. sumidai* sp. nov., and *H. akiensis*, respectively. Significant differences (P < 0.001) are shown in bold. See Materials and Methods for definitions of morphological characteristics.

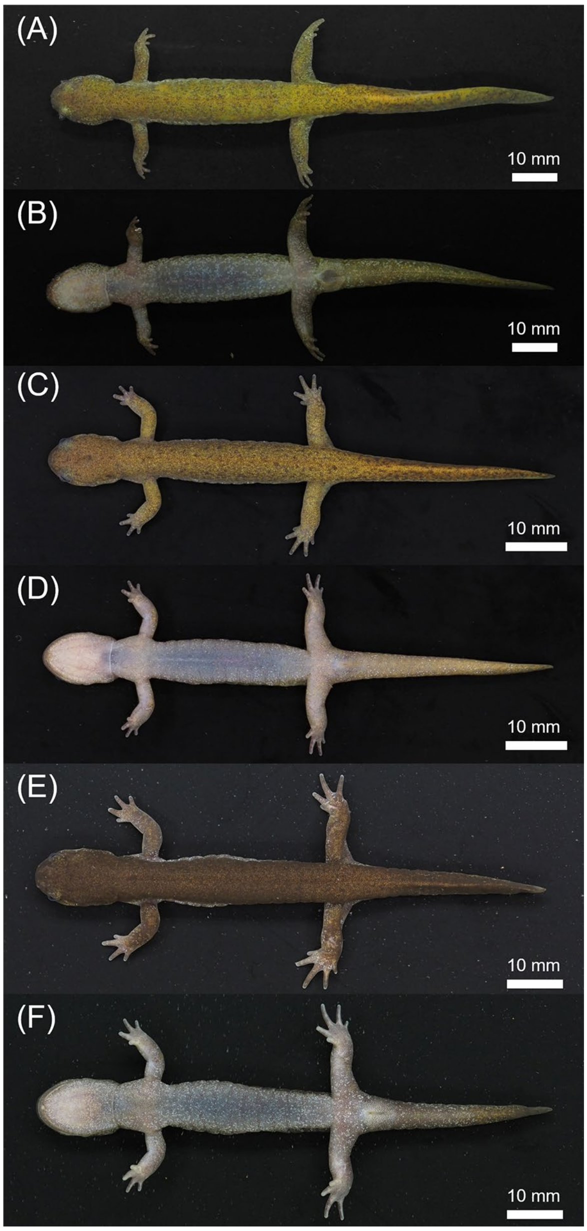

Fig. 4. Holotype of *Hynobius geiyoensis* sp. nov. (HMNH-AM-101, adult male, 58.4 mm SVL) from the (A) dorsal and (B) ventral perspective; holotype of *H. sumidai* sp. nov. (HMNH-AM-102, adult male, 48.2 mm SVL) from the (C) dorsal and (D) ventral perspective; and a specimen from the type locality (topotype) of *H. akiensis* (KPM-NFA 946, adult male, 55.3 mm) from the (E) dorsal and (F) ventral perspective.

VTW = 2.8, HW = 10.2, MXHW = 10.4, LFLL = 13.8, RFLL = 13.7, LHLL = 17.8, RHLL = 18.0, L1FL = 1.5, L2FL = 3.2, L3FL = 3.1, L4FL = 1.7, R1FL = 1.3, R2FL = 3.4, R3FL = 2.7, R4FL = 1.6, L1TL = 1.9, L2TL = 3.9, L3TL = 5.3, L4TL = 4.0, L5TL = 1.6, R1TL = 1.6, R2TL = 3.6, R3TL = 5.0, R4TL = 3.7, R5TL = 1.0, IND = 2.9, IOD = 3.8, LUEW = 1.6, RUEW = 1.4, SL =4.0, LUEL =2.5, RUEL = 2.7, LJL = 7.3, CGN = 12.

Comparisons. The new species statistically differs from *H. akiensis* in terms of the following length measurements in males: SVL, RHL, RTAL, RMTAH, RFLL, R5TL, RIOD, RUEW, RUEL, and RLJL; except for SVL, RTAL, RMTAH, and R5TL, these lengths were significantly shorter in *H. geiyoensis* sp. nov. relative to the lengths in *H. akiensis*. The new species differs from *H. akiensis* by the following characters: SVL > 57.1 mm (18/19 = 94.7 %) vs. SVL < 57.2 mm (26/28 = 92.9 %); RUEW < 3.2 % (18/19 = 94.7 %) vs. RUEW > 3.1 % (23/28 = 82.1 %); RUEL < 4.2 % (17/19 = 89.5 %) vs. RUEL > 4.1 % (27/28 = 96.4 %); usually have DTBYLD (17/19 = 89.5 %) vs. usually lack DTBYLD (25/28 = 89.3 %).

Variation. Morphometric measurements and observations are presented in Tables 2 and 4, respectively. The dorsum is uniformly yellowish brown or darkish brown. In the new species, DBDD are sometimes present (8/19 = 42.1 %), DWDL (3/19 = 15.8 %) and DTBYLD (2/19 = 10.5 %) are rarely absent, DGM is rarely present (1/19 = 5.3 %), CGN rarely 11 (2/19 = 10.5 %), and CFBALN rarely more than 0 (1/19 = 5.3 %). The iris is dark brown. When preserved, the dorsal coloration tends to fade to dark gray and DTBYLD becomes unclear after preservation.

Distribution. This new species is known from Higashihiroshima-shi (including the former Higashihiroshima-shi and Kurose-cho), Takehara-shi, and Kure-shi (including the former Kure-shi) in Hiroshima Prefecture, and Imabari-shi (including the former Imabari-shi and Namikata-cho) in Ehime Prefecture. DNA samples from the former Kurose-cho is not included in this study; however, based on previous field surveys (Okawa *et al.*, 1990), this species may be distributed in this area. This new species may also occur in the former Akitsu-cho (currently part of Higashihiroshima-shi) but there is currently no evidence to support this hypothesis. It was reported in 2018 that the populations of Kure-shi (2018) might already be extinct as. However, sufficient field surveys with objective evidences to support its extinction in Kure-shi have not been performed. Therefore, further field surveys are needed to clarify the current distribution range of the new species in greater detail.

Natural History. The dominant vegetation type of the type locality is a mixed forest of evergreen Fagaceae trees (i.e., *Quercus* and *Castanopsis* spp.) and Japanese cedar (*Cryptomeria japonica*) (Fig. 5). Larvae have distinct black dots on the lateral sides of the tail, whereas claws on the tips of the fingers and toes are absent. One pair of balancers is present during the early developmental stages of the larvae. Egg sacs are coil-shaped and are attached to fallen branches or leaves in ponds, puddles, or swamps at forest edges from February to April.

Remarks. The new species forms a monophyletic group with the Highland and Aki groups (Matsui *et al.*, 2019). The morphology of females is unclear; thus, further studies to clarify female morphology are required.

***Hynobius sumidai* sp. nov.**

(New standard Japanese name: *Hiroshima-sanshouo*)
(Figs. 4–5)

Hynobius nebulosus: Okawa *et al.*, 1990: 50, in part.
Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Etymology. The specific name "*sumidai*" is dedicated to Dr. Masayuki Sumida who was the phylogenetic

Table 4. Characteristics among the three species of *Hynobius*

		H. geiyoensis sp. nov.	*H. sumidai* sp. nov.	*H. akiensis*
Character	Condition	*n* = 19	*n* = 7	*n* = 28
DBDD	Absent	11 (57.9 %)	2 (28.6 %)	20 (71.4 %)
	Present	8 (42.1 %)	5 (71.4 %)	8 (28.6 %)
DWDV	Absent	0 (0 %)	3 (42.9 %)	5 (17.9 %)
	Present	19 (100 %)	4 (57.1 %)	23 (82.1 %)
DWDL	Absent	3 (15.8 %)	3 (42.9 %)	6 (21.4 %)
	Present	16 (84.2 %)	4 (57.1 %)	22 (78.6 %)
DTBYLD	Absent	2 (10.5 %)	0 (0 %)	25 (89.3 %)
	Present	17 (89.5 %)	7 (100 %)	3 (10.7 %)
DTBYLV	Absent	19 (100 %)	7 (100 %)	28 (100 %)
	Present	0 (0 %)	0 (0 %)	0 (0 %)
DGM	Absent	18 (94.7 %)	7 (100 %)	22 (78.6 %)
	Present	1 (5.3 %)	0 (0 %)	6 (21.4 %)
CGN	11	2 (10.5 %)	0 (0 %)	0 (0 %)
	12	17 (89.5 %)	4 (57.1 %)	24 (85.7 %)
	13	0 (0 %)	3 (42.9 %)	4 (14.3 %)
CFBALN	1	0 (0 %)	0 (0 %)	2 (7.1 %)
	0.5	1 (5.3 %)	1 (14.3 %)	1 (3.6 %)
	0	5 (26.3 %)	2 (28.6 %)	7 (25.0 %)
	-0.5	8 (42.1 %)	3 (42.9 %)	11 (39.3 %)
	-1	3 (15.9 %)	1 (14.3 %)	6 (21.4 %)
	-1.5	1 (5.3 %)	0 (0 %)	1 (3.6 %)
	-2	1 (5.3 %)	0 (0 %)	0 (0 %)

Values show the number of individuals exhibiting the characteristic (with associated percentages given in parentheses). See Materials and Methods for definitions of morphological trait

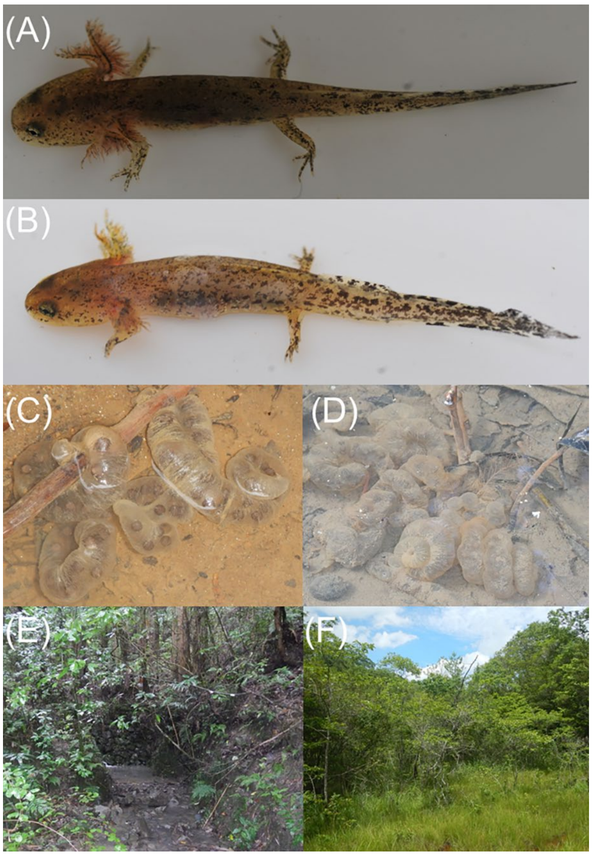

Fig. 5. Larvae, egg sacs, and habitat at the type locality of *Hynobius geiyoensis* sp. nov. (A, C, and E, respectively) and *H. sumidai* sp. nov. (B, D, and F, respectively).

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Holotype. An adult male (specimen number: HMNH-AM-102) from Tamaricho, Takehara-shi, Hiroshima Prefecture, Chugoku, Japan [34° 24′ N, 132° 47′ E; elevation = 340 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on 23 March 2021.

Paratype. An adult female (specimen number: KPM-NFA 945) from the same locality of the holotype, collected by Jun-ichi Naito on 23 March 2021.

Diagnosis. A comparatively small species (mean snout–vent length of 47.2 mm in males) within the Japanese lentic *Hynobius*; SVL usually less than 50 mm; distinct brownish–yellow stripe on the dorsal edge of the tail always present; distinct brownish–yellow line on the ventral side of the tail never present; distinct gular mottling never present; yellowish–brown to blackish–brown on dorsum; the fifth toe of hindlimb always present; V-shaped vomerine teeth series; usually 12 costal grooves (rarely 13); costal folds between adpressed limbs always less than 1.0; coil-shaped egg sacs.

Description of holotype. A moderately large individual with HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; non-expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > I > IV on left and II > III > IV > I on right; five toes on each hindlimb, order of length III > IV > II > I > V on both sides; V-shaped vomerine teeth; skin smooth and shiny; DBDD absent; DWDV and DWDL absent; DTBYLD present (it became unclear after preservation); DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL = 48.2, TRL = 36.6, AGD = 26.0, HL = 11.3, TAL = 34.6, MTAW = 2.4, MTAH = 4.7, BTAW = 5.4, BTAH = 4.4, VTL = 2.6, VTW = 2.5, HW = 8.0, MXHW = 8.2, LFLL = 11.8, RFLL = 11.9, LHLL = 14.1, RHLL = 14.0, L1FL = 1.3, L2FL = 2.1, L3FL = 1.9, L4FL = 0.9, R1FL = 0.7, R2FL = 1.6, R3FL = 1.2, R4FL = 0.8, L1TL = 1.1, L2TL = 2.0, L3TL = 3.3, L4TL = 2.3, L5TL = 0.8, R1TL = 1.3, R2TL = 1.7, R3TL = 2.9, R4TL = 1.9, R5TL = 0.7, IND = 2.3, IOD = 3.0, LUEW = 1.6, RUEW = 1.4, SL = 3.4, LUEL = 2.4, RUEL = 2.2, LJL = 6.9, and CGN = 12.

Comparisons. The new species statistically differs from *H. geiyoensis sp.* nov. in the following length measurements: SVL, RHL, RTAL, RFLL, R3FL, R3TL, R5TL, RUEW, and RUEL in males; the lengths of these measurements, except for RHL, RFLL, RUEW, and RUEL, are significantly longer in *H. sumidai* sp. nov. relative to the measurements in *H. geiyoensis* sp. nov.. The new species differs from *H. geiyoensis* sp. nov. by the following characters: SVL < 50 mm (7/7 = 100 %) vs. SVL > 50 mm (19/19 = 100 %); R5TL < 1.9 % (7/7 = 100 %) vs. R5TL > 1.8 % (18/19 = 94.7 %); RUEW > 3.1 % (7/7 = 100 %) vs. RUEW < 3.2 % (18/19 = 94.7 %); RUEL > 4.3 % (7/7 = 100 %) vs. RUEL < 4.3 % (18/19 = 94.7 %). The new species statistically differs from *H. akiensis* in the following length measurements: SVL (shorter), RVTL (longer), and R3FL (shorter) in males. The most distinct characteristic between the two species is the presence of DTBYLD: *H. sumidai* sp. nov. always has this characteristic (7/7 = 100 %), whereas *H. akiensis* usually lacks it (25/28 = 89.3 %).

Variation. Morphometric measurements and observations are presented in Tables 2 and 4, respectively. The dorsum is uniformly yellowish–brown or darkish–brown. The venter is lighter than the dorsum. DBDD rarely absent (2/7 = 28.6 %), DWDV and DWDL sometimes absent (3/7 = 42.9 %), sometimes it has 13 CGN (3/7 = 42.9 %), and rarely has more than 0 CFBALN (1/7 = 14.3 %). The iris is dark brown or light brown. When preserved, the dorsal coloration tends to fade to dark gray and DTBYLD becomes unclear after preservation.

Distribution. This new species is endemic to Hiroshima

Prefecture, and it is known from Higashihiroshima-shi (only former Higashihiroshima-shi and Akitsu-cho) and Takehara-shi. Its distribution is thought to bein the former Kurose-cho and Kure-shi areas, but no supporting evidence is available to confirm this hypothesis. Further phylogenetic studies using more samples from Higashihiroshima-shi and Kure-shi are therefore essential.

Natural History. The dominant vegetation type in the type locality is a mixed forest of evergreen oak (*Quercus* spp.) and Japanese cypress (*Chamaecyparis obtusa*). The larval and egg sac morphologies of this new species are similar to those of *H. geiyoensis* sp. nov. The breeding season of the new species is February to April.

Remarks. The new species forms a monophyletic group with the Highland and Aki groups (Matsui *et al.*, 2019). The morphology of females is unclear; hence, additional studies to clarify the morphology of females are needed.

Hynobius akiensis Matsui, Okawa et Nishikawa, 2019

(Standard Japanese name: *Aki-sanshouo*)

(Fig. 4)

Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Holotype. An adult male (specimen number: KUHE 35925) from Mirasakacho Haizuka, Miyoshi-shi, Hiroshima Prefecture, collected by Yasuchika Misawa and Kanto Nishikawa on 26 March 2005. This specimen is stored in the Graduate School of Human and Environmental Studies, Kyoto University: Yoshidahonmachi, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, 606-8501, Japan.

Diagnosis. A comparatively small species (mean snout–vent length of 51.2 mm in males) within the Japanese lentic Hynobius; distinct white dots on the ventral side of the body usually present; distinct brownish–yellow stripe on the dorsal edge of the tail usually absent; distinct brownish–yellow line on the ventral side of the tail never present; distinct gular mottling usually absent; yellowish–brown to blackish–brown on dorsum; fifth toe of hindlimb always present; V-shaped vomerine teeth series; 12 costal grooves (rarely 13); costal folds between adpressed limbs usually less than 0.5; coil-shaped egg sacs.

Description of a specimen from the type locality (Topotype). An adult male (specimen number: KPM-NFA 946) from the same locality of holotype, collected by Takayuki Iwata on 23 March 2019. A moderately large individual with HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; slightly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I in left and III > II > IV > I in right; five toes on each hindlimb, order of length III > IV > II > V > I in left and III > IV > II > I > V in right; V-shaped vomerine teeth; skin smooth and matte; DBDD absent; DWDV and DWDV present before preservation (it became unclear after preservation); DTBYLD and DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL = 55.3, TRL = 43.0, AGD = 27.8, HL = 12.8, TAL = 32.2, MTAW = 2.7, MTAH = 5.6, BTAW = 6.7, BTAH = 5.6, VTL = 2.6, VTW = 3.1, HW = 9.8, MXHW = 9.9, LFLL = 12.6, RFLL = 12.7, LHLL = 17.6, RHLL = 16.8, L1FL = 0.6, L2FL = 3.2, L3FL = 2.9, L4FL = 1.5, R1FL = 0.8, R2FL = 3.0, R3FL = 3.2, R4FL = 1.7, L1TL = 1.1, L2TL = 3.2, L3TL = 4.9, L4TL = 3.4, L5TL = 1.2, R1TL = 0.8, R2TL = 3.3, R3TL = 4.9, R4TL = 3.5, R5TL = 0.3, IND = 2.9, IOD = 3.3, LUEW = 1.5, RUEW = 1.7, SL = 4.0, LUEL = 2.3, RUEL = 2.4, LJL = 7.5, and CGN = 12.

Comparisons. This species is parapatrically distributed with *Hynobius iwami* but is distinguishable concerning for the presence of the fifth toe as well as distinct and bright yellow lines on the dorsal and ventral sides of the tail (Matsui *et al.*, 2019). Moreover, *H. utsunomiyaorum* is distributed near the range of *H. akiensis* but *H. akiensis* significantly differs from *H. utsunomiyaorum* by longer vomerine teeth series, fifth toe, and a greater degree of limb separation (Matsui *et al.*, 2019).

Variation. Morphometric measurements and observations were presented in Tables 2 and 4, respectively. The dorsum is uniformly darkish–brown or blackish–brown. The venter is lighter than the dorsum. DBDD (8/28 = 28.6 %), DGM (6/28 = 21.4 %), and DTBYLD (3/28 = 10.7 %) are rarely present. DWDV (5/28 = 17.9 %) and DWDL (6/28 = 21.4 %) rarely absent. CGN rarely 13 (4/28 = 14.3 %), and CFBALN rarely more than 0 (3/28 = 10.7 %). The iris is dark brown. When preserved, the dorsal coloration tends to fade to dark gray.

Distribution. According to Matsui *et al.* (2019), this species is endemic to Hiroshima Prefecture. However, the results of our field survey do not support this finding; we show that the species is known from Hiroshima and Shimane Prefectures as follows: Mihara-shi (only former Kui-cho and Daiwa-cho), Miyoshi-shi (only former Miyoshi-shi and Mirasaka-cho, Kisa-cho, and Miwa-cho), Akitakata (former Takamiya-cho, Koda-cho, Mukaihara-cho, Yoshida-cho, Yachiyo-cho, and Midori-cho), Higashihiroshima (only former Toyosaka-cho), and Hiroshima (only former Hiroshima-shi, Asakita-ku), and Sera-cho (only former Sera-cho and Nishisera-cho), Akiota-cho (only former Togochi-cho), and Kitahiroshima-cho(former Chiyoda-cho, Oasa-cho, Toyohira-cho, and Geihoku-cho), Hiroshima Prefecture, and Onan-cho (only former Mizuho-cho, and Hasumi-mura), Shimane Prefecture.

Remarks. *Hynobius akiensis* from Akitakata-shi and Kitahiroshima-cho is called the transition type of *H. akiensis* (Okawa *et al.*, 2019), but the common morphological character of transition type is not defined and unclear, and a type is a polyphyletic group (Fig. 2).

Discussion

According to Matsui *et al.* (2019), *H. akiensis* and the two new species described in this study can be regarded as the same species. However, the monophyly of *H. akiensis* and the two new species described here was rejected based on the criteria of Huelsenbeck and Rannala (2004) and Hillis & Bull (1993) (Matsui *et al.*, 2019; Fig. 2). Neither mitochondrial data nor phylogenetic analyses from allozyme data supported the monophyletic relationship among the species (Matsui *et al.*, 2006). Therefore, there is no evidence that *H. akiensis* and the two new species are in a monophyletic group as the closest relatives. Moreover, these species are morphologically distinguishable based on our analyses (Tables 2–4; Fig. 3). Thus, *H. akiensis* and the two new species should be distinct species based on three species concepts. Although the monophyly of *H. geiyoensis* sp. nov. and *H. sumidai* sp. nov. was supported by Matsui *et al.* (2019), our analyses did not support it (Fig. 3). The two new species were identified by mitochondrial DNA and morphologically distinct according to their differences (Tables 2-4; Fig. 3). Thus, we suggest that they should be different species based on the morphological species concept.

Okawa *et al.* (2019) suggested that the transition type of *H. akiensis* is a new species based on morphological perspectives. However, this type is included in the clade of *H. akiensis* and it cannot be recognized as a new species based on the phylogenetic and evolutionary species concepts (Fig. 2) (Matsui *et al.*, 2019). Thus, this type should be regarded as one morphotype of *H. akiensis* at present.

Following this description, the distribution area of *H. akiensis* is substantially changed; the species is not distributed in the southern part of Hiroshima Prefecture (Table1; Fig 1). However, the number of samples and localities from Higashihiroshima-shiwas not sufficient in our analyses. In Higashihiroshima-shi, *H. akiensis* has been protected by law since November 5, 1986, so permission must be granted to collect individuals. We applied to collect specimens in Higashihiroshima-shi, but our application was rejected because individuals must not be killed to ensure the conservation of *H. akiensis*. Unlike before, the current populations in Higashihiroshima-shi and the surrounding areas might be decreasing (e.g., Kure-shi, 2018), therefore morphological re-examination of living male and female individuals of *H. geiyoensis* sp. nov. and *H. sumidai* sp. nov. from Higashihiroshima-shi should be performed in the future. The conservation statuses of these three species must be reassessed after our description and management plans for the conservation of these species should be made immediately to ensure they do not become extinct.

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References

Bennett, R. A., 1991. A review of anesthesia and chemical restraint in reptiles. Journal of Zoo and Wildlife Medicine, 22: 282–303.

Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9(8): 772–772.

Hillis, D. M. & J. J. Bull, 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology, 42(2): 182–192.

Huelsenbeck, J. P. & B. Rannala, 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Systematic Biology, 53(6): 904–913.

Ihaka, R. & R. Gentleman, 1996. R: a language for data analysis and graphics. Journal of computational and graphical statistics, 5(3): 299–314.

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547–1549.

Kure-shi, 2018. Red Data Book Kure. Kure-Kankyo-Shiminnokai. Hiroshima Prefecture, Japan. (In Japanse).

Matsui, M., K. Nishikawa, T. Utsunomiya & S. Tanabe, 2006. Geographic allozyme variation in the Japanese clouded salamander, *Hynobius nebulosus* (Amphibia: Urodela). Biological Journal of the Linnean Society, 89(2): 311–330.

Matsui, M., H. Okawa, K. Nishikawa, G. Aoki, K. Eto, N. Yoshikawa, S. Tanabe, Y. Misawa & A. Tominaga, 2019. Systematics of the widely distributed Japanese clouded salamander, *Hynobius nebulosus* (Amphibia: Caudata: Hynobiidae), and its closest relatives. Current Herpetology, 38(1): 32–90.

Matsui, M., A. Tominaga, T. Hayashi, Y. Misawa & S. Tanabe, 2007. Phylogenetic relationships and phylogeography of *Hynobius tokyoensis* (Amphibia: Caudata) using complete sequences of cytochrome b and control region genes of mitochondrial DNA. Molecular Phylogenetics and Evolution, 44(1): 204–216.

Okawa, H., T. Okuno & T. Utsunomiya, 2019. Major groups of *Hynobius nebulosus* in western Japan. Bulletin of the Herpetological Society of Japan, 2019(1): 9–21. (In Japanese).

Okawa, H., T. Utsunomiya, Y. Utsunomiya & J. Naito, 1990. *Hynobius nebulosus* from Hiroshima Prefecture. Hibakagaku, 146: 49–53. (In Japanese).

Ronquist, F., M. Teslenko, P. Van Der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539–542.

Schwarz, G., 1978. Estimating the dimension of a model. Annals of Statistics, 6(2): 461–464.

Sugawara, H., T. Watabe, T. Yoshikawa & M. Nagano, 2018. Morphological and molecular analyses of *Hynobius dunni* reveal a new species from Shikoku, Japan. Herpetologica, 74(2): 159–168.

Sugiura, N., 1978. Further analysts of the data by akaike's information criterion and the finite corrections. Communications in Statistics. Theory and Methods, 7(1): 13–26.

摘　要

菅原弘貴・内藤順一・岩田貴之・永野昌博 , 2022. アキサンショウウオ *Hynobius akiensis* における分子系統学的および形態学的問題：日本の中国地方からの 2 新種の記載 . 神奈川県立博物館研究報告（自然科学）, (51): 35–46. [Sugawara, H., J. Naito, T. Iwata & M. Nagano, 2022. Molecular Phylogenetic and Morphological Problems of the Aki Salamander *Hynobius akiensis*: Description of Two New Species from Chugoku, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 35–46.]

サンショウウオ属の 2 新種を、日本の広島県南部から記載した。形態学的および分子系統学的解析に基づくと、アキサンショウウオは 3 つのグループ（広島北部グループ、広島 - 愛媛グループ、東広島グループ）に区別された。したがって、広島県南部および愛媛県北部に分布するグループと東広島市を中心に分布するグループを､それぞれ *Hynobius geiyoensis* sp. nov.（和名：ゲイヨサンショウウオ）と *Hynobius sumidai* sp. nov.（和名：ヒロシマサンショウウオ）として記載した。雄個体による形態比較の結果、前者は他の 2 種よりも頭胴長が有意に長かった。一方、後者はアキサンショウウオに似るが、尾の背面に明瞭な黄茶色の線をもっていた。本記載により、アキサンショウウオの分布域は大きく変更となるため、本種ならびに 2 新種の保全に際して、生息状況を再評価する必要がある。

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Original article

Taxonomic Re-examination of the Yamato Salamander *Hynobius vandenburghi*: Description of a New Species from Central Honshu, Japan

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Abstract. A new species of the genus *Hynobius* is described from the western part of Aichi Prefecture, Japan. *Hynobius vandenburghi* can be divided into two groups, the Aichi and Kinki groups, based on molecular and morphological analyses; thus, we described the Aichi group of *H. vandenburghi* as a new species, *H. owariensis* sp. nov.. Morphological comparisons revealed that although male *H. vandenburghi* have distinct bright yellow lines on the dorsal and ventral sides of the tail, males of the new species do not. Additionally, in males, the new species usually has fewer costal folds between its adpressed limbs than are observed in *H. vandenburghi*. Other significant differences in several morphological characteristics were also found between *H. vandenburghi* and the new species, and results of discriminant analyses between the two species in both sexes suggested that they are separated in terms of morphological data. The new species is restricted in the western part of Aichi Prefecture, which is threatened with extinction by artificial development or reformation of well-drained paddy fields.

Key words: Chita Peninsula, discriminant analysis, extinction, Kinki District, mitochondrial DNA

Introduction

The Yamato salamander, *Hynobius vandenburghi*, was originally described from Yamato Province (= Nara Prefecture) (Dunn, 1923a), and it is distributed in Kinki (Osaka, Nara, Kyoto, and Shiga Prefectures) and Tokai (Mie, Gifu, and Aichi Prefectures) Districts (Matsui *et al.*, 2019). This species is genetically separated into two distinct groups, the Aichi (excluding populations of Atsumi Peninsula) and Kinki (including populations of Atsumi Peninsula) groups, based on molecular analyses (Matsui *et al.*, 2019). Additionally, the monophyly of the two groups is strongly supported by maximum likelihood (ML) estimations and Bayesian inference (BI) (99/1.00) (Matsui *et al.*, 2019). However, these analyses were performed using only 10 populations; thus, the monophyly of the two groups should be reassessed using a lot of populations across the entire distribution range of *H. vandenburghi*. Furthermore, the morphological similarity of the two *H. vandenburghi* groups is unreliable because Matsui *et al.* (2019) did not compare the Aichi and Kinki groups despite clear genetic evidence that they are separated. Indeed, the Aichi populations of *H. vandenburghi* are morphologically more similar to *Hynobius tokyoensis* than those of *H. vandenburghi* (Sato, 1943; Nakamura & Ueno, 1963); therefore, it is doubtful that the Aichi populations satisfy the diagnosis of *H. vandenburghi*. Consequently, it is necessary to reassess the morphology of the two *H.*

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vandenburghi groups.

In the present study, we evaluated the species validity of the two *H. vandenburghi* groups using the morphological, phylogenetic, and evolutionary species concepts following Sugawara *et al.* (2018). To resolve the taxonomic problems associated with *H. vandenburghi*, we performed statistical analyses on morphological characteristics to compare the two *H. vandenburghi* groups. We also used additional DNA sequence data to reconstruct the phylogeny of *H. vandenburghi* collected from the entire distribution range of this species. Finally, we reveal the distribution ranges of the two *H. vandenburghi* groups in detail.

Materials and methods

Molecular analysis

To reconstruct a molecular phylogeny, we collected DNA samples from *H. vandenburghi* located on personal property or in fields from February 2007 to April 2021 (Table 1; Fig. 1). When sampling in fields, we obtained a single tailbud embryo from each paired egg sac or tissue samples from larvae. The tissues collected from fields were preserved in 99.5 % ethanol. Subsequently, we extracted total genomic DNA from the tissues using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For molecular analyses, we then amplified a 585-bp fragment of the cytochrome b gene from each individual using the primers L14010 (5′-TAHGGWGAHGGATTWGAWGC MACWGC-3′) and H14778 (5′-AARTAYGGGTGRAAD GRRAYTTTRTCT-3′) (Matsui *et al.*, 2007). Our methods for polymerase chain reaction (PCR) and sequencing analysis followed those of Sugawara *et al.* (2018). The obtained sequences were registered in the DNA Data Bank of Japan (DDBJ) (Table 1). Before phylogenetic analyses, DNA sequences were aligned using MEGA X (Kumar *et al.*, 2018). After alignment, molecular phylogenies were constructed using other *Hynobius* species, *Salamandrella keyserlingii* as the outgroup (Table 1), and with BI and ML estimation. The best-fit nucleotide substitution model was decided based on the Bayesian information criterion (BIC) (Schwarz, 1978) and corrected Akaike's information criterion (AICc) (Sugiura, 1978) using jModelTest 2 (Darriba *et al.*, 2012). The Hasegawa-Kishino-Yano (HKY) model was selected with a gamma distribution in BI and ML. The Bayesian and ML trees were reconstructed using MrBayes 3.2 (Ronquist *et al.*, 2012) and MEGA X (Kumar *et al.*, 2018), respectively. For Bayesian analyses, we performed two independent MCMC runs for 2,000,000 generations with a sampling frequency of 100. In the Bayesian analysis, we examined the stationarity of the likelihood scores of sampled trees using Tracer version 1.7 (http://tree.bio.ed.ac.uk/software/tracer/) and we discarded

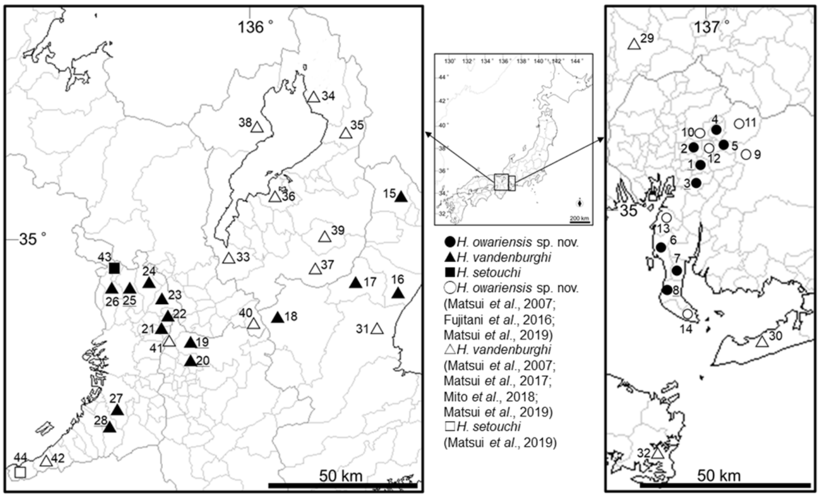

Fig. 1. Localities for populations of two species of *Hynobius* sampled in their distribution areas. Population numbers match those used for molecular analyses (see Table 1 and Fig. 2). The left and right enlarged areas include the central part of Kinki and central part of Tokai, respectively. The closed symbols correspond to each of three species sequenced in this study. The open symbols correspond to each of three species cited from other studies. For the morphological comparisons, individuals of the two species were sampled from the localities that are underlined: Pops. 1 (type locality of *H. owariensis* sp. nov.: 2 males and 1 female), 3 (18 males and 6 females), and 8 (2 males and 3 females) for *H. owariensis* sp. nov.; Pops. 19 (6 males and 3 females), 20 (7 males), 25 (3 females), and 28 (4 males) for *H. vandenburghi*.

Table 1. List of specimens used in molecular analyses

Population	Species	Sampling licality	Accession number / Label in Fig. 2
1	*Hynobius owariensis* sp. nov.	Tenpaku-ku, Nagoya-shi, Aichi	LC644689 / OWA01
2	*Hynobius owariensis* sp. nov.	Chikusa-ku, Nagoya-shi, Aichi	LC644690 / OWA02
3	*Hynobius owariensis* sp. nov.	Midori-ku, Nagoya-shi, Aichi	LC644691 / OWA03
4	*Hynobius owariensis* sp. nov.	Owariasahi-shi, Aichi	LC644692 / OWA04
5	*Hynobius owariensis* sp. nov.	Nagakute-shi, Aichi	LC644693 / OWA05
6	*Hynobius owariensis* sp. nov.	Tokoname-shi, Aichi	LC644694 / OWA06
7	*Hynobius owariensis* sp. nov.	Taketoyo-cho, Aichi	LC644695 / OWA07
8	*Hynobius owariensis* sp. nov.	Mihama-cho, Aichi	LC644696 / OWA08
9	*Hynobius owariensis* sp. nov.	Toyota-shi, Aichi	AB972617 / TYO10
10	*Hynobius owariensis* sp. nov.	Moriyama-ku, Nagoya-shi, Aichi	AB972596 / NagF7
11	*Hynobius owariensis* sp. nov.	Seto-shi, Aichi	LC225431 / H81
12	*Hynobius owariensis* sp. nov.	Meito-ku, Nagoya-shi, Aichi	LC436440 / H78
13	*Hynobius owariensis* sp. nov.	Chita-shi, Aichi	LC436441 / H79
14	*Hynobius owariensis* sp. nov.	Minamichita-cho, Aichi	AB266662 / H80
15	*Hynobius vandenburghi*	Inabe-shi, Mie	LC644697 / VAN01
16	*Hynobius vandenburghi*	Suzuka-shi, Mie	LC644698 / VAN02
17	*Hynobius vandenburghi*	Kameyama-shi, Mie	LC644699 / VAN03
18	*Hynobius vandenburghi*	Iga-shi, Mie	LC644700 / VAN04
19	*Hynobius vandenburghi*	Nara-shi, Nara	LC644701 / VAN05
20	*Hynobius vandenburghi*	Yamatokoriyama-shi, Nara	LC644702 / VAN06
21	*Hynobius vandenburghi*	Shijonawate-shi, Osaka	LC644703 / VAN07
22	*Hynobius vandenburghi*	Katano-shi, Osaka	LC644704 / VAN08
23	*Hynobius vandenburghi*	Hirakata-shi, Osaka	LC644705 / VAN09
24	*Hynobius vandenburghi*	Takatsuki-shi, Osaka	LC644706 / VAN10
25	*Hynobius vandenburghi*	Ibaraki-shi, Osaka	LC644707 / VAN11
26	*Hynobius vandenburghi*	Mino-shi, Osaka	LC644708 / VAN12
27	*Hynobius vandenburghi*	Sakai-shi, Osaka	LC644709 / VAN13
28	*Hynobius vandenburghi*	Izumi-shi, Osaka	LC644710 / VAN14
29	*Hynobius vandenburghi*	Gifu-shi, Gifu	AB972627 / GIF9
30	*Hynobius vandenburghi*	Tahara-shi, Aichi	AB266663 / H72
31	*Hynobius vandenburghi*	Tsu-shi, Mie	AB266665 / Tsu
32	*Hynobius vandenburghi*	Shima-shi, Mie	AB266666 / Shimacho
33	*Hynobius vandenburghi*	Otsu-shi, Shiga	AB266667 / Otsu
34	*Hynobius vandenburghi*	Nagahama-shi, Shiga	LC274713 / Tk2
35	*Hynobius vandenburghi*	Maibara-shi, Shiga	LC274716 / Iso12
36	*Hynobius vandenburghi*	Omihachiman-shi, Shiga	LC274709 / Mt5
37	*Hynobius vandenburghi*	Koka-shi, Shiga	LC274699 / nakahatah10
38	*Hynobius vandenburghi*	Takashima-shi, Shiga	LC274701 / Makinoh3
39	*Hynobius vandenburghi*	Hino-cho, Shiga	LC436436 / H74
40	*Hynobius vandenburghi*	Minamiyamashiro-mura, Kyoto	LC436437 / H75
41	*Hynobius vandenburghi*	Ikoma-shi, Nara	LC436438 / H76
42	*Hynobius vandenburghi*	Hannan-shi, Osaka	LC436439 / H77
43	*Hynobius setouchi*	Toyono-cho, Osaka	LC644711 / SET01
44	*Hynobius setouchi*	Misaki-cho, Osaka	LC436432 / H69
	Hynobius setouchi		LC436426 / H. setouchi (Holotype)
	Hynobius abei		LC225433 / H. abei
	Hynobius lichenatus		AB750782 / H. lichenatus
	Hynobius mikawaensis		LC225429 / H. mikawaensis
	Hynobius nigrescens		AB548378 / H. nigrescens
	Hynobius setoi		LC225432 / H. setoi
	Hynobius takedai		LC225430 / H. takedai
	Hynobius tokyoensis		AB266640 / H. tokyoensis
	Salamandrella keyserlingii		NC_026032 / S. keyserlingii

Population number corresponds to the localities in Fig. 1 from which the individuals were collected.

the first 25 % of generations as burn-in. The assessment of monophyly was performed using posterior probability (PP) and bootstrap (BS) values based on the criteria of Huelsenbeck & Rannala (2004) and Hillis & Bull (1993); thus, a monophyletic group was considered to have PP ≥ 0.95 and BP ≥ 70.

Morphological analysis

We sampled 55 individuals of *H. vandenburghi* from February 2019 to March 2021, including 32 individuals (22 males and 10 females) of the Aichi group from three populations (Pops. 1, 3, and 8) and 23 individuals (17 males and 6 females) of the Kinki group from four populations (Pops. 19, 20, 25, and 28) (Table 1; Fig. 1). The collected adults were measured after being anesthetized using ethyl 3-aminobenzoate methane sulfonate salt (Sigma-Aldrich, St. Louis, MO, USA) diluted 1,000-fold in water (Bennett, 1991). For conservation of *H. vandenburghi*, measured adults were returned to their site of capture except for the candidates of type specimens. Before we returned individuals to their site of capture, we took photos of their dorsal, ventral, and lateral sides against a black background, and we also obtained tissue samples (preserved in 99.5 % ethanol) from their tail tips for evidence of collection. All examined adults were measured using a vernier caliper with 22 measurements as follows: snout-vent length (SVL), trunk length (TRL), axilla-groin distance (AGD), head length (HL), tail length (TAL), median tail width (MTAW), median tail height (MTAH), vomerine teeth length (VTL), and vomerine teeth width (VTW), head width (HW), forelimb length (FLL), hindlimb length (HLL), second finger length (2FL), third finger length (3FL), third toe length (3TL), five toe length (5TL), internarial distance (IND), interorbital distance (IOD), upper eyelid length (UEL), snout length (SL), upper eyelid width (UEW), lower jaw length (LJL). For each individual, we also recorded data for the presence of distinct black dots on the dorsum (DBDD), the presence of distinct white dots on the venter (DWDV), the presence of distinct white dots on the lateral side of the body (DWDL), the presence of a distinct and bright yellow line on the dorsal (DBTYLD) and ventral (DBTYLV) sides of the tail, and presence of distinct gular mottling (DGM). The number of costal folds between the adpressed limbs (CFBALN) and the number of costal grooves (CGN) was counted using the method of Matsui *et al.* (2019).

Before performing morphological comparisons between the two groups, we tested for normality using Shapiro–Wilk tests. When data followed a normal distribution, we tested for homoscedasticity using F tests. When population variances were equal, we performed Student's t-tests; when variances were not equal, we performed Welch's t-tests. When data did not follow a normal distribution, we performed Brunner–Munzel tests. To examine the overall morphological variation between the two groups, we performed discriminant analyses using SVL and standardized values (R = %SVL) for 21 measurements as follows: RTRL, RAGD, RHL, RTAL, RMTAW, RMTAH, RVTL, RVTW, RHW, RFLL, RHLL, R2FL, R3FL, R3TL, R5TL, RIND, RIOD, RUEW, RSL, RUEL, RLJL. All statistical analyses were performed in R with $\alpha = 0.05$ (Ihaka & Gentleman, 1996).

For measurements of the holotype and a specimen from the type locality of *H. vandenburghi* (topotype), 43 characteristics were measured including SVL, TRL, AGD, HL, TAL, MTAW, MTAH, basal tail width (BTAW), basal tail height (BTAH), VTL, VTW, HW, maximum head width (MXHW), left forelimb length (LFLL), left hindlimb length (LHLL), right forelimb length (RFLL), right hindlimb length (RHLL), left first finger length (L1FL), left second finger length (L2FL), left third finger length (L3FL), left fourth finger length (L4FL), right first finger length (R1FL), right second finger length (R2FL), right third finger length (R3FL), right fourth finger length (R4FL), left first toe length (L1TL), left second toe length (L2TL), left third toe length (L3TL), left fourth toe length (L4TL), left fifth toe length (L5TL), right first toe length (R1TL), right second toe length (R2TL), right third toe length (R3TL), right fourth toe length (R4TL), right fifth toe length (R5TL), IND, IOD, left upper eyelid length (LUEL), right upper eyelid length (RUEL), SL, left upper eyelid width (LUEW), right upper eyelid width (RUEW), LJL.

The holotype of a new species described in this study is stored in the Toyohashi Museum of Natural History: 1-238, Oiwacho Oana, Toyohashi-shi, Aichi Prefecture, 441-3147, Japan. A single topotype of *H. vandenburghi* and two paratypes of the new species described in this study are stored in the Kanagawa Prefectural Museum of Natural History: 499, Iryuda, Odawara-shi, Kanagawa Prefecture, 250-0031, Japan. In order to avoid overcollection of these species, further details would be made available only by either contacting the corresponding author or each museum.

Results

The monophyly of the Aichi and Kinki groups of *H. vandenburghi* was supported (but not strongly supported) by PP, whereas it was rejected by BS (Fig. 2). The monophyly of each group was strongly supported by PP and BS (Fig. 2). Genetically, these groups were not divided

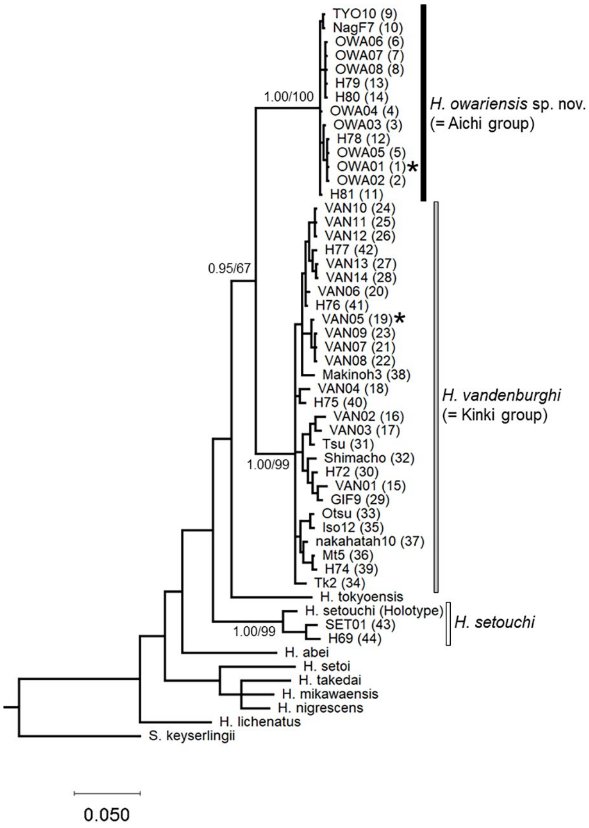

Fig. 2. Phylogenetic tree produced using Bayesian inference (BI) based on 585-bp cytochrome b sequences rooted with *Salamandrella keyserlingii* as an outgroup. Scale represents genetic distance (expect for changes per site). Numbers located near the nodes are posterior probabilities (PP) for BI and bootstrap values (BS) for maximum likelihood estimation. Values appearing in parentheses after the haplotype names correspond to population localities as indicated in Table 1 and Fig. 1. Asterisks after the parentheses (Pops. 1 and 19) indicate the type locality of each species.

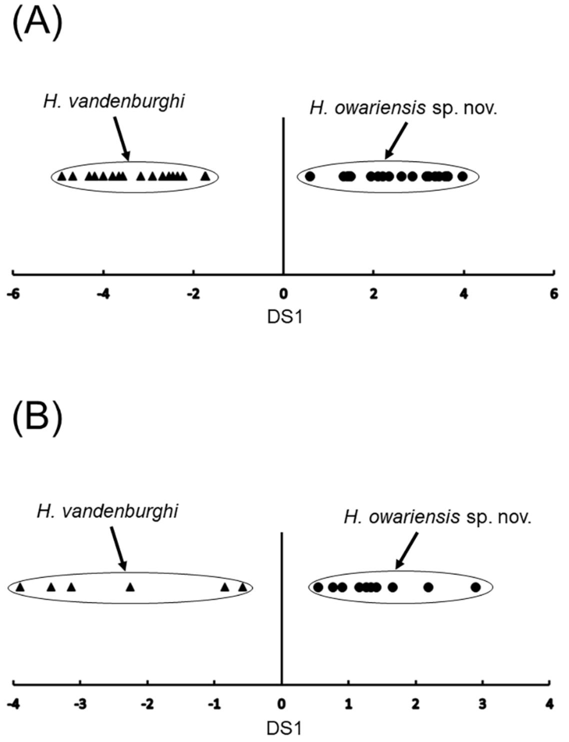

Fig. 3. Results of discriminant analyses of two species for (A) males and (B) females. The x axis indicates "discriminant score 1" (DS1).

into further populations based on PP and BS (Fig. 2).

Morphological measurements of the two groups are shown in Table 2 and the significant values of all measurements between the two groups are listed in Table 3. Males and females of the Aichi and Kinki groups differed significantly in 11 (SVL, RTRL, RAGD, RHL, RMTAH, RVTW, RHLL, R3TL, RUEW, RUEL, and RLJL) and 2 (SVL and RVTL) morphological characteristics, respectively (Table 3). Discriminant analyses indicated that the two groups were different and that the distribution areas of scores did not overlap (Fig. 3). Results of morphological observations are shown in Table 4. Males of the Aichi group almost always had DGM (20/22 = 90.9 %), almost always had no DBDD (20/22 = 90.9 %), usually had no DWDV (18/22 = 81.8 %) and DWDL (19/22 = 86.4 %), usually had 13 costal grooves (18/22 = 81.8 %) and CFBALN ≤ -1.5 (19/22 = 86.4 %), and never had DBTYLD and DBTYLV (22/22 = 100 %). Females of the Aichi group almost always had 13 CGN (9/10 = 90.0 %) and CFBALN ≤ -1.5 (9/10 = 90.0 %), almost always had no DBTYLV (9/10 = 90.0 %), and never had DGM (10/10 = 100 %). Males of the Kinki group always had DBTYLD and DBTYLV (17/17 = 100 %), and usually had 13 CGN (14/17 = 82.4 %) and CFBALN ≥ -1.0 (15/17 = 88.2 %), and usually had no DBDD (15/17 = 88.2 %). Females of the Kinki group always had DBTYLD, DBTYLV, and CFBALN ≤ -1.5 (6/6 = 100 %), usually had 13 CGN (5/6 = 83.3 %), usually had no DBDD (5/6 = 83.3 %), and never had DGM (6/6 = 100 %).

Given the results of molecular and morphological analyses, we described the Aichi group of *H. vandenburghi* as a new species based on three species concepts.

Taxonomy

Hynobius owariensis **sp. nov.**

(New standard Japanese name: *Owari-sanshouo*)
(Figs. 4–5)

Hynobius nebulosus: Kuzumi and Kakegawa, 1989; Fujitani, 2000; Fujitani *et al.*, 2016: 3, in part.
Hynobius vandenburghi: Matsui *et al.*, 2019: 49, in

Table 2. Measurement (mm) of SVL and character ratios (R = %SVL) of TRL to LJL Ranges are shown in parentheses.

	H. owariensis sp. nov.			*H. vandenburghi*		
	Holotype	Male	Female	Topotype	Male	Female
Trait		*n* = 22	*n* = 10		*n* = 17	*n* = 6
SVL	58.2	60.6±4.65	59.1±6.46	54.5	53.8±3.30	54.3±1.52
		(51.2-66.7)	(47.3-65.7)		(49.6-60.3)	(52.0-56.0)
RTRL	79.7	77.9±1.31	78.0±3.69	77.1	77.2±0.63	78.0±0.75
		(75.4-82.0)	(69.6-84.5)		(76.2-78.2)	(77.4-79.5)
RAGD	55.7	54.5±1.42	55.7±1.76	53.4	53.4±1.43	56.1±0.78
		(51.7-57.0)	(53.8-59.0)		(50.7-56.1)	(54.6-56.8)
RHL	21.5	22.8±0.89	22.8±0.97	23.3	23.5±0.91	23.0±1.08
		(21.4-24.8)	(21.2-24.0)		(22.2-24.8)	(21.6-24.5)
RTAL	76.5	74.1±7.33	71.1±5.24	74.3	77.8±5.73	71.8±8.30
		(60.9-86.9)	(60.7-77.2)		(65.7-87.3)	(58.1-81.3)
RMTAW	6.9	7.5±0.94	7.3±1.27	5.9	6.9±0.91	7.0±1.53
		(4.9-8.7)	(5.5-9.3)		(5.8-9.0)	(5.1-9.3)
RMTAH	12.0	13.8±1.87	12.5±1.82	10.5	12.2±1.20	12.0±0.79
		(9.8-17.8)	(9.9-15.7)		(10.5-14.0)	(10.5-12.5)
RVTL	4.1	4.8±0.49	4.5±0.42	5	4.8±0.46	5.0±0.31
		(3.8-5.7)	(4.1-5.2)		(3.6-5.6)	(4.6-5.4)
RVTW	5.5	5.5±0.46	5.2±0.27	5.1	5.1±0.47	4.9±0.28
		(4.6-6.3)	(4.7-5.5)		(4.2-5.9)	(4.6-5.3)
RHW	16.8	17.0±0.91	16.7±0.69	15.6	16.7±0.73	15.9±0.70
		(14.9-18.8)	(15.6-17.7)		(15.3-18.1)	(15.3-17.2)
RFLL	21.1	23.1±1.35	21.9±1.95	21.5	23.6±1.53	23.2±1.44
		(21.0-25.6)	(19.1-25.6)		(21.4-26.1)	(21.5-24.9)
RHLL	25.9	28.3±1.56	27.9±2.05	28.6	30.8±1.34	28.6±2.14
		(24.4-30.7)	(25.1-31.1)		(28.5-33.2)	(25.5-30.8)
R2FL	5.0	5.2±0.44	4.5±0.56	5.7	5.2±0.50	4.7±0.28
		(4.4-6.1)	(3.2-5.3)		(4.0-5.7)	(4.4-5.2)
R3FL	3.1	4.2±0.62	4.2±0.49	5.1	4.1±0.71	4.1±0.58
		(2.7-5.3)	(3.2-5.0)		(2.9-5.2)	(3.3-5.0)
R3TL	7.0	7.4±0.62	7.3±0.84	7.2	8.1±0.76	7.5±0.57
		(5.9-8.5)	(6.2-9.0)		(6.8-9.4)	(7.0-8.5)
R5TL	1.2	2.1±0.48	2.0±0.64	2.4	2.1±0.71	2.1±0.21
		(1.1-3.1)	(1.2-3.1)		(1.0-3.3)	(1.8-2.4)
RIND	4.1	4.2±0.52	4.5±0.63	4.6	4.3±0.54	4.4±0.48
		(3.4-5.7)	(3.9-5.7)		(2.9-5.1)	(3.8-5.1)
RIOD	6.4	5.7±0.35	5.8±0.32	6.1	5.7±0.45	5.6±0.47
		(4.9-6.4)	(5.5-6.4)		(4.9-6.9)	(4.9-6.1)
RUEW	2.6	2.8±0.20	3.0±0.31	2.8	3.3±0.31	3.3±0.23
		(2.5-3.2)	(2.5-3.6)		(2.6-3.9)	(2.9-3.6)
RSL	6.4	5.8±0.37	5.8±0.37	6.6	5.9±0.29	5.9±0.29
		(5.2-6.4)	(5.1-6.2)		(5.5-6.6)	(5.4-6.2)
RUEL	4.1	4.2±0.39	4.4±0.30	4.6	4.4±0.25	4.4±0.48
		(3.7-5.6)	(4.1-5.1)		(4.0-4.8)	(3.8-5.1)
RLJL	11.7	12.6±0.60	12.8±0.86	12.8	13.9±0.65	13.4±0.38
		(11.4-13.5)	(11.7-14.2)		(12.8-14.8)	(12.9-13.8)

See Materials and Methods section for definitions of morphological traits.

part; Ichioka *et al.*, 2021

Etymology. The specific epithet "*owariensis*" refers to the old name of the western part of Aichi Prefecture (= Owari) where the new species occurs.

Holotype. An adult male (specimen number: TMNH-AM-78) from Tenpakucho Yagotourayama, Tenpaku-ku, Nagoya-shi, Aichi Prefecture, Japan (35° 08' N, 136° 58' E; elevation = 50 m), collected by Takeshi Fujitani on 18 February 2020. This population is on private land; thus, we obtained permission from the landowner to collect the specimen.

Table 3. Significant values for the 22 morphological characteristics compared between the two species (for both sexes and between sexes of each species)

	OWA vs. VAN		Male vs. Female	
Trait	Males	Females	OWA	VAN
SVL	**P < 0.0001**	P < 0.05	NS	NS
RTRL	P < 0.05	NS	**P < 0.0001**	P < 0.05
RAGD	P < 0.05	NS	P < 0.05	**P < 0.0001**
RHL	P < 0.05	NS	NS	NS
RTAL	NS	NS	NS	NS
RMTAW	NS	NS	NS	NS
RMTAH	P < 0.01	NS	NS	NS
RVTL	NS	P < 0.05	NS	NS
RVTW	P < 0.01	NS	NS	NS
RHW	NS	NS	NS	P < 0.05
RFLL	NS	NS	P < 0.05	NS
RHLL	**P < 0.0001**	NS	NS	P < 0.01
R2FL	NS	NS	P < 0.01	P < 0.01
R3FL	NS	NS	NS	NS
R3TL	P < 0.01	NS	NS	NS
R5TL	NS	NS	NS	NS
RIND	NS	NS	NS	NS
RIOD	NS	NS	NS	NS
RUEW	**P < 0.0001**	NS	**P < 0.0001**	NS
RSL	NS	NS	NS	NS
RUEL	P < 0.01	NS	P < 0.01	NS
RLJL	**P < 0.0001**	NS	NS	NS
P < 0.05	3	2	2	2
P < 0.01	4	0	2	2
P < 0.001	0	0	0	0
P < 0.0001	4	0	2	1
Total	11	2	6	5

OWA and VAN are abbreviations of *Hynobius owariensis* sp. nov. and *H. vandenburghi*, respectively. Larger significant difference values are shown in bold. See Materials and Methods section for definitions of morphological traits.

Fig. 4. Holotype of *Hynobius owariensis* sp. nov. (TMNH-AM-78, adult male, 58.2 mm SVL): (A) dorsal and (B) ventral views.

Paratypes. An adult female (specimen number: KPM-NFA 940) from the same locality of the holotype, collected by Takeshi Fujitani on 28 February. 2020. An adult male (specimen number: KPM-NFA 941) from Odakacho Takayama, Midori-ku, Nagoya-shi, Aichi Prefecture, Japan (35° 03’ N, 136° 56’ E; elevation = 30 m), collected by Takeshi Fujitani on 4 March 2019. This population is also on private land; therefore, we again obtained permission from the landowner to collect specimens.

Diagnosis. A comparatively large species (mean SVL = 60.6 mm in males and 59.1 mm in females) within the Japanese lentic *Hynobius* species: SVL usually > 56 mm in males; ratio of hindlimb length almost always < 30 %SVL in males; distinct and bright yellow stripe on the ventral edge of tail always absent in males and almost always absent in females; distinct and bright yellow stripe on the dorsal edge of tail always absent in males; distinct black dots on the dorsum almost always absent in males; distinct white dots on the ventral and lateral sides of the body usually absent in males; DGM almost always present in males and never present in females; fifth toe of hindlimb always present; U-shaped or V-shaped vomerine teeth series; 13 (rarely 12 or 14) costal grooves; number of costal folds between adpressed limbs usually −3.0 to −1.5 in males and almost always −4.0 to −1.5 in females.

Description of holotype. A moderately large individual: HL slightly larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on left and III > II > IV > I on right; five toes on each hindlimb, order of length III > II > IV > I > V on left and III > IV > II > V > I on right; U-shaped vomerine teeth; skin smooth and shiny; DWDV and DWDL absent; DBDD absent; DBTYLD and DBTYLV absent; DGM present. The holotype had the following measurements (in mm): SVL =58.2, TRL = 46.4, AGD = 32.4, HL = 12.5, TAL = 44.5, MTAW = 4.0, MTAH = 7.0, BTAW = 7.6, BTAH = 6.4, VTL = 2.4, VTW = 3.2, HW = 9.8, MXHW = 10.1, LFLL = 12.3, RFLL = 10.7, LHLL = 15.1, RHLL = 14.6, L1FL = 1.3, L2FL = 2.9, L3FL = 1.8, L4FL = 1.5, R1FL = 0.9, R2FL = 1.4, R3FL = 1.8, R4FL = 1.3, L1TL = 1.5, L2TL = 3.2, L3TL = 4.1, L4TL = 2.9, L5TL = 0.7, R1TL = 0.9, R2TL

= 2.9, R3TL = 3.8, R4TL = 3.1, R5TL = 1.2, IND = 2.4, IOD = 3.7, LUEW = 1.5, RUEW = 1.1, SL =3.9, LUEL =2.4, RUEL = 2.6, LJL = 6.8, CGN = 13.

Comparisons. The new species resembles *H. vandenburghi* in morphology but differs statistically from it in the following length measurements: SVL, RTRL, RAGD, RHL, RMTAH, RVTW, RHLL, R3TL, RUEW, RUEL, and RLJL in males and SVL and RVTL in females; the lengths of these measurements, except for SVL, RTRL, RAGD, RMTAH, and RVTW in males and SVL in females, are significantly shorter in *H. owariensis* sp. nov. than in *H. vandenburghi*. In males, *H. owariensis* sp. nov. differs from *H. vandenburghi* by the following combination of characters: SVL > 56 mm (18/22 = 81.8 %) vs. SVL < 56 mm (14/17 = 82.4 %); RHLL shorter than 30 % (20/22 = 90.9 %), vs. RHLL of 30 % or longer (14/17 = 82.4 %); usually have CFBALN ≤ -1.5 (19/22 = 86.4 %) vs. usually have CFBALN ≥ -1.0 (15/17 = 88.2 %); always lack DBTYLD and DBTYLV (22/22 = 100 %) vs. always have DBTYLD and DBTYLV (17/17 = 100 %). In females, *H. owariensis* sp. nov. almost always lacks DBTYLV (9/10 = 90 %), whereas *H. vandenburghi* always have DBTYLV (6/6 = 100 %).

Table 4. Characteristics of skin markings between the two species of *Hynobius*

		H. owariensis sp. nov.		*H. vandenburghi*	
		Male	Female	Male	Female
Character	Condition	*n* = 22	*n* = 10	*n* = 17	*n* = 6
DBDD	Absent	20 (90.9%)	7 (70.0%)	15 (88.2%)	5 (83.3%)
	Present	2 (9.1%)	3 (30.0%)	2 (11.8%)	1 (16.7%)
DWDV	Absent	18 (81.8%)	4 (40.0%)	11 (64.7%)	3 (50.0%)
	Present	4 (18.2%)	6 (60.0%)	6 (35.3%)	3 (50.0%)
DWDL	Absent	19 (86.4%)	7 (70.0%)	11 (64.7%)	3 (50.0%)
	Present	3 (13.6%)	3 (30.0%)	6 (35.3%)	3 (50.0%)
DBTYLD	Absent	22 (100%)	5 (50.0%)	0 (0%)	0 (0%)
	Present	0 (0%)	5 (50.0%)	17 (100%)	6 (100%)
DBTYLV	Absent	22 (100%)	9 (90%)	0 (0%)	0 (0%)
	Present	0 (0%)	1 (10%)	17 (100%)	6 (100%)
DGM	Absent	2 (9.1%)	10 (100%)	10 (58.8%)	6 (100%)
	Present	20 (90.9%)	0 (0%)	7 (41.2%)	0 (0%)
CGN	12	2 (9.1%)	0 (0%)	3 (17.6%)	0 (0%)
	13	18 (81.8%)	9 (90.0%)	14 (82.4%)	5 (83.3%)
	14	2 (9.1%)	1 (10.0%)	0 (0%)	1 (16.7%)
CFBALN	1.0	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
	0.5	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
	0.0	0 (0%)	0 (0%)	5 (29.4%)	0 (0%)
	-0.5	1 (4.5%)	1 (10.0%)	1 (5.9%)	0 (0%)
	-1.0	2 (9.1%)	0 (0%)	7 (41.2%)	0 (0%)
	-1.5	10 (45.5%)	2 (20.0%)	2 (11.8%)	3 (50.0%)
	-2.0	4 (18.2%)	2 (20.0%)	0 (0%)	0 (0%)
	-2.5	4 (18.2%)	0 (0%)	0 (0%)	0 (0%)
	-3.0	1 (4.5%)	1 (10.0%)	0 (0%)	3 (50%)
	-3.5	0 (0%)	3 (30.0%)	0 (0%)	0 (0%)
	-4.0	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)

Values indicate the number of individuals exhibiting that characteristic with percentages show for each condition in parentheses. See Materials and Methods section for definitions of morphological characteristics.

Variation. Morphometric measurements are presented in Table 2 and the significant values of all measurements between sexes are listed in Table 3. Males have relatively longer RFLL and R2FL than females, whereas males have relatively shorter RTRL, RAGD, RUEW, and RUEL than those of females. The skin markings of *H. owariensis* sp. nov. are listed in Table 4. The dorsum is uniformly yellowish-brown or darkish-brown. DBDD is rarely present in males (2/22 = 9.1 %) and sometimes present in females (3/10 = 30 %). The venter is lighter than the dorsum. DWDV is rarely present in males (4/22 = 18.2 %) and sometimes absent in females (4/10 = 40 %). DWDL is rarely present in males (3/22 = 13.6 %) and rarely present in females (3/10 = 30.0 %). In females, DBTYLD is sometimes present (5/10 = 50 %) and DBTYLV is rarely present (1/10 = 10 %). DGM is rarely absent in males (2/22 = 9.1 %). CGN is rarely 12 (2/22 = 9.1 %) or 14 (2/22 = 9.1 %) in males and rarely 14 in females (1/10 = 10.0 %). CFBALN is rarely > -1.5 in males (3/22 = 13.6 %) and rarely > -1.0 (1/10 = 10 %) in females. The iris is dark brown. When preserved in 70 % ethanol, the dorsal coloration tends to fade to dark gray. The indistinct yellowish line on the dorsal and ventral sides of the tail (e.g.,

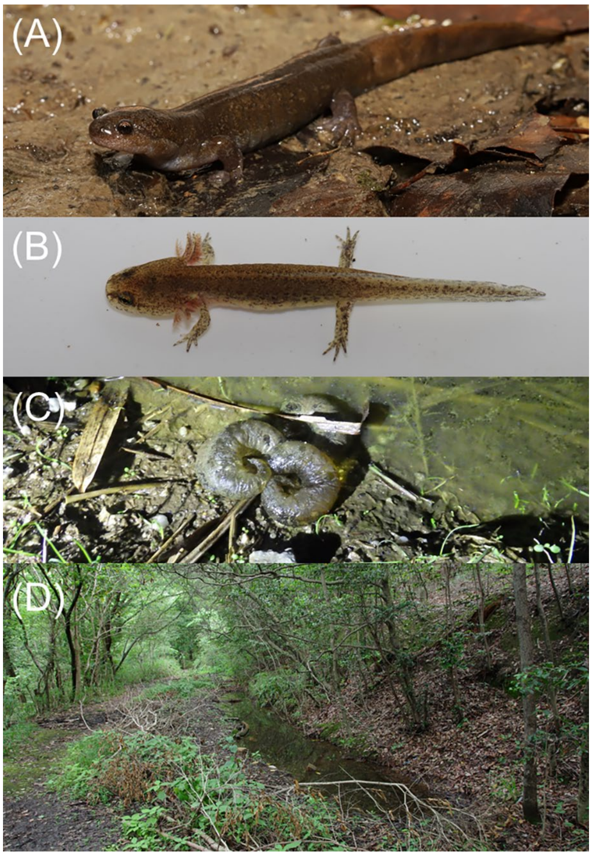

Fig. 5. (A) Live holotype of *Hynobius owariensis* sp. nov. (TMNH-AM-78), and the (B) larva, (C) banana-shaped egg sacs, and (D) type locality of the new species.

Fig. 4) is difficult to confirm after preservation.

Natural History. The main vegetation in the surrounding habitat of the new species is an evergreen forest of Fagaceae trees (i.e., *Castanopsis* and *Quercus* spp.) (Fig. 5). Larvae have black spots on the lateral sides of their body and tail, and they have no claws on the tips of their fingers and toes. In the early developmental stages of the larvae, they have one pair of balancers. Egg sacs are banana-shaped and are attached to fallen leaves or branches in puddles or ponds at forest edges from February to April.

Distribution. It is known from Nagoya-shi (including the Midori-ku, Tenpaku-ku, Moriyama-ku, Chikusa-ku, and Meito-ku), Tokai-shi, Chita-shi, Tokoname-shi, Seto-shi, Toyota-shi (only former Fujioka-cho), Nagakute-shi, and Owariasahi-shi, and Higashiura-cho, Agui-cho, Mihama-cho, Taketoyo-cho, and Minamichita-cho (Matsui *et al.*, 2019) in Aichi Prefecture. In this study, DNA data from Higashiura-cho and Agui-cho were not included, but adult specimens from these towns are stored in the Toyohashi Museum of Natural History (confirmed by T. Fujitani). It is possible that this species may also be distributed in Handa-shi, but reliable records on its inhabitation are not available. Probably, the populations of Tokai-shi, Chita-shi, and Tokoname-shi, as well as Higashiura-cho, Agui-cho, and Taketoyo-cho, were already extinct by 2021 based on our field surveys.

Remarks. The new species forms a monophyletic group with *H. vandenburghi* (Matsui *et al.*, 2019). The results of our study support this hypothesis by BI, but the posterior probability is not high (Fig. 2).

Hynobius vandenburghi Dunn, 1923

(Standard Japanese name: *Yamato-sanshouo*)

(Fig. 6)

Hynobius nebulosus: Fujitani *et al.*, 2016: 3, in part.

Holotype. An adult male (specimen number: CAS 26714) from Nara, Yamato Province, Hondo, collected by Victor Kühne (an alias used by John Cheesman Thompson) (Beolens *et al.*, 2011) in May 1907 (Dunn, 1923a; Dunn, 1923b). This specimen is stored in the California Academy of Sciences: 55 Music Concourse Drive, San Francisco, California, 94118, United States.

Diagnosis. A comparatively small species (with a mean SVL of 53.8 mm in males and 54.3 mm in females) within the Japanese lentic salamander species complex of Hynobius: SVL usually < 56 mm in males; the ratio of hindlimb length usually ≥ 30 %SVL in males; distinct and bright yellow stripe on the dorsal and ventral edges of the tail always present in both sexes; distinct black dots on dorsum usually absent in both sexes; distinct gular mottling never present in females; fifth toe of hindlimb always present; V-shaped or U-shaped vomerine teeth series; 13 (rarely 12 or 14) costal grooves; the number of costal folds between adpressed limbs usually > −1.5 in males and always < −1.0 in females.

Fig. 6. A specimen of *Hynobius vandenburghi* (KPM-NFA 942, adult male) from the type locality: (A) dorsal, (B) ventral, and (C) lateral views.

Description of a specimen from the type locality (Topotype). An adult male (specimen number: KPM-NFA 942) from Nakamachi, Nara-shi, Nara Prefecture, Japan (34° 40’ N, 135° 43’ E; elevation = 230 m), collected by Shota Seguchi on 1 March 2020. This specimen was collected after obtaining collection permission from The Unit of Natural Environment, Division of Landscape and Natural Environment, Department of Water Cycle, Forest, and Landscape Environment, Nara Prefecture. A moderately large individual: HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; slightly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on left and II > III > I > IV on right; five toes on each hindlimb, order of length III > IV > II > I > V on left and III > II > IV > V > I on right; V-shaped vomerine teeth; skin smooth and shiny; DWDV and DWDL absent; DBDD absent; DBTYLD and DBTYLV present; DGM absent (but indistinct gular mottling present). This specimen had the following measurements (in mm): SVL =54.5, TRL = 42.0, AGD = 29.1, HL = 12.7, TAL = 40.5, MTAW = 3.2, MTAH = 5.7, BTAW = 5.1, BTAH = 5.0, VTL = 2.7, VTW = 2.8, HW = 8.5, MXHW = 8.8, LFLL = 11.7, RFLL = 11.8, LHLL = 15.6, RHLL = 14.9, L1FL = 1.1, L2FL = 3.1, L3FL = 2.8, L4FL = 1.3, R1FL = 1.2, R2FL = 3.4, R3FL = 2.7, R4FL = 1.1, L1TL = 1.5, L2TL = 3.3, L3TL = 3.9, L4TL = 3.4, L5TL = 1.3, R1TL = 1.2, R2TL = 3.3, R3TL = 3.7, R4TL = 3.1, R5TL = 1.7, IND = 2.5, IOD = 3.3, LUEW = 1.5, RUEW = 1.4, SL = 3.6, LUEL =2.5, RUEL = 2.4, LJL = 7.0, CGN = 13.

Variation. Morphometric measurements are presented in Table 2 and the significant values of all measurements between sexes are listed in Table 3. Males have relatively longer RHW, RHLL, and R2FL than those of females, whereas males have relatively shorter RTRL and RAGD than those females. Skin markings are listed in Table 4. The dorsum is uniformly yellowish-brown or darkish-brown. DBDD are rarely present in males (2/17 = 11.8 %) and females (1/6 = 16.7 %). The venter is lighter than the dorsum. DWDV are often lacking in males (11/17 = 64.7 %) and sometimes present in females (3/6 = 50.0 %). DWDL are frequently lacking in males (11/17 = 64.7 %) and sometimes present in females (3/6 = 50.0 %). DGM sometimes lacking in males (10/17 = 58.8 %). CGN rarely 12 in males (3/17 = 17.6 %) and rarely 14 in females (1/6 = 16.7 %). CFBALN rarely < −1.0 in males (2/17 = 11.8 %) and sometimes > 2.0 in females (3/6 = 50 %). Iris is dark brown. When preserved in 70 % ethanol, dorsal coloration tends to fade to dark gray. DBTYLD and DBTYLV can be confirmed after preservation.

Distribution. It is known from Tahara-shi (only former Tahara-cho and Atsumi-cho), Aichi Prefecture (Matsui *et al.*, 2019), Gifu-shi (only former Gifu-shi), Seki-shi (only former Seki-shi), Kakamigahara-shi (only former Kakamigahara-shi), and Kaizu-shi (only former Nanno-cho), and Ibigawa-cho (only former Tanigumi-mura), Gifu Prefecture (Matsui *et al.*, 2019; Sakai *et al.*, 2019; Gifu High School, 2018), Nagahama-shi (only former Nagahama-shi, and Azai-cho and Kinomoto-cho), Maibara-shi (former Maibara, Omi-cho, and Santo-cho), Hikone-shi, Omihachiman-shi (only former Omihachiman-shi), Konan-shi (only former Kosei-cho), Higashiomi-shi (only former Yokaichi-shi and Gamo-cho), Koka-shi (only former Minakuchi-cho, Konan-cho, Koka-cho, and Tsuchiyama-cho), Ritto-shi, Kusatsu-shi, Otsu-shi (only former Otsu-shi), Takashima-shi (only former Adogawa-cho, Shin-asahi-cho, Imazu-cho, and Makino-cho), and Hino-cho and Ryuo-cho , Shiga Prefecture (Tago, 1931; Kokashi-Minakuchi-Kodomonomori-Shizenkan, 2013; Mito *et al.*, 2018; Matsui *et al.*, 2019), Kuwana-shi (only former Tado-cho), Inabe-shi (only former Inabe-cho), Suzuka-shi, Kameyama-shi (only former Kameyama-shi), Iga-shi (only former Ueno-shi), Tsu-shi (only former Tsu-shi and Hisai-shi, and Kawage-cho, Ano-cho, Hakusan-cho, and Ichishi-cho), Matsusaka-shi (only former Matsusaka-shi and Ureshino-cho), and Shima-shi (only former Ago-cho, Daio-cho, and Shima-cho), Mie Prefecture (Miyamoto, 2001; Shimizu, 2014; Matsui *et al.*, 2019), Nara-shi (only former Nara-shi), Yamatokoriyama-shi, and Ikoma-shi, and Oyodo-cho, Nara Prefecture (Matsui *et al.*, 2019), Kyoto-shi (only former Kyoto-shi of Nishikyo-ku, Higashiyama-ku, and Fushimi-ku), Nagaokakyo-shi, Kyotanabe-shi, Kizugawa-shi (only former Kizu-cho and Kamo-cho), Uji-shi and Kameoka-shi, Oyamazaki-cho, Seika-cho, and Ujitawara-cho, and Minamiyamashiro-mura, Kyoto Prefecture (Tanabe & Matsui, 2002; Matsui *et al.*, 2019), Toyonaka-shi, Mino-shi, Ibaraki-shi, Takatsuki-shi, Hirakata-shi, Katano-shi, Shijonawate-shi, Higashiosaka-shi, Tondabayashi-shi, Sakai-shi (only former Sakai-shi), Izumi-shi, and Hannan-shi, Osaka Prefecture (Osaka Prefecture, 1978; Matsui *et al.*, 2019). Populations from Nantan-shi (only former Sonobe-cho) of Kyoto Prefecture (Tanabe & Matsui, 2002) are lacking DNA data. This population is adjacent to the distribution area of *Hynobius setouchi*, so there is a possibility that this is the *H. setouchi* population. DNA analyses including samples from this population are essential for deciding the distribution range of *H. vandenburghi.*

Remarks. We examined a male specimen (specimen number: TMNH-AM-70) from Tahara-shi stored in the Toyohashi Museum of Natural History (1-238, Oiwacho Oana, Toyohashi-shi, Aichi Prefecture, 441-3147), but DBTYLD was not clear. The line of this specimen may have already faded; thus, the presence of DBTYLD still requires confirmation in several living individuals from the area after obtaining *Hynobius vandenburghi* collection permission from Tahara-shi. *H. vandenburghi* is also parapatrically distributed with *H. setouchi*, but it has no DBTYLD and DBTYLV.

Discussion

According to Matsui *et al.* (2019), *H. owariensis* sp. nov. and *H. vandenburghi* are the same species. In our study, the monophyly of the two species was also supported by BI based on the criteria of Huelsenbeck & Rannala (2004), but the probability was very low (Fig. 2). However, the monophyly was rejected by ML based on the criteria of Hillis & Bull (1993) (Fig. 2). Therefore, the monophyly of the two species was not strongly supported by our molecular analyses. On the other hand, the two species were morphologically distinguishable based on our analyses (Tables 2–4; Fig. 3). Males of *H. vandenburghi* usually have yellow stripes on the edges of their tail based on the diagnosis of *H. vandenburghi* (Matsui *et al.*, 2019); however, all examined males from *H. owariensis* sp. nov. did not have clear yellow stripes on the dorsal and ventral sides of their tail (Table 4). Therefore, *H. owariensis* sp. nov. does not strictly satisfy the diagnosis of *H. vandenburghi* described by Matsui *et al.* (2019). Furthermore, statistical analyses using morphological data suggested that these two groups were distinguishable (Table 3; Fig. 3). Furthermore, they are also genetically distinguishable based on the phylogenetic analyses using allozyme and mitochondrial data (Fig. 2) (Matsui *et al.*, 2006; Matsui *et al.*, 2019). Thus, we conclude that *H. owariensis* sp. nov. should be distinct species based on the morphological, phylogenetic, and evolutionary species concepts.

The distribution area of *H. vandenburghi* was limited to Tokai and Kinki Districts (Matsui *et al.*, 2019); it is not distributed in the Chita Peninsula and surrounding areas of Nagoya-shi, Aichi Prefecture (Figs 1–2). Following this description, the eastern, northern, southern, and western limits of the distribution range for *H. vandenburghi* are as follows: the Tahara-shi (Aichi Prefecture) and Seki-shi (Gifu Prefecture) line, Nagahama-shi (Shiga Prefecture) and Ibigawa-cho (Gifu Prefecture) line, Shima-shi (Mie Prefecture) and Hannan-shi (Osaka Prefecture) line, and Mino-shi (Osaka Prefecture) and Hannan-shi line, respectively. The distribution areas of *H. vandenburghi* and *H. setouchi* are adjacent in the northwestern and southwestern parts of Osaka Prefecture (Fig. 1; Table 1). According to Matsui *et al.* (2019), the boundary of the two species in the southwestern part of Osaka Prefecture is located in Hannan-shi (Pop. 42) and Misaki-cho (Pop. 44) (See Fig. 1). In addition, our analyses suggested that the boundary for the two species' distributions in the northwestern part of Osaka Prefecture is located in Mino-shi (Pop. 26) and Toyono-cho (Pop. 43) (Fig. 1). In our molecular analyses, samples from Kyoto Prefecture only included single sequencing data (Pop. 40) obtained by Matsui *et al.* (2019) because *H. vandenburghi* is protected by law in Kyoto Prefecture and collection is not allowed without permission. According to Tanabe & Matsui (2002), *H. nebulosus* (which currently is *H. vandenburghi*) is distributed in Nantan-shi of Kyoto Prefecture, but this species is not distributed in the adjacent area. Further molecular analyses, including samples from Nantan-shi, are required to clarify the boundary of *H. vandenburghi* and *H. setouchi*.

Hynobius vandenburghi is widely distributed in Tokai to Kinki regions, but our analyses only included individuals from Nara and Osaka Prefecture. Thus, there is a possibility that additional morphological variation exists. However, populations from Aichi (only Atsumi Peninsula), Gifu, Shiga, and Kyoto Prefectures are protected by the laws of the local governments; hence, we were unable to include these populations in our morphological analyses. Additionally, the present study did not include the Mie populations, which are not protected by local government law, because we could not obtain normal individuals from the Mie populations. Further morphological analyses, including individuals from the Mie and other populations, are therefore needed (following *H. vandenburghi* collection permission being granted by each local government) to reach a final decision on the diagnosis of *H. vandenburghi.* Based on our field surveys and previous reports (e.g., Sakai *et al.*, 2018), populations of *H. vandenburghi* are threatened with extinction. Considering that *H. vandenburghi* has a wider distribution range than *H. owariensis* sp. nov., some populations might already be extinct (e.g., former Tahara-cho of Aichi Prefecture, Kuwana-shi of Mie Prefecture, Toyonaka-shi, Tondabayashi-shi, and Higashi-osaka-shi of Osaka Prefecture). To save this species from extinction, reassessment of the conservation status of *H. vandenburghi* should be performed after widely incorporating the opinions of actors who conserve this species locally and without being overly focused on the apparent scale of the distribution area of *H. vandenburghi.*

Hynobius owariensis sp. nov. is found only at the Chita Peninsula and surrounding areas of Nagoya-shi (Fig. 1); however, the introduction of *H. vandenburghi* to Nagoya-shi has been confirmed in a specific park of the city (Fujitani *et al.*, 2016). To avoid a more artificial introduction of *H. vandenburghi*, information on the taxonomic revision of *H. vandenburghi* should be widely and immediately disseminated. This new species exists in very fragmented habitats and all populations are unsustainable because of their small population size or location close to development areas. Therefore, the viability of *H. owariensis* sp. nov. may be strongly affected by catastrophes or human activities such as deforestation and development. As previously mentioned, several populations (e.g., Tokai-shi, Chita-shi, and Tokoname-shi, and Higashiura-cho, Agui-cho, and Taketoyo-cho) of *H. owariensis* sp. nov. might already be extinct based on evidence from our field surveys. Following this description, the conservation status of the new species must therefore be reassessed immediately to ensure that essential conservation strategies are implemented and *H. owariensis* sp. nov. is saved from extinction.

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References

Bennett, R. A., 1991. A review of anesthesia and chemical restraint in reptiles. Journal of Zoo and Wildlife Medicine, 22: 282–303.

Beolens, B., M. Watkins & M. Grayson, 2011. The Eponym dictionary of reptiles. xiii+296 pp. Johns Hopkins University Press, Maryland.

Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9(8): 772–772.

Dunn, E. R., 1923a. New species of Hynobius from Japan. Proceedings of the California Academy of Sciences, 4th Series, 12(2): 27–29.

Dunn, E. R., 1923b. The salamanders of the family Hynobiidae. Proceedings of the American Academy of Arts and Sciences, 58(13): 445–523.

Fujitani, T., 2000. Research on *Hynobius nebulosus tokyoensis* in Higashiyama Park, Nagoya City. Amphibian History, (4): 9–12.

Fujitani, T., M. Notohara & Y. Kumazawa, 2016. Genetic diversity of *Hynobius nebulosus* in Nagoya City and its neighboring regions revealed using mitochondrial DNA sequences. Bulletin of the Herpetological Society of Japan, 2016(1): 1–12.

Gifu High School, 2018. Outstanding Performance Award at 20th Japan Water Award. Online. Available from internet: http://www.japanriver.or.jp/sjwp/sjwpj_history/jyusyou/jyusyou_pdf/no20_gufukoukou.pdf (downloaded on 2021-7-25).

Hillis, D. M. & J. J. Bull, 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology, 42(2): 182–192.

Huelsenbeck, J. P. & B. Rannala, 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Systematic Biology, 53(6): 904–913.

Ichioka, Y., M. Takikawa, R. Yamada, K. Miwa, Y. Shibara, T. Noro & T. Fujitani, 2021. A case of predation on *Hynobius vandenburghi* by *Procambarus clarkii* in Nagoya, Japan. Bulletin of Nagoya Biodiversity Center, 8: 65–69.

Ihaka, R. & R. Gentleman, 1996. R: a language for data analysis and graphics. Journal of computational and graphical statistics, 5(3): 299–314.

Kokashi-Minakuchi-Kodomonomori-Shizenkan, 2013. Red List of Koka City 2012. Online. Available from: https://www.city.koka.lg.jp/secure/10943/kokacityredlist2012all.pdf (downloaded on 2021-7-25).

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547–1549.

Kuzumi, S. & M. Kakegawa, 1989. Phylogenetic position of *Hynobius tokyoensis* from Aichi Prefecture. In Matsui M., T. Hikida & R. C. Goris (eds.), Current Herpetology in East Asia, p. 156. Herpetological Society of Japan, Kyoto, Japan.

Matsui, M., Y. Misawa, K. Nishikawa & T. Shimada, 2017. A new species of lentic breeding salamander (Amphibia, Caudata) from Central Japan. Current Herpetology, 36(2): 116–126.

Matsui, M., K. Nishikawa, T. Utsunomiya & S. Tanabe, 2006. Geographic allozyme variation in the Japanese clouded salamander, *Hynobius nebulosus* (Amphibia: Urodela). Biological Journal of the Linnean Society, 89(2): 311–330.

Matsui, M., H. Okawa, K. Nishikawa, G. Aoki, K. Eto, N. Yoshikawa, S. Tanabe, Y. Misawa & A. Tominaga, 2019. Systematics of the widely distributed Japanese clouded salamander, *Hynobius nebulosus* (Amphibia: Caudata: Hynobiidae), and its closest relatives. Current Herpetology, 38(1): 32–90.

Matsui, M., A. Tominaga, T. Hayashi, Y. Misawa & S. Tanabe, 2007. Phylogenetic relationships and phylogeography of *Hynobius tokyoensis* (Amphibia: Caudata) using complete sequences of cytochrome b and control region genes of mitochondrial DNA. Molecular Phylogenetics and Evolution, 44(1): 204–216.

Mito, N., K. Ohshima & O. Saitoh, 2018. Genetic Diversity among Clouded Salamanders (*Hynobius nebulosus*) in Shiga Prefecture. Zoological Science, 5(5): 427–435.

Miyamoto, Y., 2001. Regarding egg depositions of the Clouded

Salamander in the Chusei area of Mie Prefecture. Annual Research Report of Mie Prefecture Environmental Conservation Agency, (7): 135–148. (In Japanese).

Nakamura, K. & S. Ueno, 1963. Japanese Reptiles and Amphibians in Color. Hoikusha, Osaka Prefecture, Japan. (In Japanese).

Osaka Prefecture, 1978. Clouded Salamander. In Osaka Prefecture (eds.), The Second Natural Environmental Conservation Basic Survey, The Animal Distribution Survey Report (Amphibians and Reptiles), Osaka Prefecture III, pp. 24–25. Osaka, Japan. (In Japanese).

Ronquist, F., M. Teslenko, P. Van Der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539–542.

Sakai, Y., A. Kusakabe, K. Tsuchida, Y. Tsuzuku, S. Okada, T. Kitamura, S. Tomita, T. Mukai, M. Tagami, M. Takagi, Y. Yaoi & T. Minamoto, 2019. Discovery of an unrecorded population of Yamato salamander (*Hynobius vandenburghi*) by GIS and eDNA analysis. Environmental DNA, 1(3): 281–289.

Sato, I., 1943. A Monograph of the tailed batrachians of Japan. xxxvi+536 pp. Nippon Shuppan-sya, Osaka, Japan. (In Japanese).

Schwarz, G., 1978. Estimating the dimension of a model. Annals of Statistics, 6(2): 461–464.

Shimizu, Z., 2014. The distribution of Hynobiidae in Mie prefecture. Mie Natural History, (14): 51–61. (In Japanese).

Sugawara, H., T. Watabe, T. Yoshikawa & M. Nagano, 2018. Morphological and molecular analyses of *Hynobius dunni* reveal a new species from Shikoku, Japan. Herpetologica, 74(2): 159–168.

Sugiura, N., 1978. Further analysts of the data by akaike's information criterion and the finite corrections. Communications in Statistics, Theory and Methods, 7(1): 13–26.

Tago, K., 1931. The Salamanders of Japan. xxviii+218 pp. Unsodo, Kyoto, Japan. (In Japanese).

Tanabe, S. & M. Matsui, 2002. *Hynobius nebulosus* (Temminck et Schlegel, 1838). In Kyoto Prefecture (eds.), Red Data Book of Kyoto Prefecture. Vol. 1 (Wildlife), p. 119. Kyoto, Japan. (In Japanese).

摘　要

菅原弘貴・藤谷武史・瀬口翔太・澤畠拓夫・永野昌博, 2022. ヤマトサンショウウオ *Hynobius vandenburghi* の分類学的再検討：日本の本州中部からの1新種の記載. 神奈川県立博物館研究報告（自然科学）, (51): 47–59. [Sugawara, H., T. Fujitani, S. Seguchi, T. Sawahata & M. Nagano, 2022. Molecular Phylogenetic and Morphological Problems of the Aki Salamander *Hynobius akiensis*: Description of Two New Species from Chugoku, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 47–59.]

サンショウウオ属の1新種を、日本の愛知県西部から記載した。分子遺伝学的および形態学的解析の結果、ヤマトサンショウウオは愛知グループと近畿グループの二つに分けられることが示唆された。このため、ヤマトサンショウウオの愛知グループを、新種 *Hynobius owariensis* sp. nov.（和名：オワリサンショウウオ）として記載した。形態比較の結果、調査した雄個体において、ヤマトサンショウウオが尾の上下縁に明瞭かつ鮮明な黄色線をもつのに対して、本新種ではこの形質が確認できなかった。さらに、雄個体において、体側に沿って前肢と後肢を伸ばした時、本新種は多くの個体が肋皺1個分よりも離れるが、ヤマトサンショウウオでは多くの個体が肋皺1個分以内（個体によっては重複する）に収まっていた。その他、両種間には有意に異なる形質が複数存在していることに加えて、判別分析の結果においても、雌雄共に形態的に区別可能であることが示唆された。本新種は愛知県の西部（知多半島から名古屋市周辺部）に固有であるが、既に絶滅したと考えられる集団も複数存在し、現在も開発や乾田化によって、絶滅の危機に瀕している。

Bull. Kanagawa Pref. Mus. (Nat. Sci.), no. 51, pp. 61-72, Mar. 2022

Original article

Distribution Records and Re-descriptions of Some Japanese Species of the Subfamily Phygadeuontinae (Hymenoptera, Ichneumonidae)

Kyohei WATANABE[1)]

Abstract. New distribution records of six species of Japanese Phygadeuontinae, *Acrolyta spola* Momoi, 1970 (from Tokunoshima Is.), *Diatora lissonota* (Viereck, 1912) (from Tokunoshima Is.), *Mastrus oshimensis* (Uchida, 1930) (from Honshu), *Phygadeuon elongatus* (Uchida, 1930) (from Honshu), *P. yonedai* Kusigemati, 1986 (from Honshu and Izuoshima Is.), and *Theroscopus maruyamanus* (Uchida, 1930) (from Honshu), are recorded. These species except *A. spola* and *P. yonedai* are re-described. In addition, *P. akaashii* Uchida, 1930 and *T. fukuiyamensis* (Uchida, 1936) are also re-described. *Mastrus oshimensis* and *T. maruyamanus* have only been recorded in the original description and these are the second record for both species.

Key words: Asia, fauna, new record, parasitoid wasps, zoogeograph

Introduction

The subfamily Phygadeuontinae Förster, 1869 is a large-sized subfamily of the family Ichneumonidae, consisting of over 120 genera and 1900 species from the world (Yu *et al.*, 2016). They are idiobiont parasitoid, while the strategy and the host preference are highly varied. In Japan, 41 genera and 126 species of this subfamily have been recorded (Watanabe, 2021). In this study, I provide additional distribution data of the Japanese Phygadeuontinae. Furthermore, some re-descriptions of Japanese species (e.g., species described by Dr. Toichi Uchida (1898–1974)) are provided.

Materials and methods

Dried specimens deposited in Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa, Japan (KPM-NK), Museum of Nature and Human Activities, Sanda, Hyogo, Japan (MNHAH), and Systematic Entomology, Hokkaido University, Sapporo, Japan (SEHU) were examined.

A stereomicroscope (Nikon SMZ800) was used for morphological observation. Photographs were taken using OLYMPUS TG-4 digital camera joined with the stereomicroscope. Digital images were edited using Adobe Photoshop® CC. Morphological terminology follows Broad *et al.* (2018). Eady (1968) is also referred to for the description of micro-sculpture. The following abbreviations are used in this paper: female (F), the segment of antennal flagellum (FL), Malaise trap (MsT), the diameter of lateral ocellus (OD), ocello-ocular line (OOL), postocellar line (POL), standard Japanese name (SJN), the segment of tarsus (TS), and metasomal tergite (T).

In this study, I treat the subtribes of Gelini *sensu* Townes (1970) as a genus-group because the monophyly of these groups is still debated and there is little reliable evidence.

Results and discussion

New distribution records are found for the following six species of Japanese Phygadeuontinae: *Acrolyta spola* Momoi, 1970; *Diatora lissonota* (Viereck, 1912); *Mastrus oshimensis* (Uchida, 1930); *Phygadeuon elongatus* (Uchida, 1930); *P. yonedai* Kusigemati, 1986; *Theroscopus maruyamanus* (Uchida, 1930). All species except *A. spola* and *P. yonedai* were insufficiently described. Therefore, they are re-described below.

Phygadeuon akaashii Uchida, 1930 and *T. fukuiyamensis* (Uchida, 1936) have been recorded from several areas of Japan. Similar to the case of the above-mentioned species, however, the descriptions of these species were

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insufficient. Therefore, these species are also re-described based on the additional materials below.

Mastrus oshimensis and *T. maruyamanus* have only been recorded in the original description (Uchida, 1930), and the additional specimens reported in this study are the second records of both species.

Subfamily Phygadeuontinae Förster, 1869

Acrolyta genus group
(subtribe Acrolytina *sensu* Townes (1970))

Genus ***Acrolyta*** Förster, 1869

Acrolyta Förster, 1869: 174. Type: *Acrolyta empretiae* Ashmead, 1896 (= *Ischnoceros nigricapitatus* Cook & Davis, 1891). Designated by Viereck (1914).
Rhadinocera Förster, 1869: 177. Type: *Hemiteles* (*Rhadinocera*) *algonquinus* Viereck, 1917 (= *Ischnoceros nigricapitatus* Cook & Davis, 1891). Included by Viereck (1922).
Mosia Seyrig, 1952: 69. Type: *Mosia crassicornis* Seyrig, 1952. Original designation.
Parhemiteles Seyrig, 1952: 82. Type: *Parhemiteles flaviger* Seyrig, 1952. Original designation.

Since Watanabe (2021), a single specimen of *A. spola* Momoi, 1970 has been collected from Tokunoshima Island and its record is reported here.

Acrolyta spola Momoi, 1970
(SJN: *Munebuto-mame-togari-himebachi*)

Acrolyta spola Momoi, 1970: 344.

Description. See Momoi (1970).

Material examined. JAPAN: KPM-NK 81409, F, Kagoshima Pref., Tokunoshima Is., Isen Town, Itokina, 30. V. 2007, K. Watanabe leg.

Distribution. Japan (Yakushima Is., Amamioshima Is., Tokunoshima Is., Okinawajima Is., and Ishigakijima Is.).

Remarks. This is the first record of this species from Tokunoshima Island.

Genus ***Diatora*** Förster, 1869

Diatora Förster, 1869: 180. Type: *Diatora prodeniae* Ashmead, 1904. Included by Ashmead (1904).
Microtoridea Viereck, 1912: 150. Type: *Microtoridea lissonota* Viereck, 1912. Original designation.
Zaparaphylax Viereck, 1913: 647. Type: *Zaparaphylax perinae* Viereck, 1913 (= *Microtoridea lissonota* Viereck, 1912). Original designation.
Apanteloctonus Seyrig, 1952: 135. Type: *Apanteloctonus albiscapus* Seyrig, 1952. Original designation.

A single species, *D. lissonota* (Viereck, 1912), has been recorded from Japan. In this study, I newly record this species from Tokunoshima Island and re-describe it herein.

Diatora lissonota (Viereck, 1912)
(SJN: *Okinawa-mame-togari-himebachi*)
(Figs 1A–D)

Microtoridea lissonota Viereck, 1912: 150.
Zaraparaphylax perinae Viereck, 1913: 647.
Microtoridea secunda Cushman, 1934: 1.
Hemiteles guamensis Fullaway, 1946: 223.

Description. Female (n=1). Body length 3.7 mm. Body polished, covered with silver setae.

Head 0.57 times as long as wide in dorsal view. Clypeus 0.5 times as wide as maximum length, sparsely punctate, its anterior margin thin. Face densely punctate laterally, punctate medially. Length of malar space 0.95 times as long as basal width of mandible. Frons, gena, and vertex sparsely punctate. OD: POL: OOL = 0.5: 0.75: 0.7. Occipital carina complete except for dorsal part narrowly absent, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible slightly shorter than lower tooth. Base of mandible flat, with a weak transverse concavity near base. Antenna with 19 flagellomeres, subapical part not widened. Length of FL I 5.0 times as maximum depth of FL I in lateral view and 1.05 times as long as length of FL II.

Mesosoma. Upper side of collar with a weak median carina. Lateral part of pronotum sparsely punctate. Epomia present. Mesoscutum smooth (Fig. 1C), with a few, very sparse punctures. Notaulus distinct and sharp, its posterior end beyond the centre of mesoscutum. Scutellum smooth, with a few, sparse punctures, without a lateral longitudinal carina except for base. Mesopleuron covered with fine longitudinal striae and fine punctures except for smooth area on speculum. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus complete. Posterior transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a weak, complete juxtacoxal carina. Propodeal carinae and areas complete. Postero-lateral corner of area densipara not

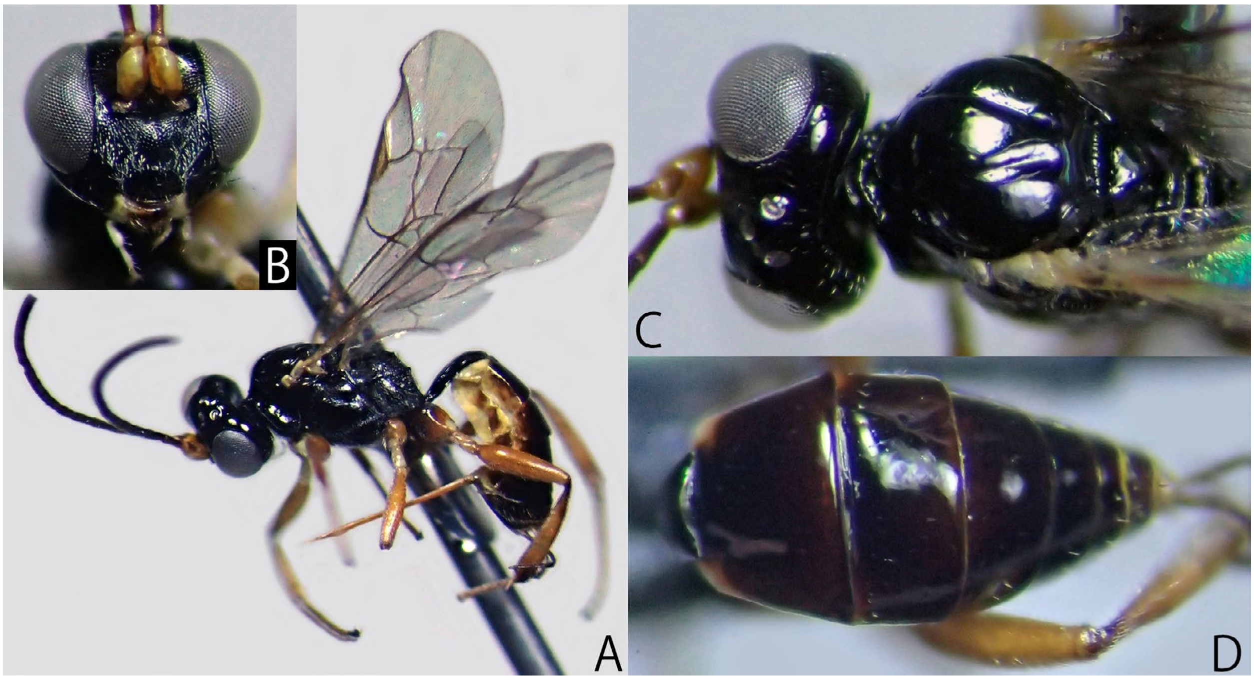

Fig. 1. *Diatora lissonota* (Viereck, 1912), female, KPM-NK 81410. A: lateral habitus; B: head, frontal view; C: head, mesoscutum, and scutellum, dorsal view; D: T II to T IV, dorsal view.

projected. Area basalis wider than area superomedia. Area superomedia slightly wider than long, receiving lateral section of anterior transverse carina near the antero-lateral corner (thus shape of area superomedia rectangle). Length of fore wing 3.0 mm. Areolet absent. Vein 1cu-a interstitial to vein M&RS. Nervellus subvertical, intercepted near middle. Hind femur 3.9 times as long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 0.8: 0.6: 0.3: 0.5. Tarsal claws simple.

Metasoma. T I to T VII smooth (Fig. 1D), with a few, fine punctures. T I 2.0 times as long as maximum width. Dorso-lateral carina of T I present except for posterior part. T II 0.55 times as long as maximum width. Ovipositor sheath 1.0 times as long as hind tibia. Ovipositor straight, with a nodus and ventral teeth.

Coloration (Figs. 1A–D). Body (excluding wings and legs) black to blackish brown. Mandible except for apex, postero-dorsal corner of pronotum, tegula, and membranous part of metasomal sternites yellow. Scape, pedicel, and ovipositor yellowish brown. Flagellum more or less tinged with brown. Wings hyaline. Veins and pterostigma brown to yellowish brown except for yellow wing base. Legs yellowish brown. Fore coxa and fore and mid trochanters and trochantelli yellow. Base and apex of hind tibia darkened.

Male. Not examined in this study.

Material examined. JAPAN: KPM-NK 81410, F, Kagoshima Pref., Tokunoshima Is., Tokunoshima Town, Kedoku, 20. V. 2008, K. Watanabe leg.

Distribution. Japan (Tokunoshima Is. and Okinawajima Is.); China, Guam, India, Malaysia, Palau, Philippines, Taiwan, and Truk Island.

Bionomics. Unknown in Japan. According to Yu *et al.* (2016), some lepidopterous and braconid hosts are recorded in other countries.

Remarks. KPM-NK 81410 has been compared with a specimen deposited in MNHAH (1 F, Taiwan, Hsien, III. 1966, K. Yano & H. Kajita leg., det. by S. Momoi). By the result of the comparison, no noteworthy morphological differences were observed. In Japan, only a single male collected from Okinawa Island was recorded (Momoi, 1970). Therefore, the KPM-NK 81410 is the second record of this species from Japan. This is also the first record of this species from Tokunoshima Island.

Mastrus genus group
(subtribe Mastrina *sensu* Townes (1970))

Genus ***Mastrus*** Förster, 1869

Mastrus Förster, 1869: 176. Type: *Phygadeuon* (*Mastrus*) *neodiprioni* Viereck, 1911. Included by Viereck (1911).

Daictes Förster, 1869: 176. Type: *Phygadeuon* (*Daictes*) *fukaii* Viereck, 1911 (= *Hemiteles aciculatus* Provancher, 1886). Included by Viereck (1911).

Aenoplex Förster, 1869: 176. Type: *Aenoplex betulaecola* Ashmead, 1896 (= *Orthocentrus pilifrons* Provancher, 1879). Included by Ashmead (1896).

Seven species, *M. ecornutus* Momoi, 1970, *M. fukaii* (Viereck 1911), *M. molestae* (Uchida, 1933), *M. oshimensis* (Uchida, 1930), *M. sugiharai* (Uchida, 1936), *M. takadai* Momoi, 1970, and *M. tenuibasalis* (Uchida, 1940), have been recorded from Japan. I found more than ten undetermined species from Japan. The taxonomic treatment of these species requires the additional specimens and comparison with European species. In this study, I newly record *Ma. oshimensis* from Honshu and re-describe it herein.

Mastrus oshimensis (Uchida, 1930)
(SJN: *Ooshima-chibi-togari-himebachi*)
(Figs 2A–C)

Hemiteles oshimensis Uchida, 1930: 342.

Description. Female (n=2). Body length 4.6–5.6 mm. Body covered with silver setae.

Head matt (Fig. 2B), 0.6 times as long as wide in dorsal view. Clypeus 0.45 times as wide as maximum length, sparsely punctate dorsally, its anterior margin narrowly marginate, with a minute median concavity. Length of malar space 0.9–0.95 times as long as basal width of mandible. OD: POL: OOL = 0.5: 0.65: 0.8. Occipital carina complete, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible longer than lower tooth. Base of mandible flat. Antenna with 20–21 flagellomeres, subapical part not widened. Length of FL I 4.0 times as maximum depth of FL I in lateral view and 0.9 times as long as length of FL II.

Mesosoma polished. Lateral part of pronotum coriaceous, sparsely punctate dorsally. Epomia present. Mesoscutum matt. Notaulus distinct and weak, its posterior end not beyond the centre of mesoscutum. Scutellum densely punctate, without a lateral longitudinal carina except for base. Mesopleuron covered with fine longitudinal striae with coriaceous surface and fine punctures except for a smooth area on speculum and its anterior area. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus present except for posterior 0.4. Posterior transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a complete juxtacoxal carina. Propodeal carinae and areas complete. Postero-lateral corner of area densipara not projected. Area basalis as wide as area superomedia. Area superomedia longer than wide, receiving lateral section of anterior transverse carina just in front of middle (Fig. 2C). Length of fore wing 4.3–4.9 mm. Areolet absent. Vein 1cu-a slightly postfurcal to vein M&RS. Nervellus inclivous, intercepted posterior to middle. Hind femur 4.8 times as

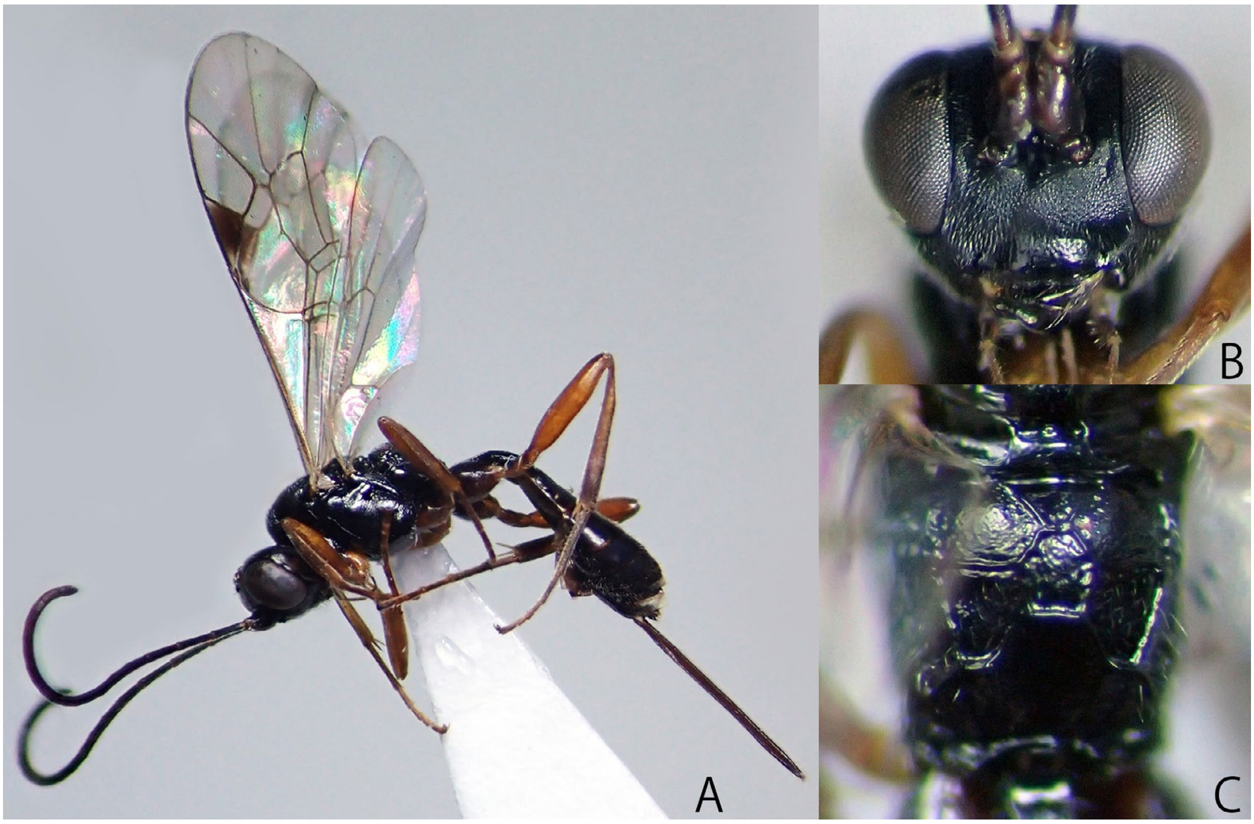

Fig. 2. *Mastrus oshimensis* (Uchida, 1930), female, KPM-NK 81411. A: lateral habitus; B: head, frontal view; C: propodeum, dorsal view.

long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 0.9: 0.6: 0.35: 0.5. Tarsal claws simple.

Metasoma. T I 1.9–2.05 times as long as maximum width, coriaceous. Median dorsal carina of T I present except for posterior part. Dorsolateral carina of T I complete. T II 0.7 times as long as maximum width, coriaceous except for smooth posterior margin. T III to T VII polished and finely punctate. Ovipositor sheath 1.3–1.45 times as long as hind tibia. Ovipositor straight, with a weak nodus and ventral teeth.

Coloration (Figs. 2A–C). Body (excluding wings and legs) black to blackish brown. Ventral surface of pedicel and base of FL I tinged with yellowish brown. Posterior areas of T VI and T VII white. Membranous part of metasomal sternites whitish brown. Posterior margins of T I to T V narrowly tinged with reddish yellow. Median area of T III sometimes slightly tinged with reddish brown. Ovipositor reddish brown. Wings hyaline. Veins and pterostigma brown to blackish brown except for base and apex of pterostigma yellowish brown. Legs reddish brown. Mid and hind coxae, hind trochanter, hind trochantellus, and apical part of hind tibia blackish brown to black.

Male. Unknown.

Materials examined. JAPAN: SEHU (lectotype), F, "Oshima", 2. IV. 1928, K. Sato leg.; KPM-NK 81411, F, Kanagawa Pref., Hiratsuka City, Okazaki, Hiraokanomori, 14. XI. 2018, Y. Hotta leg.

Distribution. Japan (Honshu and Izuoshima Is.).

Bionomics. Unknown.

Remarks. KPM-NK 81411 is the second record of this species and also the first record of this species from Honshu.

Phygadeuon genus group
(subtribe Phygadeuontina *sensu* Townes (1970))

Genus ***Phygadeuon*** Gravenhorst, 1829

Phygadeuon Gravenhorst, 1829: 635. Type: *Phygadeuon flavimanus* Gravenhorst, 1829. Designated by Westwood (1840).

Apterophygas Förster, 1869: 172. Type: *Apterophygas paradoxus* Bridgman, 1889. Included by Schmiedeknecht (1897).

Gunopaches Förster, 1869: 174. Type: *Gunopaches crassus* Perkins, 1962. Designated by Perkins (1962).

Habromma Förster, 1869: 176. Type: *Habromma nigrum* Ashmead, 1902 (= *Isochresta unicincta* Ashmead, 1902). Included by Ashmead (1902).

Pantolispa Förster, 1869: 178. Type: *Gunopaches crassus* Perkins, 1962. Designated by Perkins (1962).

Isochresta Förster, 1869: 181. Type: *Isochresta unicincta* Ashmead, 1902. Included by Ashmead (1902).

Bathymetis Förster, 1869: 182. Type: *Phygadeuon* (*Bathymetis*) *cylindricus* Brischke, 1891 (= *Phygadeuon dimidiatus* Thomson, 1884). Designated by Viereck (1914).

Iselix Förster, 1869: 182. Type: *Phygadeuon nitidus* Gravenhorst, 1829. Designated by Viereck (1914).

Homelys Förster, 1869: 182. Type: *Phygadeuon lapponicus* Thomson, 1884. Designated by Viereck (1914).

Ernoctona Förster, 1869: 183. Type: *Phygadeuon rugulosus* Gravenhorst, 1829. Designated by Perkins (1962).

Zaphleges Förster, 1869: 184. Type: *Phygadeuon leucostigmus* Gravenhorst, 1829. Designated by Ashmead (1900).

Ischnocryptus Kriechbaumer, 1892: 351. Type: *Phygadeuon nitidus* Gravenhorst, 1829. Designated by Viereck (1914).

Eight species, *P. akaashii* Uchida, 1930, *P. bidentata* (Uchida, 1930), *P. elongatus* (Uchida, 1930), *P. kiashii* Uchida, 1930, *P. kochiensis* Uchida, 1936, *P. sapporoensis* (Ashmead, 1906), *P. similis* (Uchida, 1930), and *P. yonedai* Kusigemati, 1986, have been recorded from Japan. I found more than 20 undetermined species from Japan. The taxonomic treatment of these species requires the additional specimens and comparison with European species. In this study, I re-describe *P. akaashii* and *P. elongatus* and record some distribution data of these species and *P. yonedai*.

Phygadeuon akaashii Uchida, 1930
(SJN: *Akaashi-futakobu-chibi-togari-himebachi*)
(Figs 3A–D)

Phygadeuon akaashii Uchida, 1930: 338.

Description. Female (n=6). Body length 4.6–6.4 mm. Body polished, covered with silver setae.

Head 0.65–0.7 times as long as wide in dorsal view. Clypeus 0.4 times as wide as maximum length, sparsely punctate dorsally (Fig. 3C), its anterior margin narrowly marginate except for a pair of median teeth. Face densely punctate. Frons with smooth areas above antennal sockets. Length of malar space 0.7–0.75 times as long as basal width of mandible. Maximum width of gena as wide as eye in lateral view. OD: POL: OOL = 0.5: 0.6–0.8:

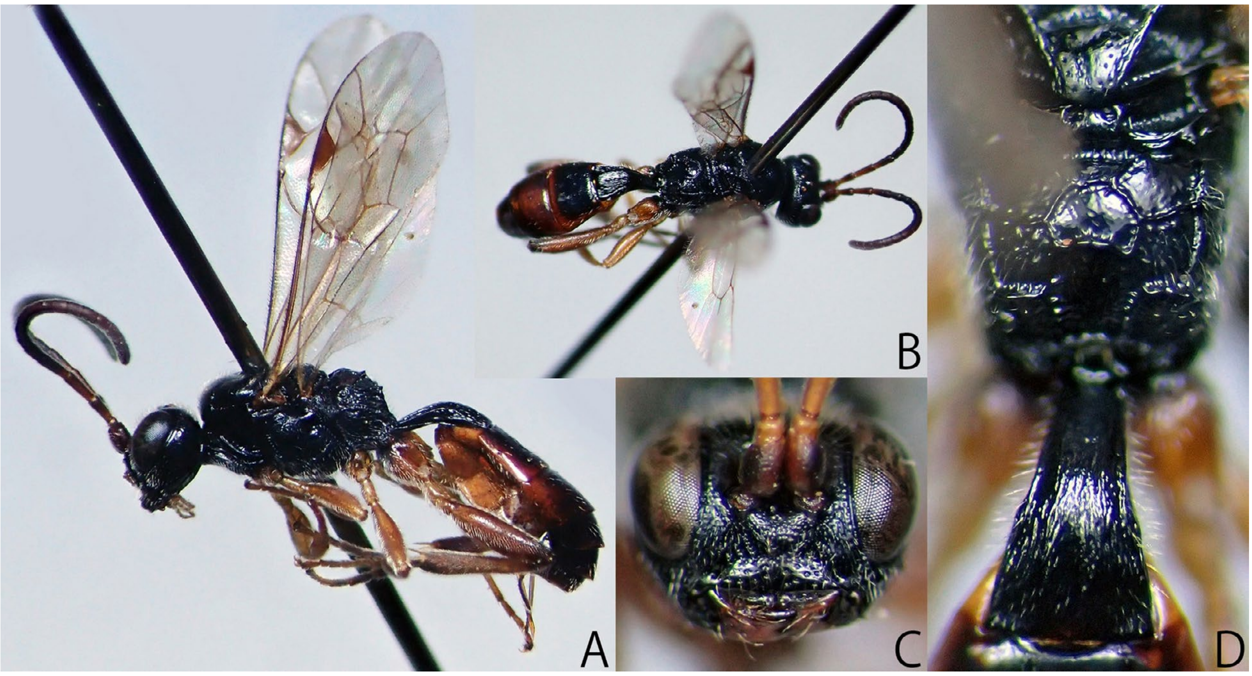

Fig. 3. *Phygadeuon akaashii* Uchida, 1930, females (A, B, D: KPM-NK 81851; C: KPM-NK 81853). A: lateral habitus; B: dorsal habitus; C: head, frontal view; D: scutellum, propodeum, and T I, dorsal view.

0.85–1.0. Occipital carina complete, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible slightly longer than lower tooth. Base of mandible flat. Antenna with 17–18 flagellomeres, subapical part slightly widened. Length of FL I 2.2–2.5 times as maximum depth of FL I in lateral view and 0.7–0.9 times as long as length of FL II.

Mesosoma. Lateral part of pronotum punctate dorsally, with oblique and longitudinal rugae postero-ventrally. Epomia weakly present. Mesoscutum punctate. Notaulus distinct and weak, its posterior end not beyond the centre of mesoscutum. Scutellum punctate, without a lateral longitudinal carina except for base. Mesopleuron covered with sparse punctures except for a large smooth area on speculum and its anterior area. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus complete. Posterior transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a complete juxtacoxal carina. Propodeal carinae and areas complete. Postero-lateral corner of area densipara weakly projected. Area basalis as wide as area superomedia. Area superomedia wider than long, receiving lateral section of anterior transverse carina near or just behind of middle (Fig. 3D). Length of fore wing 4.1–5.0 mm. Areolet present. Vein 1cu-a interstitial to vein M&RS. Nervellus subvertical, intercepted near posterior 0.75. Hind femur 3.9–4.1 times as long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 0.9: 0.7: 0.3: 0.8. Tarsal claws simple.

Metasoma. T I 1.8–1.9 times as long as maximum width, smooth except for postero-lateral area with a few longitudinal striae (Fig. 3D). Median dorsal carina of T I present except for area near posterior margin. Dorsolateral carina of T I complete except for area near spiracle. T II 0.55–0.73 times as long as maximum width, largely smooth. T III largely smooth. T V to T VI smooth basally and finely punctate posteriorly. T VI and T VII usually concealed under T V in dried specimen. Ovipositor sheath 0.4–0.5 times as long as hind tibia. Ovipositor straight, with an indistinct nodus and ventral teeth.

Coloration (Figs. 3A–D). Body (excluding wings and legs) black to blackish brown. Basal segments of flagellum, pedicel, mandible except for apex and base, tegula, and apical margin of T II tinged with reddish brown. Membranous part of metasomal sternites yellowish brown. Ovipositor reddish brown. Metasomal tergites sometimes partly tinged with dark reddish brown. Wings hyaline. Veins and pterostigma brown to blackish brown except for base of pterostigma whitish yellow. Legs reddish brown. Apex of hind femur, base and apical part of hind tibia, and hind tarsus blackish brown.

Male. Not examined in this study.

Materials examined. JAPAN: KPM-NK 81851, F, Yamanashi Pref., Ichinose, 7. IX. 1982, T. Murota leg.; KPM-NK 81854, F, Fukui Pref., Arashi, 23. IX. 1973, T. Tano leg.; KPM-NK 81855, F, ditto, 29. IX. 1973.; KPM-NK 81853, 81856, 81857, 3 F, ditto, 4. X. 1973.

Distribution. Japan (Hokkaido, Honshu, and Shikoku).

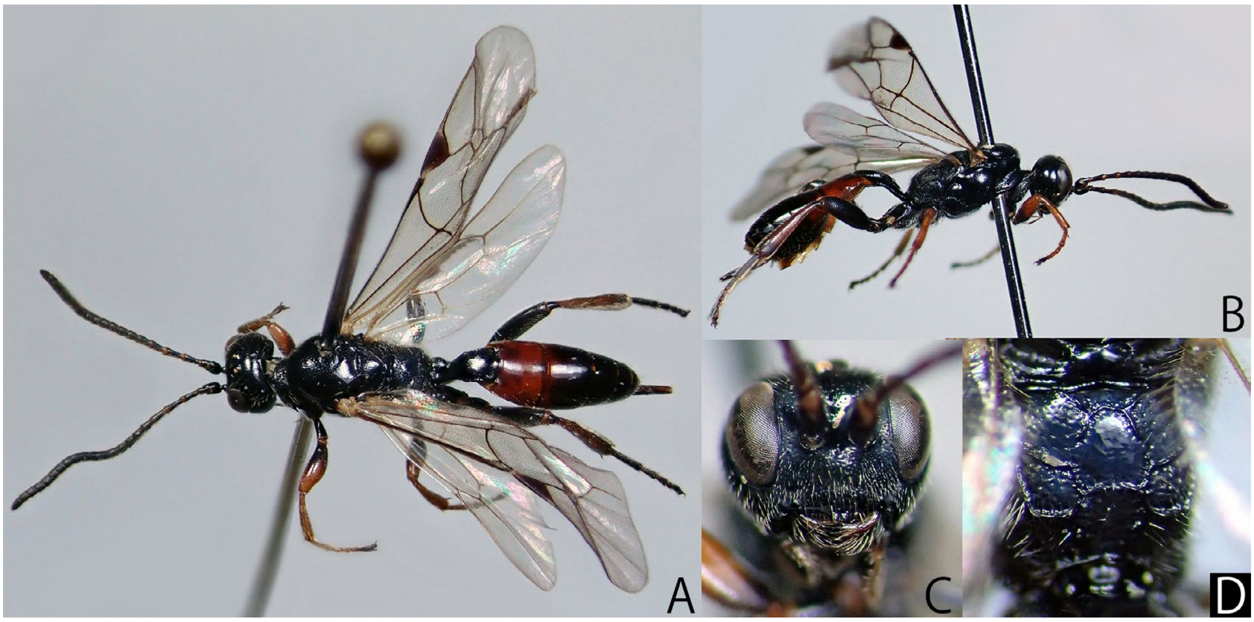

Fig. 4. *Phygadeuon elongatus* (Uchida, 1930), female, KPM-NK 81801. A: dorsal habitus; B: lateral habitus; C: head, frontal view; D: propodeum, dorsal view.

Bionomics. Host record: *Istochaeta aldrichi* (Mesnil, 1953) (Diptera, Tachinidae) (Kato, 1935); *Sarcophaga* sp. (Diptera, Sarcophagidae) (Minamikawa, 1969).

Remarks. KPM-NK 81853 has been compared with the lectotype deposited in SEHU.

Phygadeuon elongatus (Uchida, 1930)
(SJN: *Naga-futakobu-chibi-togari-himebachi*)
(Figs 4A–D)

Ischnocryptus elongatus Uchida, 1930: 338.

Description. Female (n=3). Body length 6.9–8.0 mm. Body polished, covered with silver setae.

Head 0.65–0.7 times as long as wide in dorsal view. Clypeus 0.4 times as wide as maximum length, sparsely punctate dorsally, punctate along anterior margin except for a pair of median teeth. Face densely punctate. Frons largely smooth above antennal sockets. Length of malar space 0.7–0.75 times as long as basal width of mandible. Maximum width of gena distinctly wider than eye in lateral view. OD: POL: OOL = 0.5: 0.7–1.0: 1.1–1.2. Occipital carina complete, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible slightly longer than lower tooth. Base of mandible shallowly concave. Antenna with 18–20 flagellomeres, subapical part slightly widened. Length of FL I 2.2 times as maximum depth of FL I in lateral view and 0.7–0.85 times as long as length of FL II.

Mesosoma. Lateral part of pronotum punctate dorsally, with oblique and longitudinal rugae postero-ventrally. Epomia weakly present. Mesoscutum sparsely punctate. Notaulus indistinct and weak. Scutellum sparsely punctate, without a lateral longitudinal carina except for base. Mesopleuron covered with sparse punctures except for a smooth area on speculum. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus present except for posterior 0.1. Posterior transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a complete juxtacoxal carina. Propodeal carinae and areas complete (sometimes carinae partly and narrowly indistinct: Fig. 4D). Postero-lateral corner of area densipara not projected. Area basalis as wide as area superomedia. Area superomedia as long as wide, receiving lateral section of anterior transverse carina just behind of middle (Fig. 4D). Length of fore wing 5.9–6.7 mm. Areolet present. Vein 1cu-a interstitial to vein M&RS. Nervellus subvertical, intercepted near posterior 0.6–0.7. Hind femur 3.2–3.6 times as long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 0.9–1.0: 0.7: 0.3–0.35: 0.8–0.9. Tarsal claws simple.

Metasoma. T I 1.9–2.4 times as long as maximum width, largely covered with minute and fine striae. Median dorsal carina of T I present except for posterior part. Dorsolateral carina of T I present in front of spiracle. T II 0.8–1.1 times as long as maximum width, largely smooth. T III to T VII smooth, with minute punctures. Ovipositor sheath (HT: 0.55) 0.65–0.75 times as long as hind tibia. Ovipositor straight to slightly decurved, without nodus and indistinct ventral teeth.

Coloration (Figs. 4A–D). Body (excluding wings and legs) black to blackish brown. Basal segments of flagellum and pedicel partly tinged with brown. Mandible except for base and apex, T II, and T III reddish brown. Membranous part of metasomal sternites and ovipositor dark yellowish brown. Wings hyaline. Veins and pterostigma brown to blackish brown except for apex and base of pterostigma whitish yellow. Legs black to blackish brown. Fore and mid trochantelli, femora, tibiae, and tarsus reddish brown. Tibial spurs yellowish brown. Subbasal part of hind tibia tinged with reddish brown.

Male. Unknown.

Materials examined. JAPAN: KPM-NK 81800, F, Gunma Pref., Katashina Vil., Marunuma, Yuzawa, 12. VII. 2014, K. Watanabe leg.; KPM-NK 81801, F, Nagano Pref., Outaki Vil., Mt. Ontakesan, Hakkaisan, 17. VII. 2007, K. Watanabe leg.; KPM-NK 81802, F, Fukui Pref., Ikeda town, Mizuumi Mt. Heko-san, 18. VI. 2016, S. Shimizu leg.

Distribution. Japan (Hokkaido and Honshu); Far East Russia.

Bionomics. Unknown.

Remarks. KPM-NK 81802 has been compared with the holotype deposited in SEHU. The above additional specimens differ from the holotype in the body length (6.9–8.0 mm in the additional specimens, "11 mm" in holotype) (Uchida, 1930), while I judged that this character state is an intraspecific variation. The above record is the first of this species from Honshu.

Phygadeuon yonedai Kusigemati, 1986
(SJN: *Yoneda-futakobu-chibi-togari-himebachi*)

Phygadeuon yonedai Kusigemati, 1986: 257.

Description. See Kusigemati (1986).

Materials examined. JAPAN: KPM-NK 81841–81843, 3 F, Tokyo, Izuoshima Is., Ohshima Town, Mt. Omaru, 17. VIII. – 5. X. 2012, K. Tsujii leg. (MsT); KPM-NK 81844, F, Tokyo, Izuoshima Is., Ohshima Town, Kandachi, 12. IX. 2012, K. Tsujii leg. (MsT); KPM-NK 81850, F, Kanagawa Pref., Ebina City, Sagamigawa-Riv., 20. IX. 1992, H. Nagase leg.; KPM-NK 81848, 81849, 2 F, ditto, 25. V. 2006, M. Ooishi & R. Watanabe leg. (Yellow pan trap); KPM-NK 81845, 81846, 2 F, Toyama Pref., Toyama City, Arimine, Inonedani, 1–8. IX. 2009, M. Watanabe *et al.* leg. (MsT); KPM-NK 81847, F, ditto, 15–22. IX. 2009, M. Watanabe *et al.* leg. (MsT); SEHU, F (holotype), Saga Pref., Miyuki, Kitashigeyasu, 18. XII. 1984 m Y. Yoneda leg. (Host: puparium of *Sepedon aenescens*).

Distribution. Japan (Honshu, Izuoshima Is., and Kyushu); Taiwan.

Bionomics. Host: *Sepedon aenescens* Wiedemann, 1830 (Diptera, Sciomyzidae) (Kusigemati, 1986).

Remarks. This is the first record of this species from Honshu and Izu-oshima Is.

Genus ***Theroscopus*** Förster, 1850

Theroscopus Förster, 1850: 72. Type: *Ichneumon pedestris* Fabricius, 1775. Designated by Viereck (1914).

Chamerpes Förster, 1869: 172. Type: *Pezomachus hemipterus* Gravenhorst, 1829 (= *Ichneumon hemipterus* Fabricius, 1793). Designated by Viereck (1914).

Eriplanus Förster, 1869: 180. Type: *Hemiteles* (*Eriplanus*) *metacomet* Viereck, 1917 (= *Ichneumon rufulus* Gmelin, 1790). Designated by Viereck (1917).

Phyrtus Förster, 1869: 181. Type: *Pezomachus hemipterus* Gravenhorst, 1829 (= *Ichneumon hemipterus* Fabricius, 1793). Designated by Ashmead (1900a).

Thysiotorus Förster, 1869: 181. Type: *Thysiotorus brevipennis* Brischke, 1891 (= *Ichneumon hemipteron* Riche, 1791). Designated by Viereck (1914).

Aenoplegimorpha Viereck, 1912: 147. Type: *Aenoplegimorpha phytonomi* Viereck, 1912 (= *Ichneumon rufulus* Gmelin, 1790). Original designation.

Seven species, *T. akanensis* (Uchida, 1930), *T. daisetsuzanus* (Uchida, 1930), *T. fukuiyamensis* (Uchida, 1936), *T. maruyamanus* (Uchida, 1930), *T. pennulae* (Uchida, 1932), *T. shanaensis* (Uchida, 1936), and *T. striatus* (Momoi, 1970), have been recorded from Japan. I found more than ten undetermined species from Japan. The taxonomic treatment of these species requires the additional specimens and comparison with European species. In this study, I re-describe *Th. fukuiyamensis* and *Th. maruyamanus*.

Theroscopus fukuiyamensis (Uchida, 1936)
(SJN: *Fukuiyama-chibi-togari-himebachi*)
(Figs 5A–D)

Hemiteles (*Aenoplex*) *fukuiyamensis* Uchida, 1936: 13.

Description. Female (n=11). Body length 6.1–8.5 mm. Body polished, covered with silver setae.

Head 0.6 times as long as wide in dorsal view. Clypeus 0.45 times as wide as maximum length, sparsely punctate, its anterior margin with a pair of minute median teeth. Face almost flat, densely punctate. Length of malar space 0.85–0.95 times as long as basal width of mandible. OD: POL:

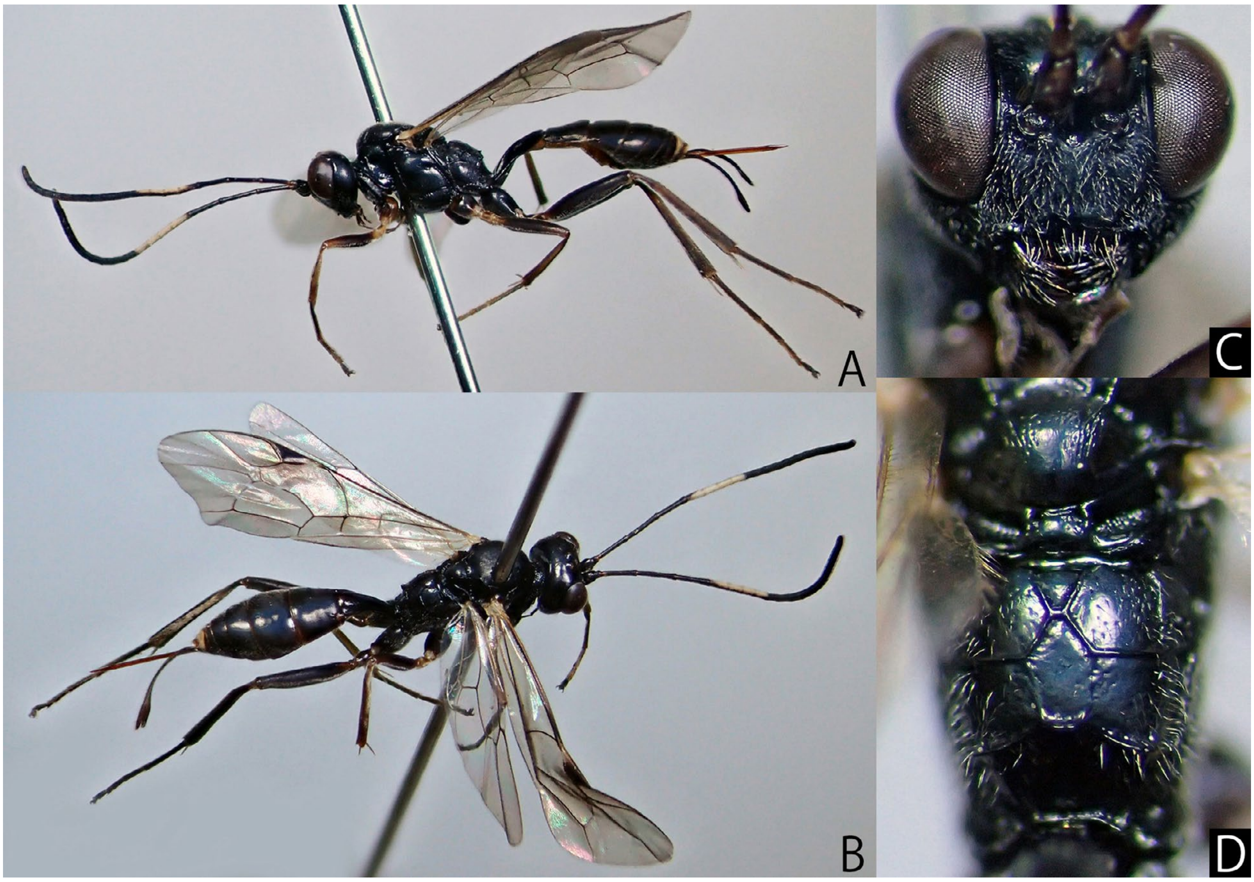

Fig. 5. *Theroscopus fukuiyamensis* (Uchida, 1936), female, KPM-NK 81812. A: lateral habitus; B: dorsal habitus; C: head, frontal view; D: scutellum and propodeum, dorsal view.

OOL = 0.5: 0.5–0.7: 1.0–1.2. Occipital carina complete, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible slightly longer than lower tooth. Base of mandible flat. Antenna with 19–20 flagellomeres, subapical part slightly widened. Length of FL I 5.7–6.7 times as maximum depth of FL I in lateral view and 0.83–0.9 times as long as length of FL II.

Mesosoma. Lateral part of pronotum coriaceous and sparsely punctate dorsally, longitudinally striate posteriorly, smooth along sharp epomia. Mesoscutum matt, densely punctate. Notaulus distinct, its posterior end not beyond the centre of mesoscutum. Scutellum finely punctate, without a lateral longitudinal carina except for base. Mesopleuron covered with fine and dense punctures, with fine longitudinal striae ventrally, without a conspicuous smooth area. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus present except for posterior 0.35. Posterior transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a complete juxtacoxal carina. Propodeal carinae and areas complete or sometimes lateromedian longitudinal carina and lateral longitudinal carina partly obtuse (Fig. 5D). Propodeum finely punctate except for area superomedia, area dentipara except lateral part, and area postero smooth. Postero-lateral corner of area densipara not projected. Area basalis narrower than area superomedia. Area superomedia longer than wide, receiving lateral section of anterior transverse carina just in front of middle (Fig. 5D). Length of fore wing 5.0–6.6 mm. Areolet absent. Vein 1cu-a interstitial to vein M&RS. Nervellus subvertical, intercepted posterior 0.75. Hind femur 4.95–5.2 times as long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 0.9: 0.6: 0.2–0.3: 0.4–0.5. Tarsal claws simple.

Metasoma. T I 2.0–2.6 times as long as maximum width, largely coriaceous. Median dorsal carina of T I present except for posterior part. Dorsolateral carina of T I complete. T II 0.7–0.85 times as long as maximum width. T II to T VII covered with minute punctures. Ovipositor sheath 0.85–0.95 times as long as hind tibia. Ovipositor straight, with a weak nodus and ventral teeth.

Coloration (Figs. 5A–D). Body (excluding wings and legs) black to blackish brown. FL IV to FL VII (sometimes also base of FL VIII) and posterior part of T VII white. Posterior margins of T II and T III tinged with reddish brown. Ovipositor reddish brown. Wings hyaline, slightly darkened behind of pterostigma. Veins and pterostigma brown to blackish brown except for base of pterostigma whitish brown. Wing base whitish yellow. Fore and mid legs reddish brown (coxae and femora more or less darkened. Hind leg black to blackish brown. Hind trochanter, trochantellus, base of femur, and subbasal part of tibia tinged with reddish brown.

Male. Unknown.

Materials examined. JAPAN: KPM-NK 81809, F, Kanagawa Pref., Yokosuka City, Mt. Oogusu-yama, 7. V. 2014, K. Watanabe leg.; KPM-NK 81810, F, Kanagawa Pref., Hadano City, Mt. Koubou-yama, 29. IV. 2007, K. Watanabe leg.; KPM-NK 81811, 81812, F, ditto, 1. V. 2016, K. Watanabe & H. Utsugi leg.; KPM-NK 81803, F, Nagano Pref., Ueda City, Sugadairakogen, Tsukuba University, 3–26. IX. 2014, S. Shimizu leg. (MsT); KPM-NK 81807, 81808, 2 F, Toyama Pref., Nanto City, Togamura-kamimomose, 21–28. VII. 2009, M. Watanabe *et al.* leg. (MsT); KPM-NK 81805, 81806, 2 F, ditto, 25. VIII. 2009, 1. IX. 2009; KPM-NK 81804, F, ditto, 15–29. IX. 2009; SEHU, F (holotype), Kochi Pref., Kochi City, Fukuiyama, 18. V. 1931, Y. Sugihara leg.

Distribution. Japan (Honshu and Shikoku).

Bionomics. Unknown.

Remarks. KPM-NK 81812 has been compared with the holotype deposited in SEHU. The above additional specimens differ from the holotype in the body length (6.1–8.5 mm in the additional specimens, "10 mm" in holotype) (Uchida, 1936), while I judged that this character state is an intraspecific variation.

Theroscopus maruyamanus (Uchida, 1930)
(SJN: *Kitaguni-chibi-togari-himebachi*)
(Figs 6A–E)

Phygadeuon maruyamanus Uchida, 1930: 336.

Description. Female (n=1). Body length 5.9 mm. Body covered with silver setae.

Head matt, 0.6 times as long as wide in dorsal view. Clypeus 0.45 times as wide as maximum length, sparsely punctate dorsally, its anterior margin with a pair of minute median teeth. Frons with smooth areas above antennal sockets. Length of malar space 1.1 times as long as basal width of mandible. OD: POL: OOL = 0.5: 0.8: 0.95. Occipital carina complete, its lower end joined with hypostomal carina distant from mandibular base. Upper tooth of mandible slightly longer than lower tooth. Base of mandible flat, with a shallow concavity. Antenna broken in KPM-NK 81838, see remarks.

Mesosoma polished. Lateral part of pronotum punctate dorsally, longitudinally rugulose along posterior margin. Epomia present. Mesoscutum punctate, matt medially. Notaulus weak and short. Scutellum densely punctate, without a lateral longitudinal carina except for base. Mesopleuron covered with fine longitudinal striae and fine punctures except for smooth area on speculum and its anterior area. Epicnemial carina present laterally, its dorsal end not reaching anterior margin of mesopleuron. Sternaulus present except for posterior 0.45. Posterior

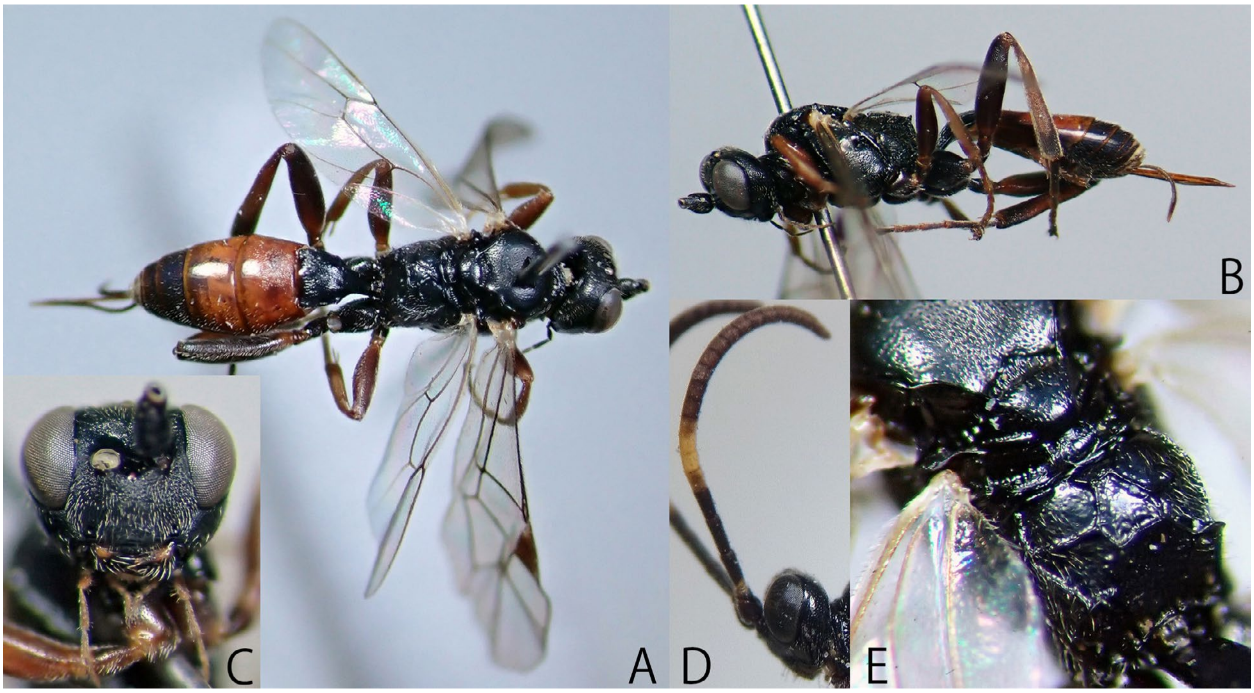

Fig. 6. *Theroscopus maruyamanus* (Uchida, 1930), females (A–C, E: KPM-NK 81838; D: lectotype deposited in SEHU) — A: dorsal habitus; B: lateral habitus; C: head, frontal view; D: head and antenna, lateral view; E: scutellum and propodeum, dorsal view.

transverse carina of mesosternum largely absent in front of middle coxa. Metapleuron punctate, with a complete juxtacoxal carina. Propodeal carinae and areas complete. Postero-lateral corner of area densipara projected. Propodeum punctate except for area superomedia largely smooth. Area basalis slightly narrower than area superomedia. Area superomedia as long as wide, receiving lateral section of anterior transverse carina just behind of middle (Fig. 6E). Length of fore wing 4.9–6.0 mm. Areolet absent. Vein 1cu-a interstitial to vein M&RS. Nervellus subvertical, intercepted posterior 0.75. Hind femur 4.0 times as long as maximum depth in lateral view. Hind TS I: II: III: IV: V = 2.0: 1.0: 0.7: 0.3: 0.7. Tarsal claws simple.

Metasoma polished. T I 1.5 times as long as maximum width, coriaceous anteriorly, longitudinally striate posteriorly. Median dorsal carina of T I present except for posterior part. Dorsolateral carina of T I complete except it on spiracle. T II 0.55 times as long as maximum width, coriaceous except for smooth posterior area. T III to T VII polished and finely punctate. Ovipositor sheath 0.8 times as long as hind tibia. Ovipositor straight, with a nodus and ventral teeth.

Coloration (Figs. 6A–C, E). Body (excluding wings and legs) black to blackish brown (pedicel and flagellum see remarks below). Basal spot of mandible yellowish brown. Tegula brown. T II, T III, and posterior margins of T IV and T V reddish brown. Posterior parts of T VI and T VII white. Membranous part of metasomal sternites and ovipositor reddish brown. Wings hyaline. Veins and pterostigma brown to blackish brown except for base of pterostigma yellowish brown. Legs reddish brown to dark reddish brown. Coxae and trochanters black. Hind trochantellus, femur, base and apical part of tibia, and tarsus darkened.

Male. Unknown.

Material examined. JAPAN: KPM-NK 81838, F, Tokyo, Hinohara Vil., Mt. Outakyama, 1. VI. 2008, T. Ban leg.

Distribution. Japan (Hokkaido and Honshu).

Bionomics. Unknown.

Remarks. KPM-NK 81838 has been compared with the lectotype deposited in SEHU. Although the antennae of KPM-NK 81838 are lost, I conclude that this specimen is *T. maruyamanus* by the other characteristics. KPM-NK 81838 is the second record of this species and is the first record of this species from Honshu. The character states of the antenna of lectotype: antenna with 20 flagellomeres, subapical part slightly widened; length of FL I 2.7 times as maximum depth of FL I in lateral view and 1.0 times as long as length of FL II; FL IV to FL VII white (Fig. 6D). KPM-NK 81838 differs from the lectotype in the following three characteristics: body length 5.9 mm (7.0 mm in lectotype); T I 1.5 times as long as maximum width (1.3 times in lectotype); ovipositor sheath 0.8 times as long as hind tibia (ca. 0.55 times in lectotype), while I judged that these character states are intraspecific variations.

Acknowledgements

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References

Ashmead, W. H., 1896. Descriptions of new parasitic Hymenoptera. Transactions of the American Entomological Society, 23: 179–234.

Ashmead, W. H., 1900. Classification of the Ichneumon flies, or the superfamily Ichneumonoidea. Proceedings of the United States National Museum, 23(1206): 1–220.

Ashmead, W. H., 1902. Papers from the Harriman Alaska Expedition XXVIII. Hymenoptera. Proceedings of the Washington Academy of Science, 4: 117–268.

Ashmead, W. H., 1904. Descriptions of new genera and species of Hymenoptera from the Philippine Islands. Proceedings of the United States National Museum, 28(1387): 127–158.

Broad, G. R., M. R. Shaw & M. G. Fitton, 2018. Ichneumonid Wasps (Hymenoptera: Ichneumonidae): their classification and biology. Handbooks for the Identification of the British Insects, 7(12): 1–418 + vi.

Cushman, R. A., 1934. New Ichneumonidae from India and China. Indian Forest Records, 20: 1–8.

Eady, R. D., 1968. Some illustrations of microsculpture in the Hymenoptera. Proceedings of the Royal Entomological Society of London, 43: 66–72.

Förster, A., 1850. Monographie der Gattung *Pezomachus*, Grav. Archiv für Naturgeschichte, 16(1): 49–232.

Förster, A., 1869. Synopsis der Familien und Gattungen der Ichneumonen. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens, 25: 135–221.

Fullaway, D. T., 1946. Ichneumonidae, Evaniidae, and Braconidae of Guam. Bernice P. Bishop Museum Bulletin, 189: 221–227.

Gravenhorst, J. L. C., 1829. Ichneumonologia Europaea. Pars II. Sumtibus Auctoris, Vratislaviae, 989 pp.

Kato, S., 1935. About *Centeter cinerea* Aldrich exported to New Zealand. Kontyu, 9: 7–24. [In Japanese.]

Kriechbaumer, J., 1892. Cryptiden-Studien. Entomologische Nachrichten, 18(22): 340–352.

Kusigemati, K., 1986. A new Ichneumonid parasite of Sciomyzid fly, *Sepedon aenescens*, in Japan (Hymenoptera). Kontyu, 54: 257–260.

Minamikawa, J., 1969. Host records of Ichneumonidae (Hymenoptera). Kontyu, 37: 220–232.

Momoi, S., 1970. Ichneumonidae (Hymenoptera) of the Ryukyu Archipelago. Pacific Insects, 12: 327–399.

Perkins, J. F., 1962. On the type species of Förster's genera (Hymenoptera: Ichneumonidae). Bulletin of the British Museum (Natural History), 11: 385–483.

Schmiedeknecht, O., 1897. Die Ichneumoniden-Gattung *Hemiteles*. Mit einer Übersicht der europäischen Arten. Természetrajzi Füzetek, 20: 103–135.

Seyrig, A., 1952. Les Ichneumonides de Madagascar. IV Ichneumonidae Cryptinae. Mémoires de l'Académie Malgache. Fascicule XIX, 213 pp. Académie Malgache, Antananarivo.

Townes, H., 1970. The genera of Ichneumonidae, Part 2. Memoirs of the American Entomological Institute, 12: 1–537.

Uchida, T., 1930. Fuenfter Beitrag zur Ichneumoniden-Fauna Japans. Journal of the Faculty of Agriculture, Hokkaido University, 25: 299–347.

Uchida, T., 1936. Zur Ichneumonidenfauna von Tosa (II.) Subfam. Cryptinae. Insecta matsumurana, 11: 1–20.

Viereck, H. L., 1911. Descriptions of six new genera and thirty-one new species of Ichneumon flies. Proceedings of the United States National Museum, 40(1812): 173–196.

Viereck, H. L., 1912. Descriptions of five new genera and twenty-six new species of Ichneumon-flies. Proceedings of the United States National Museum, 42(1888): 139–153.

Viereck, H. L., 1913. Descriptions of six new genera and twelve new species of Ichneumon-flies. Proceedings of the United States National Museum, 44(1974): 639–648.

Viereck, H. L., 1914. Type species of the genera of Ichneumon flies. United States National Museum Bulletin, 83: 1–186.

Viereck, H. L., 1917. Guide to the insects of Connecticut. Part III. The Hymenoptera, or wasp-like insects of Connecticut. Ichneumonoidea. State of Connecticut. State Geological and Natural History Survey, Bulletin, 22: 1–824.

Viereck, H. L., 1922. First supplement to "Type species of the genera of Ichneumon-flies." Proceedings of the United States National Museum, 59(2364): 129–150.

Watanabe, K., 2021. Taxonomic and zoogeographic study of the Japanese Phygadeuontinae (Hymenoptera, Ichneumonidae), with descriptions of 17 new species. Bulletin of the Kanagawa Prefectural Museum (Natural Science), (50): 55–136.

Westwood, J. O., 1840. Introduction to the modern classification of insects. Vol. II. Synopsis of the genera of British insects. London, 587 + 158 pp.

Yu, D. S., K. van Achterberg & K. Horstmann, 2016. World Ichneumonoidea 2015. Taxonomy, biology, morphology and distribution. [Flash drive]. Taxapad®, Vancouver, Canada.

摘　要

渡辺恭平, 2022. 日本産チビトガリヒメバチ亜科（ハチ目, ヒメバチ科）数種の分布記録と再記載. 神奈川県立博物館研究報告（自然科学）, (51): 61–72. [Watanabe, K., 2022. Distribution Records and Re-descriptions of Some Japanese Species of the Subfamily Phygadeuontinae (Hymenoptera, Ichneumonidae). *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 61–72.]

日本産のチビトガリヒメバチ亜科 Phygadeuontinae の 6 種について、新分布記録を報告した。すなわち、徳之島からムネブトマメトガリヒメバチ *Acrolyta spola* Momoi, 1970 とオキナワマメトガリヒメバチ *Diatora lissonota* (Viereck, 1912) を、本州と伊豆大島からヨネダフタコブチビトガリヒメバチ *Phygadeuon yonedai* Kusigemati, 1986 を、本州からオオシマチビトガリヒメバチ *Mastrus oshimensis* (Uchida, 1930) 、ナガフタコブチビトガリヒメバチ *P. elongatus* (Uchida, 1930) およびキタグニチビトガリヒメバチ *Theroscopus maruyamanus* (Uchida, 1930) を新たに記録した。これらの種のうち、ムネブトマメトガリヒメバチとヨネダフタコブチビトガリヒメバチを除いた種は、先行研究で形態形質の十分な記載がされていないため、新たに再記載を行った。これらに加えて、同様に記載が不十分なアカアシフタコブチビトガリヒメバチ *P. akaashii* Uchida, 1930 とフクイヤマチビトガリヒメバチ *T. fukuiyamensis* (Uchida, 1936) についても、追加標本の情報とともに再記載を行った。オオシマチビトガリヒメバチとキタグニチビトガリヒメバチはいままで原記載による記録のみが知られており、今回の記録はそれぞれの種において 2 例目の記録となる。

Bull. Kanagawa Pref. Mus. (Nat. Sci.), no. 51, pp. 73-80, Mar. 2022

原著論文

神奈川県内の絶滅危惧種キバネツノトンボの生態的知見

Ecological Knowledge of Endangered Species *Libelloides ramburi* (M'Lachlan, 1875) in Kanagawa Prefecture

苅部治紀 [1)]・加賀玲子 [1)]

Haruki Karube & Reiko Kaga

Abstract. *Libelloides ramburi* is an endangered species whose distribution in Kanagawa Prefecture is currently limited to a corner of the northern part of the prefecture. In this study, we report the results of the ecological survey conducted in 2020 and 2021. We newly observed that this species rested on dead herbal stems such as Japanese pampas grass in the nocturnal habitat. In addition to the preference for resting places of the *L. ramburi* (i.e., Japanese pampas grass in the early stage, rice grass in the latter half), we also found that the specie has the preference for the height of the resting position. In 2021, we marked 174 adults of *L. ramburi* for individual identification, of which 6 % were recaptured. The result suggests that adult members in the populations interchange rather frequently. The number of days to survive was as follows: male: 34 days, female: 24 days. The ratio of males was high in the early stage, but it was remarkably decreased in the later stage. Other new ecological knowledge are 1) Two species of spiders were recorded as predators of this species, 2) The average number of eggs in a mass was 60.4, 3) The majority of spawning targets were dead stems of alien plants, *Solidago canadensis* var. *scabra* (74 % of the total spawning targets).

Key words: marking survey, night rest, oviposition, population dynamic, predator

序　論

キバネツノトンボ *Libelloides ramburi* (M' Lachlan,1875)（図 1）は、良好な草地環境に生息する種とされ、国内では本州、九州に分布するが（日本昆虫目録 , 2016）、その分布は局所的である。近年多くの地域で減少が顕著になっており、現在 15 都道府県で地域のレッドリストに掲載されている。神奈川県でも、最近の記録がほとんどなくなっていることから、2006 年のレッドリストでは絶滅危惧 I 類に指定されている。

筆者らは、神奈川県における本種の現状に興味を持ち、2017 年から調査に着手し、分布的な知見については、昨年度報告した（苅部・加賀 , 2021）。近年の本種の公表記録はごく少数だが、苅部・加賀（2021）は相模原市緑区の旧藤野町地域には多産地を含めて産地が複数現存することを明らかにした。本報では、一連の調査で確認できた多産地において、これまでほとんど知見のない本種の生態と生活史を解明することを目的として、個体マーキングなどの手法を用いて調査を実施したので、結果を報告する。

調査目的と方法

筆者らは、神奈川県内での本種の分布調査を展開する中で、相模原市緑区の旧藤野町地域には多産地を含めて産地が複数現存することを明

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Fig. 1. *Libelloides ramburi*, male.
図 1. キバネツノトンボ　オス.

Fig. 2. Individual stationary in the evening.
図 2. 夕方静止する個体.

Fig. 3. Resting individual.
図 3. 休止した個体.

らかにした（苅部・加賀, 2021）。今回の調査は、産地群の中でも個体数がとくに多く、立地が周囲と地形的に隔離されている場所を選び、個体群動態や生態の調査地として継続観察を実施した。

調査については、1) これまで全く知見のない、本種の夕方から夜間の休止生態を静止位置や雌雄の別を記録する。この手法を利用することで個体群動態も調査した。よく静止が見られるススキ株には番号をつけて、静止株の選択の有無や季節推移を記録した。また、産卵数や位置などは他の産地を含めて調査した。これらの調査は、2020 年 5 月 8 日、11 日、17 日、24 日、29 日、6 月 4 日の夕方に行った。2020 年 5 月 8 日と 11 日は、個体への影響を考えて個体数チェックにとどめたが、影響が無いことを確認し、同 5 月 17 日以降は静止状態の個体の翅を開かせた状態で尾端を目視することで、雌雄の判別も行った。

2) 同産地で、個体マーキングを実施し、夕方の静止時に確認できた全個体の前翅に数字マーキングを継続して行い、その動態を追跡した。2020 年度に調査した夜間休止個体の推移を追跡する手法では、個体識別や確認個体群の産地間の出入りが確認できないため、個体を識別し追跡するための新たな手法として導入した。調査は、2021 年 5 月 4 日、5 月 14 日、5 月 28 日、6 月 7 日に実施した。

これらの手法によって、断片的な生態情報しかなかった本種の生態を解明した。

結　果

1）夜間休止

本種の生態については、日中の行動については断片的な報告があるが、夕方からの休止の生態についてはこれまで報告されたことはない。2020 年度に筆者らは調査地において、日中活発に飛翔していた個体が夕方になると草地のススキ枯れ枝などに静止し（図 2）、以降飛翔しないことを確認した。本種は鮮黄色の目立つ色調をしていることから、確認は容易に思われたが、実際には薄茶色の枯れ葉や枯れ枝に静止すると、発見は困難であり、確認には習熟が必要であった。

夕方飛翔を終えて休止に入った個体は、最初は調査者の接近に反応して再飛翔する個体も見られたが、気温の低下とともに活動は終息し、休止個体を刺激しても翅を広げる威嚇姿勢と思われる行動は示しても（図 3）、飛翔することはなくなった。また、接触刺激にはヨコバイ類のように掴まった茎をぐるぐる回る逃避行動も見られた。

今回の調査で得られたデータを図 4 に示す。2020 年 5 月 8 日の最初の詳細生態調査は、本調査地が生態調査に好適と考えてから着手したもので、初回調査を実施した 5 月 1 日には、すでに多数が見られたこと、成虫の成熟状況から判断すると初回調査時には、発生中盤に入っていたものと考えられる。なお、接触刺激に対する反応を確認したところ個体の影響はほとんどないこと

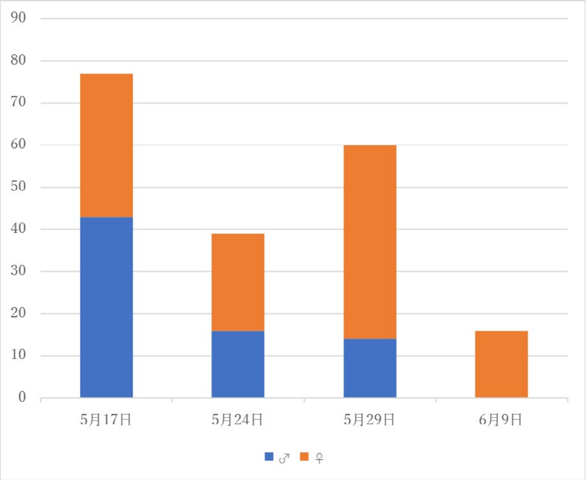

Fig. 4. 2020 confirmed population and male/female ratio.
図4. 2020年確認個体数と雌雄比.

Fig. 5. Landscape of habitat in May.
図5. 5月の調査地の景観.

Fig. 6. Landscape of habitat in June.
図6. 6月の調査地の景観.

が確認できたため、調査後半は静止個体を刺激し翅を開かせることで雌雄の判別を実施した。

まず、毎回の総確認個体数は、5月8日41頭、11日57頭、17日77頭（43♂34♀）、24日39頭（16♂23♀）、29日60頭（14♂46♀）、6月9日16頭（16♀）と推移した。本種はオスが最初に出現し、末期にはメスのみになることは東（2018）で指摘されているが、本調査地の詳細調査でも、初期に雌雄の判別調査を実施できなかったが、出現後半の動向は明らかになったと言える。性比は調査に着手した5月17日以降では、オスの比率は、5月17日56 %、24日41 %、29日23 %、6月9日は0 %と推移した。発生初期のデータはないが、発生後期にむけてメスの比率が急速に上昇した。今回の調査時の個体数のピークは5月17日の77頭であったが、5月下旬まで性比を変えながら高水準を保った。

本種の夜間休止は枯れ茎に静止するが、初回調査時は、調査地はまだ早春の景観で枯れ残った植物が主で、ススキ *Miscanthus sinensis* Andersson やセイタカアワダチソウ *Solidago canadensis* var. scabra の枯れ茎が目立った（図5）。発生終盤には急速に植物が成長し、イネ科草本（アズマガヤ *Hystrix duthiei* (Stapf) Bor subsp. *longearistata* (Hack.) Baden, Fred. et Seberg、オニウシノケグサ *Festuca arundinacea* Schreb. など）の被覆された緑の草地に枯れ茎が点在する景観になった（図6）。

静止する植物別の個体数は、詳細を調査するようになった5月17日以降のデータとなるが、図7に示す。

本種がおもに静止する植物は、5月17日、24日、29日の調査で、オスでは、ススキ枯れ茎の選択が76.7 %→81.2 %→28.6 %と後期にむけて減少、一方、イネ科を選択するケースは、4.7 %→18.8 %→64.3 %と急上昇している。メスでは、5月17日，24日、29日、6月9日の調査で、ススキ枯れ茎を選択するケースは50 %→82.6 %→8.7 %→0 %と後期にむけて急減し、イネ科の選択率は、0 %→0 %→76.1 %→50.0 %と後期にむけて増加した。

静止位置は、地表から10 cmピッチでカウントした。その結果、静止位置は地上50–110 cmに集中し、80–90 cmの位置がピークになった（図8）。

集中して静止個体の利用がみられるススキ株については、ナンバリングして調査期間中の追跡を実施した。その結果追跡した30株のうち、5回の調査中毎回利用があったのが2株、4回利用があったのが11株であった。このことから、特定のススキ株を選択的に利用していることが示唆された（図9）。

２）産卵

今回発生後期に、多くの産卵も確認できたので、

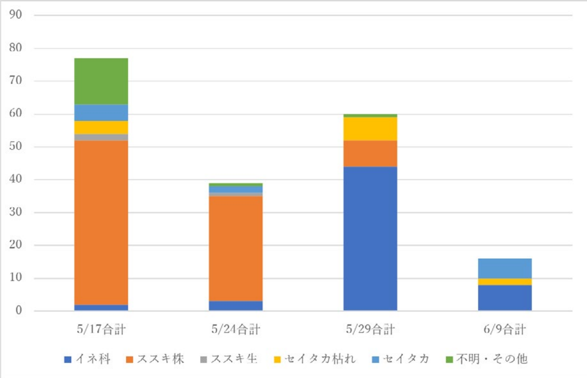

Fig. 7. Changes in stationary plants due to seasonal changes.
図 7. 季節の推移による静止植物の変化.
凡例
イネ科 : イネ科草本　Gramineae plants
ススキ株 : ススキ株枯れ茎 : Withered stocks of Japanese pampas grass
ススキ生 : ススキ株生葉 : Living Japanese Pampas grass
セイタカ枯れ : セイタカアワダチソウ枯れ茎 : Withered *Solidago canadensis*
セイタカ : セイタカアワダチソウ生茎 : Living *Solidago canadensis*
不明・その他 : 植物種不明・その他 : Unknown

Fig. 9. Japanese pampas grass where multiple resting.
図 9. 複数が休止するススキ株（矢印は静止しているキバネツノトンボ）.

産卵位置や卵数についても調査を行った。本種の産卵はおもに枯れ茎に左右一列ずつ付着する形状で行われる。産卵対象には好みがあるようで、複数の卵塊がみられる枯れ茎もあった（図 10）。18 卵塊の調査によると、各卵塊の最小数は 28 卵、最大数は 107 卵となり 4 倍の開きがあった。 1 卵塊あたりの平均卵数は 60.4 個であった。卵塊の左右での卵数はほぼ同一になっていた（表 1）。

産卵対象の植物種別内訳は、5 月 24 日の調査では、ススキ 3 卵塊、イネ科が 16 卵塊、セイタカアワダチソウ 54 卵塊（74 %）と圧倒的にセイタカアワダチソウ枯れ枝が好まれていた。卵塊の付着位置の地上からの平均高は、ススキが 120.7 cm、イネ科が 43.7 cm、セイタカアワダチソウが 80.1 cm であった。この日確認された 73 卵塊のうち、14 例計 36 卵塊が同一の茎に付着していた（図 11）。

幼虫はいっせいに孵化し、しばらく卵塊上に

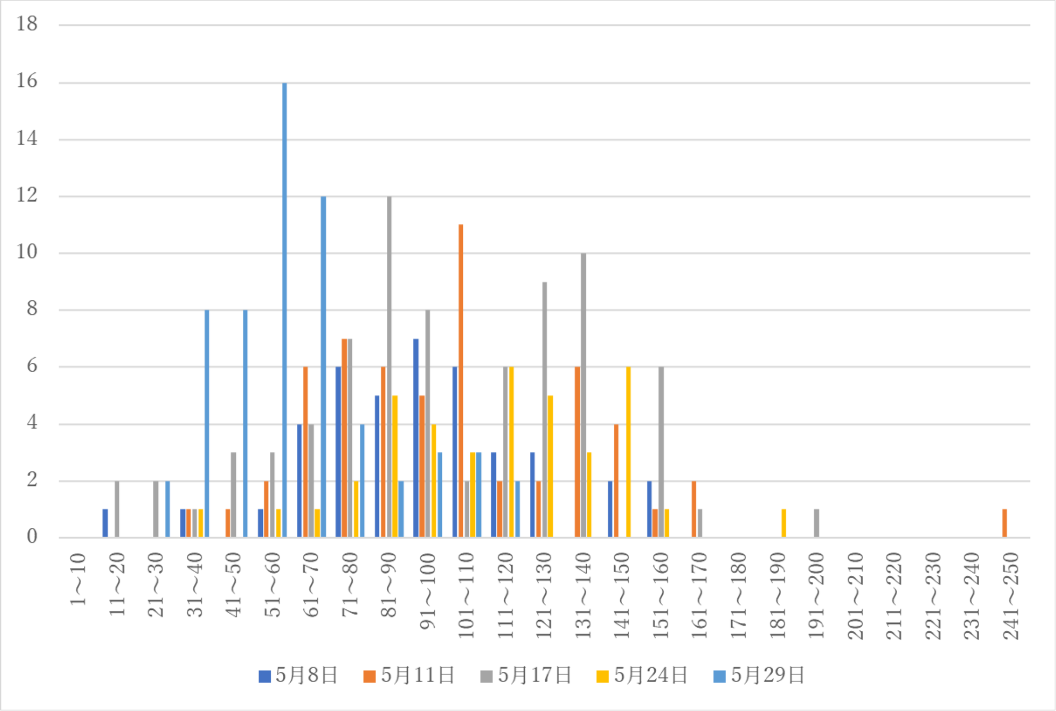

Fig. 8. Resting Height and Population 2020.
図 8. 2020 年休息場所高さと個体数.

Fig. 10. Multiple egg masses.
図 10. 複数の卵塊の例.

Table 1. Number of eggs on May 24, 2021
表 1. 一卵塊あたりの卵数（2021 年 5 月 24 日）

卵塊番号	サンプル番号	左列個数	右列個数	備　　考	合　計
1	1	27	30	一枝に2卵塊	57
	2	23	22		45
2	3	34	35	一枝に2卵塊	69
	4	52	55		107
3	5	35	33		68
4	6	31	30		61
5	7	29	28		57
6	8	30	31		61
7	9	32	35		67
8	10	27	28	一枝に3卵塊	55
	11	18	17		35
	12	31	31		62
9	13	37	34		71
10	14	25	26		51
11	15	32	35		67
12	16	17	11		28
13	17	32	32	卵塊上に 重ねて産卵	64
	18	32	31		63

* 太枠は 1 卵塊 , 細枠は 1 枝当たりの卵数を表す
* The thick frame indicates the number of eggs, and the thin frame indicates the number of eggs laid in one branch.

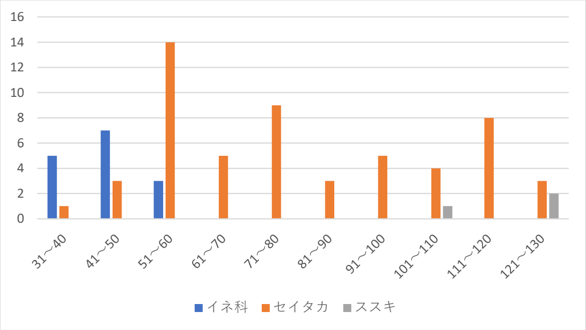

Fig. 11. Differences in egg count by plants.
図 11. 植物種による卵数の違い.

Fig. 12. Hatched larvae.
図 12. 孵化した幼虫.

Fig. 13. Predation by *Larinia argiopiformis.*
図 13. コガネグモダマシによる捕食.

Fig. 14. Predation by *Oxyopes badius.*
図 14. クリチャササグモによる捕食.

とどまる（図 12）。

３）捕食者

観察中に本種が捕食された死体を確認した。直接確認された事例はすべてクモ類によるものであった。捕食者はコガネグモダマシ *Larinia*

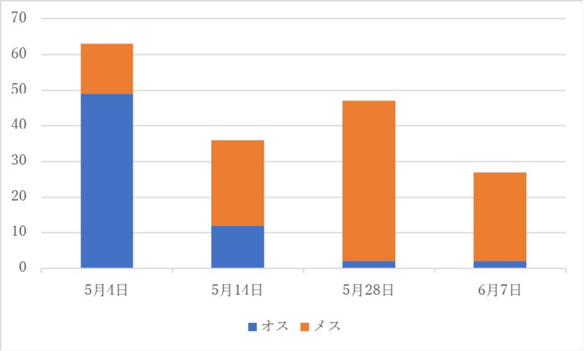

Fig. 15. 2021 confirmed population and male/female ratio.
図 15. 2021 年個体数と雌雄比.

argiopiformis（図 13）とクリチャササグモ *Oxyopes badius*（図 14）であった。観察時期の後半には、毎回数個体ずつの被捕食個体を確認し、観察時にはまだ生存している個体もあった。

４）マーキング調査による個体群動態調査

2021 年の調査では、詳細な個体群動態を把握するために、夕方草地に静止する全個体に個体識別のマーキングを行い、追跡を実施した。マーキングは右の前翅の透明部分に油性黒色サインペン（商品名サクラマイネーム）使用し通しナンバーを付けた。捕獲時に雌雄の別とメスについては腹部の状態を記録した。

前年の調査では、毎回の確認個体数は記録できたが、今回初めて調査地における総確認数（174 頭）を把握できた。個体群としては、オスが最初に出現し、徐々にメスの比率が上がっていくことが昨年の調査でも明らかになっていたが、本調査では個体識別ができるので、調査時の重複のない完全なデータとしても実証できた。それによると、5 月 4 日の初回調査でオスの比率は 78 %、5 月 14 日は同 33 % とこの 10 日間で急減し、5 月 28 日は 4 %、6 月 7 日は 7 % となった。なお、今回の初回調査時はすでに多数の個体が出現しており、初期の様相は不明である（図 15）。

Fig. 16. Male individuals reconfirmed after marking.
図 16. マーキング後再確認された個体（オス）.

Fig. 17. Female individuals reconfirmed after marking.
図 17. マーキング後再確認された個体（メス）.

Table 2. Days took for reconfirmation of marking individuals.
表 2. マーキング個体の再確認までの日数.

番号	雌雄	マーキング日	再捕獲日	再確認までの日数
47	オス	5月4日	5月14日	10日
60	オス	5月4日	5月14日	10日
33	不明	5月4日	5月28日	24日
67	メス	5月4日	5月28日	24日
94	メス	5月4日	5月28日	24日
25	オス	5月4日	6月7日	34日
27	オス	5月4日	6月7日	34日
87	メス	5月14日	5月28日	14日
128	メス	5月28日	6月7日	10日
番号不明	メス	不明	6月7日	不明

また、今回マーキングによって再捕獲による追跡が初めて成功したが、期間中の再捕獲例は 10 例（6 %）のみであった。このうち一例は翅のマーキング位置で破損しており、数字が読み取れず、不明の 1 例（No.33）は再捕獲時に重複マーキングに気が付かず個体判別ができなかったためである。再捕獲はオス 4 例（図 16）、メス 5 例（図 17）、不明 1 例であった。また、今回再捕獲された個体は、すべてが一回のみの確認であり、継続して、あるいは複数回の再確認例はなかった。再捕獲までの日数はマーキング時の次回調査（10 日後 : 3 例・14 日後 : 1 例）、2 回後（24 日後 : 3 例）、3 回後（34 日後 : 2 例）となった。（表 2）。

考　察

過去の生態的知見

本種の生態的な知見はごく少ない。過去の文献では、東（2018）に筆者らの知る限りもっとも詳細な記述があるので、以下に紹介する。それによると、兵庫県の観察地では、

- 本種の卵数と卵期については、一回の産卵数は枯れ枝に 2 列に並べて 22 から 24 で卵数にすると 44 個から 48 個になる、卵期は 29 日。
- 成虫の発生について、まずオスが現れ、4 ～ 5 日してからメスが姿を見せ、早い時期に飛翔しているのは殆どオスで、5 月も半ばを過ぎるとメスばかりになる。
- 成虫の飛翔には、摂食活動は地上よりおおむね 1 m くらいの高さを水平に飛びながら餌を捉えてそのまま食する。2–3 m の高さを餌が飛ぶとそれに飛翔して捉える。
- 4–5 m の高さを同種の個体が飛翔してくるとそれに向かって上昇し、これは雌雄を確認するための行動でオスであれば下降してくる。一頭も飛翔しない時間帯があり、一頭が飛翔し始めると周りから飛び出してくる。
- 成虫の生存期間についてはオスでは 20 日ぐらい、メスでは 25 日ぐらい、出現期間は約一ヶ月半で長くても二ヶ月。

などの記述がある。

今回の生態調査によって、本種の生態について多くの新知見が得られた。

１）夜間休止観察による知見

本種の飛翔行動が終了する夕方以降の休止状況が初めて明らかになった。夕方遅くに休止を始める静止個体の調査だけでも、ある程度の個体群動態が把握できる可能性が示せた。本種は、日中は盛んに飛翔するため産地における正確な個体数カウントや雌雄の判別は極めて困難であったが、本調査のような夕方観察の方法をとれば、正確な個体数データが得られる手法が確立できたことは今後の調査についても重要である。

静止する植物は、初期のススキから後期はイネ科に推移したが、これは、観察初期から中期は草地の芽吹きの枯れ茎が目立つ時期で、静止場所としてもススキが選択されやすく、後期はイネ科草本が急速に伸長する時期にあたり、ススキ枯れ茎が目立たなくなることと相関するものと思われる。

本産地では、産卵対象は外来植物であるセイタカアワダチソウの枯れ茎（74 %）を圧倒的に選択していた。在来種の外来種利用としても興味深い。

２）個体群動態

個体数の推移については、2020 年は、静止個体の観察で、個体数は後期に向けて急減し、またメスの比率が上昇することを明らかにできたが、2021 年に実施したマーキング調査でより詳細な動向が判明した。2021 年の個体数は上下動しながら、オスは初期の 78 % から 10 日後には 33 %、24 日後には 4 % と急減していった。調査日は等間隔では実施できなかったが、いったん減少した総個体数がその後回復したように見えることは、メスの羽化参入によるものと思われる。また、調査地は毎回数十頭が確認できる多産地であるが、今回のマーキング調査で、その構成員は、再確認率は 5 % とほとんどが入れ替わっていることが初めて明らかになったことも注目される。

本種の成虫の寿命データはこれまで存在していなかったが、本調査で少なくともオスで 34 日以上、メスで 24 日以上生存し得ることも初めて明らかにできた。

本種の県内における生息環境は、定期的な環境かく乱が生じる（草地としてはリセットが繰り返される）ところに生息しており、こうした環境は自然状態では崩壊地や山火事の跡地などに不定期に生じていたものと思われ、本種はそうした環境を渡りあるいて生存してきたメタ個体群構造をもつ種と言え、広域の草地探索と移動によって維持されてきたものと考えられる。本調査での定着率の低さ＝個体群の出入りの多さは、そのことを強く示唆する。

一連の調査で明らかになったのは、本種の現在の生息環境は耕作地に隣接する定期的に草刈りが実施される里山環境であることである。このため、生息環境は外来植生が卓越する場所が多く、本種が好んで利用する産卵対象種は、外来植物であるセイタカアワダチソウやイネ科雑草であったことも注目される。とくにセイタカアワダチソウは本種の産卵対象として重要な役割を持っている。在来のススキの枯れ茎はほとんど利用されていないことと対照的であった。国内には在来植生を主とする自然草原の本種の生息環境はほとんど残されていないものと考えられるが、本種の本来の利用植物にも興味がもたれる。

今回二年間の野外調査の結果を報告したが、近年継続する暖冬や春の訪れの早期化は、本種の出現時期に影響をあたえ、過去の記録と比較すると、一カ月近く出現が早まっていることを指摘した（苅部・加賀 , 2021）。本年度も調査開始時期が 5 月頭になり、出現初期の様相をとらえることができなかった。

次年度以降の調査では、おそらく4月末には始まっているであろう出現初期の状況を把握すること、多くの成果をもたらしたマーキング調査を継続していきたい。また、現状野外での知見がほとんどない幼虫や蛹時期の生態、とくに幼虫の餌の解明、さらに、成虫の日周活動や交尾産卵などの繁殖行動の解明も重要な課題である。これら生態調査から得られる情報によって、今後の本種の保全管理の手法の構築も期待できよう。

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引用文献

東 輝弥, 2018. キバネツノトンボに関する知見. きべりはむし, 41 (1): 9–11

苅部治紀・加賀玲子, 2021. 神奈川県内の絶滅危惧種キバネツノトンボの現状. 神奈川県立博物館研究報告（自然科学）, 49: 137–141.

関本茂行，吉澤和徳, 2016. 日本昆虫目録第5巻, 脈翅目群, 長翅目, 隠翅目, 毛翅目, 撚翅 目（日本昆虫目録編集委員会編），pp. 7–40，櫂歌書房，福岡.

摘要

苅部治紀・加賀玲子, 2022. 神奈川県内の絶滅危惧種キバネツノトンボの生態的知見. 神奈川県立博物館研究報告（自然科学）, (51): 73–80. [Karube, H. & R. Kaga, 2021. Ecological Knowledge of Endangered Species *Libelloides ramburi* (M'Lachlan, 1875) in Kanagawa Prefecture. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 73–80.]

キバネツノトンボは、現在、神奈川県内での分布が県北部の一角に極限される絶滅危惧種である。本報では、これまでほとんど報告のなかった本種の生態を解明するために、2020年、2021年に継続観察を実施した生態調査の結果を報告した。まず、今回本種が夜間生息地の草地のススキなどの枯れ茎に静止して休止することを初めて明らかにした。この中で発生初期はススキ、後期はイネ科草本を利用するなどの休止場所の選択や休止位置などの知見が得られた。また、休止時調査によりその時点の生息個体数や性比の確認、本種の天敵としての2種のクモ類についても明らかにできた。産卵対象は、外来植物のセイタカアワダチソウの枯れ茎が74 %と多数を占めた。今回本種では初めて実施された個体識別マーキング調査により、総計174頭にマークし、再捕獲率は6 %と個体群の出入りが激しい種であることや、発生初期にオスが多く、後期に向かって減少する性比の変動、オスで34日、メスで24日の個体寿命など、これまで未知であった多くの新知見を得ることができ、この調査手法の有効性を示すことができた。

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原著論文

自動撮影カメラで確認された神奈川県小田原市入生田地区の哺乳類相

Mammal Fauna Survey Using Camera Traps in Iryuda, Odawara City, Kanagawa Prefecture, Japan

鈴木　聡 1)

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Abstract. I conducted a mammal fauna survey using a camera trap in Iryuda, Odawara City, Kanagawa Prefecture, from April 2020 to May 2021. Cameras set at 10 sites took a total of 2,500 photographs of eight species of middle to large mammals and unidentified species of Rodentia Muridae and Chiroptera. The most photographed species was *Sus scrofa*, followed by *Paguma larvata*, *Nyctereutes procyonoides* and *Cervus nippon*, in this order. In correlation analyses of relative abundance index (RAI) between eight taxa (seven species and Muridae) that were photographed at more than seven sites, the correlation between *S. scrofa* and *C. nippon* was especially high and the correlations between *Mu. itatsi* and Muridae, and between *Me. anakuma* and Muridae, were present. Several species pairs showed negative correlations, such as between *Mu. itatsi* and *P. larvata*. Correlations of RAI between species could partly reflect the similarity of habitats and strength of competition between species.

Key words: abandoned cultivated land, *Cervus nippon, Nyctereutes procyonoides, Paguma larvata, Sus scrofa*

はじめに

神奈川県の陸生哺乳類相（外来種と翼手目を除く）は､絶滅種も含めて 29 種で構成される（中村 , 2003）。哺乳類の生息状況は年々変化しており、1995 年と 2006 年の神奈川県版レッドデータブックでは、評価される種が異なっているが、絶滅の危険性が増加している種は少なくない。一方で、ニホンジカ *Cervus nippon* のように個体数を増加させ、生態系に影響を与えている種も見られる。野生哺乳類が関わる様々な問題解決のためには、まず哺乳類の生息状況を把握する必要がある。そのためのアプローチの一つは、特定の種を対象にした分布研究である（塩沢ほか , 1984; 古内ほか , 1988; 有馬ほか , 1993; 青木ほか , 2006; 岡田ほか , 2007）。もう一つのアプローチが地域の哺乳類相を調査する方法である。神奈川県において、哺乳類の種多様性の高い地域は、丹沢と箱根である。丹沢地域では動植物を対象に総合調査が 3 回行われている（神奈川県 , 1964, 1997; 丹沢大山総合調査団 , 2007）。一方、箱根では、特定の種を対象にした分布調査は行われているものの（例 : 山口・山口 , 2005）、哺乳類相調査はほとんど行われてこなかった。自然度の高い山地以外では里山において、地元の大学などが中心となり、哺乳類相の調査が行われてきた（藤吉ほか , 2007; 岩崎ほか , 2021）。

哺乳類の生息調査の方法は分類群によって異なる。例えば、ネズミ類やモグラやジネズミなどの真無盲腸類を含む小型哺乳類の生息を確認す

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るためには、捕獲して標本を作製しないと同定が困難な場合もあるため、捕獲調査を実施することが多い。中大型哺乳類では、捕獲調査以外に糞や足跡などの痕跡調査がしばしば実施されている（金子ほか , 2009）。近年では、自動撮影調査も多く実施されており、日本国内でも各地の哺乳類相の解明に貢献している（例 : 遠藤・北村 , 2014; 幸田 , 2016; 香川ほか , 2020）。近年、自動撮影装置の入手がコスト的に容易になったことが大きく影響している。

上述のとおり、箱根では哺乳類相調査がほとんど行われてこなかったが、自動撮影調査を実施することにより、この地域における中大型哺乳類相を明らかにすることが期待できる。これまでに、箱根町畑宿の玉川大学箱根自然観察林や同湯本の早雲寺林で自動撮影調査が行われている（關・鈴木 , 2018; 關 , 2019; 鈴木 , 2020）。近年、箱根ではニホンジカの生息数が増加しており（永田 , 2019）、林床植生の退行やそれに伴う動物相への影響が懸念される。また、箱根の山麓ではイノシシ *Sus scrofa* が、年々増加する耕作放棄地周辺の耕作地の農作物に被害を与えるため、箱罠を用いた捕獲が行われている。箱根の哺乳類の生息状況は、特定の哺乳類の個体数の増加や人間活動の縮小など、様々な要因により年々変化している可能性がある。哺乳類の生息状況の変遷を把握するためには、定期的に同じ方法で調査を行う必要がある。今回、これまで自動撮影調査が実施されたことのない小田原市入生田地区で調査を行った。本稿では調査から明らかになったこの地域の中大型哺乳類相を報告するとともに、箱根の他の地域の哺乳類相との比較および生息種の種間関係についての考察を行う。

小田原市入生田は、西端に箱根外輪山の東側外壁の塔ノ峰の山頂があり、東端は国道 1 号線が通る早川の左岸河川敷で、わずかに早川の右岸が含まれる地域で、標高はおおよそ 45 m から 566 m である。標高約 80 m から 300 m の範囲の南向き斜面には耕作地があり、ミカンなどが栽培されているが、耕作放棄地も多い。

材料と方法

1．調査方法

2020 年 4 月から 2021 年 5 月まで、自動撮影カメラを小田原市入生田の耕作地周辺部および山林（35°14’37”N, 139°07’00”E 周辺、標高 90–200 m）に設置し、この地域に生息する哺乳類を調査した。調査に用いた自動撮影カメラの機種は、FOSITAN HC-1 である。

環境の異なる 10 定点にカメラを設置した（図１）。各定点の環境は、定点 1: 耕作地の外縁部 ; 定点 2: 耕作地の隣にある耕作放棄地 ; 定点 3: 沢沿い ; 定点 4: 耕作放棄地周辺の針広混交林 ; 定点 5: 季節によって流水がある針葉樹林と耕作放棄地の境界部 ; 定点 6: 斜面の耕作放棄地の下にある針葉樹林 ; 定点 7: 直径の大きい杉のある針葉樹林 ; 定点 8: 針広混交林 ; 定点 9: 沢から 20 m ほど北の斜面にある針葉樹林 ; 定点 10: 斜面の落葉広葉樹林である。

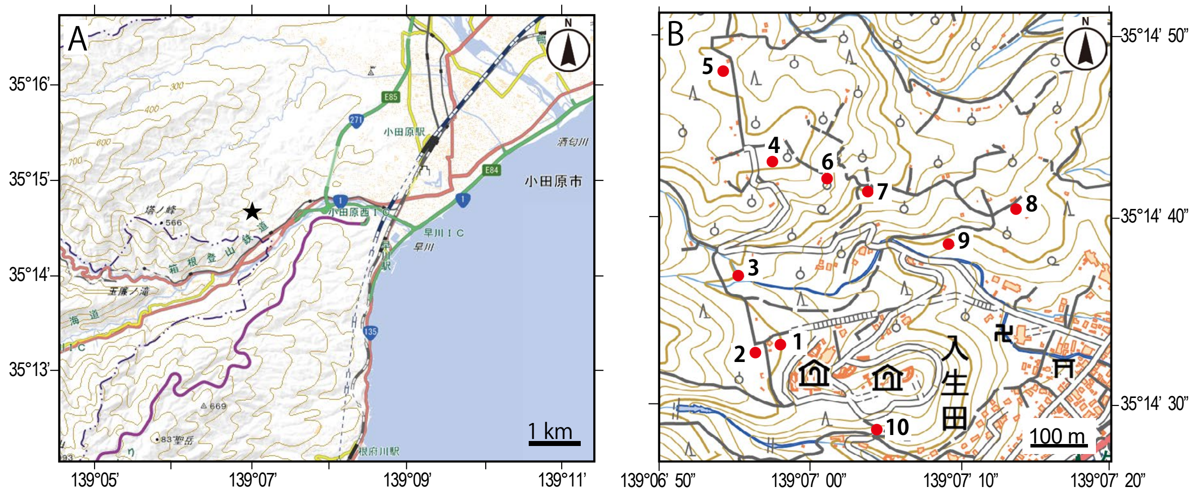

Fig. 1. Maps showing study area based on the Digital Topographic Maps published by Geospatial Information Authority of Japan. A: wide-range map with an asterisk showing the study area; B: detailed map with circles showing camera trapping sites.

図 1. 本研究の調査地を示した図（国土地理院電子地形図を加工して作成）. A: 星印で調査地周辺を示した広域図 ; B: 丸印でカメラの設置定点を示した詳細図 .

２．解析方法

カメラ回収後、撮影された動物種を同定し、撮影された回数を集計した。種同定が困難な小型哺乳類については、目または科まで同定し、以下の分析では種と同じレベルの扱いとした。本研究ではO'Brian *et al.* (2003) に従って、個体識別が困難な同一種が30分以内に連続して撮影されていた場合には、一連の行動による撮影と判断して集計から除外した。なお、撮影画像1枚あたり親子やペアなどと推測される複数個体が含まれている場合があったが、個体数ではなく撮影回数として集計した。全定点の合計および各定点について各動物種の撮影頻度指数（以下、RAI: Relative Abundance Index）を以下の式で算出した（O'Brian *et al.*, 2003）。

RAI =（総撮影回数［回］）／（カメラ稼働台・日数［日］）

種間の生息環境の簡易的な類似度の指標として、定点間におけるRAIの変化の種間における共通性を示すことができると考えられる相関係数を用いた。相関係数は7定点以上で撮影された種について算出した。

なお、哺乳類の和名および学名は川田ほか（2018）に従った。ただし、*Meles anakuma* の和名については、近年多くの文献で採用されており（例えばOhdachi et al. 2015）、かつて同種とされていたほかのアナグマ属の他種と区別する意味で使用される「ニホンアナグマ」を採用した。

結果

2,710台・日（使用した自動撮影カメラの稼働日数の合計）の稼働期間で種同定ができた中大型哺乳類8種および種同定の困難なネズミ科動物 Muridae gen. et sp. indet. および翼手目動物 Chiroptera fam., gen. et sp. indet. が、合計で2,500回撮影された（表1）。最も多く撮影されたのはイノシシでのべ806回、次いでハクビシン *Paguma larvata* のべ568回、タヌキ *Nyctereutes procyonoides* のべ562回、ニホンジカのべ233回であった。定点別にみると、定点1、2、5ではタヌキ、定点3、9ではハクビシン、定点4、6、7、8、10ではイノシシがそれぞれ最も多く撮影された。イノシシ、ニホンアナグマ、タヌキおよびハクビシンは全定点で撮影された。ニホンジカは定点1以外の9定点、ニホンテン *Martes melampus* は定点2以外の9定点、ニホンイタチ *Mustela itatsi* は定点1、2、4以外の7定点、ネズミ科動物は定点4、7、10以外の7定点と全定点の半分以上で撮影された。一方でイエネコ *Felis catus* は3定点（定点4、9、10）で撮影され、そのうちの1つの定点で撮影された個体には首輪が装着されていたことから飼育個体の可能性がある（定点番号および画像は非公開）。

撮影回数と稼働日数（表2）をもとに計算したRAIを表3に示す。全定点のRAIは、イノシシ0.297、ハクビシン0.210、タヌキ0.207、ニホンジカ0.086の順に大きかった。定点別にみると、各定点で最もRAIの大きい種はそれぞれ最も多く撮影された種であった。種ごと（7定点以上で撮影された種）にみると、RAIが最大の定点はイノシシおよびニホンジカでは定点4、ニホンアナグマでは定点9、ニホンテン、タヌキおよびハクビシンでは定点1、ニホンイタチでは定点5、ネズミ科動物では定点9であった。

種間のRAIの相関係数は、イノシシとニホンジカの間で最大であった（r = 0.868; 表4）。それ以外の7定点以上で撮影された種（ネズミ科を含む）の間の相関係数は絶対値が0.7以下で、0.603（ニホンアナグマとネズミ科）、0.522（ニホンイ

Table 1. Number of photographs of mammals in each trapping site
表1. 撮影された哺乳類の定点ごとの撮影数

	イノシシ	ニホンジカ	ニホンアナグマ	ニホンテン	ニホンイタチ	タヌキ	ハクビシン	イエネコ	ネズミ科	翼手目	Total
	S. scrofa	*C. nippon*	*Me. anakuma*	*Ma. mealmpus*	*Mu. itatsi*	*N. procyonoides*	*P.larvata*	*F. catus*	Muridae	Chiroptera	
No.1	21	0	1	4	0	103	65	0	5	0	199
No.2	51	5	1	0	0	63	19	0	3	0	142
No.3	73	3	1	3	5	13	106	0	15	0	219
No.4	143	69	13	1	0	55	53	3	0	0	337
No.5	54	37	23	3	16	65	17	0	21	2	238
No.6	107	53	19	4	11	78	48	0	15	1	336
No.7	171	40	4	5	1	42	87	0	0	1	351
No.8	28	2	22	4	1	26	13	0	7	0	103
No.9	101	14	39	1	6	69	118	2	54	0	404
No.10	57	10	7	2	1	48	42	4	0	0	171
Total	806	233	130	27	41	562	568	9	120	4	2500

Table 2. Working days of camera trap for each trapping site
表 2. 各定点における自動撮影カメラの稼働日数

Month Site	Apr. 2020	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan. 2021	Feb	Mar	Apr	May	**Total**
No.1	0	0	0	0	28	23	31	30	15	29	28	4	0	0	188
No.2	0	2	22	30	16	16	31	7	5	19	18	2	0	0	168
No.3	29	16	30	31	28	30	31	25	5	29	28	31	27	0	340
No.4	29	17	30	22	0	16	31	30	11	0	0	0	0	0	186
No.5	29	17	30	22	31	24	31	15	5	25	0	18	30	4	281
No.6	29	17	30	31	31	24	31	9	5	14	0	17	30	28	296
No7	29	17	30	10	31	30	31	17	0	0	12	31	30	28	296
No.8	1	31	30	31	10	0	0	0	5	31	28	31	30	28	256
No.9	29	17	30	31	27	30	31	28	5	31	30	31	30	28	378
No.10	0	3	30	27	31	30	31	18	5	29	28	31	30	28	321

Table 3. Relative abundance indices (RAI) of mammals at each trapping site. Underline: maximum value for each site; bold: maximum value for each species
表 3. 各定点における哺乳類の撮影頻度指数 (RAI). 下線 : 各定点における最大値 ; 太字 : 各種における最大値

	イノシシ *S. scrofa*	ニホンジカ *C. nippon*	ニホンアナグマ *Me. anakuma*	ニホンテン *Ma. mealmpus*	ニホンイタチ *Mu. itatsi*	タヌキ *N. procyonoides*	ハクビシン *P.larvata*	イエネコ *F. catus*	ネズミ科 Muridae	翼手目 Chiroptera
No.1	0.112	0	0.005	**0.021**	0	**0.548**	**0.346**	0	0.027	0
No.2	0.304	0.030	0.006	0	0	0.375	0.113	0	0.018	0
No.3	0.215	0.009	0.003	0.009	0.015	0.038	0.312	0	0.044	0
No.4	**0.769**	**0.371**	0.070	0.005	0	0.296	0.285	**0.016**	0	0
No.5	0.192	0.132	0.082	0.011	**0.057**	0.231	0.060	0	0.075	**0.007**
No.6	0.361	0.179	0.064	0.014	0.037	0.264	0.162	0	0.051	0.003
No.7	0.578	0.135	0.014	0.017	0.003	0.142	0.294	0	0	0.003
No.8	0.109	0.008	0.086	0.016	0.004	0.102	0.051	0	0.027	0
No.9	0.267	0.037	**0.103**	0.003	0.016	0.183	0.312	0.005	**0.143**	0
No.10	0.178	0.031	0.022	0.006	0.003	0.150	0.131	0.012	0	0
Total	0.297	0.086	0.048	0.010	0.015	0.207	0.210	0.003	0.044	0.001

Table 4. Correlation coefficients of RAI between species. Bold: absolute value larger than 0.3
表 4. 撮影頻度指数の種間の相関係数 . 太字：絶対値が 0.3 以上の値

	イノシシ *S. scrofa*	ニホンジカ *C. nippon*	ニホンアナグマ *Me. anakuma*	ニホンテン *Ma. mealmpus*	ニホンイタチ *Mu. itatsi*	タヌキ *N. procyonoides*	ハクビシン *P.larvata*
ニホンジカ *C. nippon*	**0.868**						
ニホンアナグマ *Me. anakuma*	0.055	**0.318**					
ニホンテン *Ma. melampus*	-0.197	-0.116	-0.150				
ニホンイタチ *Mu. itatsi*	-0.190	0.143	**0.450**	0.047			
タヌキ *N. procyonoides*	0.003	0.087	-0.216	0.166	-0.144		
ハクビシン *P. larvata*	**0.339**	0.102	-0.293	0.144	**-0.371**	0.180	
ネズミ科 Muridae	**-0.317**	-0.226	**0.603**	-0.239	**0.522**	-0.118	0.095

タチとネズミ科）、0.450（ニホンアナグマとニホンイタチ）、-0.371（ニホンイタチとハクビシン）の順に大きかった。

なお、撮影された静止画および動画を神奈川県立生命の星・地球博物館の哺乳類画像データベース（KPM-NQM）に登録し、神奈川県立生命の星・地球博物館収蔵資料データベース（https://nh.kanagawa-museum.jp/kpmnh-collections/）で公開する予定である。

考　察

本研究では、中大型哺乳類 8 種および同定困難な小型哺乳類の齧歯目ネズミ科と翼手目の動物が記録された。箱根の他の地域や大磯丘陵の西縁など周辺地域ではこれらの 8 種に加え、中大型哺乳類ではアライグマ *Procyon lotor*、ムササビ *Petaurista leucogenys*、ニホンリス *Sciurus lis*、ニホンノウサギ *Lepus brachyurus*、アカギツネ *Vulpes vulpes*、ツキノワグマ *Ursus thibetanus*、ニホンザル *Macaca fuscata* が確認されている（葉山ほか, 2005; 青木ほか, 2006; 田畑ほか, 2006; 山口, 2015; 關・鈴木, 2018; 關, 2019）。これらのうち、ムササビは自動撮影カメラで撮影されることが少ないため、本研究の結果からは生息の可能性を否定できない。地域の生息種確認調査を新規に行う際の目安は、200–300 台・日 / km^2 と考えられている（金子ほか, 2009）。本研究で自動撮影カメラを設置した場所を直線で結んだ最外郭凸多角形の面積はおおよそ 0.16 km^2 であることから、本研究の調査努力量は 16,900 台・日 / km^2 であり、目安を大幅に上回っている。このことから、今回の自動撮影カメラ調査で記録された種以外の中大型哺乳類は、現時点でこの地域に生息している可能性は低いと考えられる。

今回の調査では、イノシシが最も多く撮影され、RAI も最大であり、ハクビシン、タヌキ、ニホンジカが次いで撮影回数と RAI が大きかった。RAI を生息個体数の指標とすることには批判があるものの（Sollmann *et al.,* 2013）、入生田地区に生息する中大型哺乳類で最も個体数が多いのはイノシシであり、ハクビシンおよびタヌキも個体数が多いと考えられる。ニホンジカは住宅地から離れた定点（4–7）で多く出現していることから、現時点での主な生息地はより標高の高い場所であると考えられる。2000 年には、塔ノ峰の稜線沿いで糞が発見されるようになり、入生田および箱根町山崎でも人家近くまでニホンジカが来ているという情報があった（広谷, 2008）。今回の調査結果からは、ニホンジカの 20 年間での生息状況の変化は分からない。しかし、箱根山麓部の植生破壊や農作物への被害等の人との軋轢といった問題に対処するための基礎的な情報を得るため、定期的に自動撮影調査によるモニタリングを行うことが必要だと考えられる。その他の中大型哺乳類 4 種（ニホンアナグマ、ニホンテン、ニホンイタチ、イエネコ）が上記 4 種とくらべ RAI が極端に小さいことは、個体数が少ない、あるいは行動圏が小さいことを示唆する。生息状況を把握するためには、行動圏についても考慮する必要がある。

著者は 2018 年 10 月から 2019 年 1 月まで、今回の調査地から西南西に約 1.8 km 離れた早雲寺林において自動撮影調査を行った（鈴木, 2020）。ここでは、今回確認された中大型哺乳類のうち 6 種が確認され、ニホンジカとニホンテンの 2 種が確認されなかった。調査期間が短く、カメラの台数も少なかったため（7 台）、確認できなかった可能性もあるが、早雲寺林は早川と住宅、学校、旅館等の建物に囲まれ孤立した森林であるため、これらの 2 種の定住は困難かもしれない。イノシシが最も多く撮影され、次にハクビシンとタヌキが多かったという点は、本研究の結果と一致している。

入生田の 7 km 南西にあり、箱根南部の玉川大学箱根自然観察林（標高 410–990 m）では、今回確認された 8 種に加え、ニホンリス、ニホンノウサギ、アカギツネ、ツキノワグマ、ニホンザルの 5 種が確認されている（關・鈴木, 2018; 關, 2019）。小田原市入生田と箱根南部の間には、旧東海道（県道 732 号線）および国道 1 号線があるものの、ほぼ連続した森林でつながっている。それにも関わらず、ニホンリス、ニホンノウサギ、アカギツネの 3 種が確認されなかったことの理由として、今回の調査地域が人の利用環境（人家等の建物、畑地）内またはそれらと隣接していることが考えられる。これら 3 種の生息の有無に関わる要因を明らかにするためには、より広範囲での調査が必要である。ツキノワグマはかつて箱根町および湯河原町に生息していたが、1945 年以前に絶滅したと考えられている（野崎ほか, 1979）。關・鈴木（2018）は、箱根南部において 2017 年 6 月から 10 月にかけて少なくとも 3 個体を確認しており、複数個体が継続的に撮影された状況から、この地域にツキノワグマが恒常的に生息している可能性が高いと考えた。今後、個体数が増えれば入生田など東側の標高の低い地域まで生息域を拡大する可能性がある。關・鈴木（2018）においてニホンザルは 1 回しか確認されていない。この個体の詳細は不明だが、西湘地域個体群の S 群

またはH群に属する個体（神奈川県県西地域県政総合センター, 2021）と考えられる。西湘地域個体群は小田原市、箱根町および湯河原町の周辺に生息しており、神奈川県レッドデータブック2006において絶滅の恐れのある地域個体群（LP）に選定されている（広谷, 2006）。その中で入生田地区を含む地域に生息していたS群は2020年12月までに除去されたことから（神奈川県県西地域県政総合センター, 2021）、箱根南部で撮影された個体はすでに除去されている可能性がある。今後、S群に隣接していたH群の個体が入生田に出没する可能性がある。ツキノワグマおよびニホンザルは、人的被害をもたらすことがある一方で、神奈川県西部では絶滅が危惧されている。これらの種の保護管理は難しい問題であるが、自動撮影調査により定期的にモニタリングすることが保護管理に役立つだろう。

本研究では、入生田に生息する中大型哺乳類の種間関係を予測するための簡易的な分析として、RAIの相関分析を行った（表4）。イノシシとニホンジカの間の相関が最大であった。このことは2種の生息環境の類似性の高さを示していると考えられる。著者はイノシシとニホンジカが多く撮影された定点周辺で、イノシシの休息場所を確認しているが、そのような場所には低木や草本の密度が高くニホンジカの餌場にもなっている可能性がある。坂田ほか（2008）では、兵庫県においてニホンジカの目撃効率が高い地域でイノシシの目撃効率が下がることから、ニホンジカの食害による植生の衰退がイノシシの生息数を減らし目撃効率を下げるという間接的な競争関係が示唆されている。研究の地理的スケールは異なるが、今後ニホンジカの生息密度が高くなれば、イノシシの生息密度は減少する可能性がある。現時点では、各定点のニホンジカのRAIはイノシシのRAIの7.1–68.5 %であることから（表3）、ニホンジカの生息密度は相対的に低いと考えられる。また､これら2種の関係については、入生田では有害捕獲や狩猟が行われているため、それらの影響も考慮する必要があるだろう。それ以外の種間の相関はあまり大きくなかったが、ネズミ科動物との間でニホンアナグマ（0.60）とニホンイタチ（0.52）がやや高い相関を示した（表4）。ネズミ類は多くの食肉目動物の採食物となっているが、ニホンアナグマの主な採食物はミミズであり、ネズミ類などの哺乳類は春にわずかに利用されるのみであるため（金子, 2018）、ニホンアナグマとネズミ科動物間のRAIの相関は生息環境の類似性によるところが大きいと考えられる。一方で、ニホンイタチはネズミ類を多く捕食するため（鈴木, 2018）、このような傾向を示した可能性が考えられる。また､負の相関（例えば、ニホンイタチとハクビシン）は生息環境の類似性のほかに種間競争も反映している可能性がある。

本研究では10定点で調査を行ったが、相関分析を行う上ではやや定点数が少ない。また、相関分析は種の生息環境や種間関係を評価するためには、簡易的すぎる分析であるが、数少ない定点からのデータを用いて傾向を予測することができた。定点数を増やし、密度推定に必要なカメラの設置方法を取り、適切な解析を行うことで、哺乳類の生息数や種間関係のより正確な推定が可能になるだろう。

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引用文献

青木雄司･重昆達也･繁田真由美･柳川美保子･蓮田弘美･山口尚子･竹内時男･小林俊元･佐藤　健･二宮孝子･早川広美, 2006. 神奈川県におけるムササビの分布. 神奈川自然誌資料, (27): 27–40.

有馬征二・野口光昭・鈴木一子, 1993. 神奈川県における中型哺乳類（タヌキ・キツネ・ハクビシン）の生息状況について（３）. 神奈川県立自然保護センター報告, (10): 101–114.

遠藤　拓・北村俊平, 2014. 自動撮影カメラによる石川県林業試験場内の中・大型哺乳類相の調査. 石川県立自然史資料館研究報告, (4): 23–36.

藤吉敬子・宇山　智・井上和宏・浅野嗣三・渋谷香奈子・瀧澤　恵・菅原野花・岩本　順・藤原怜史・黒島祥一・竹村和記・石川康裕・藤吉正明, 2007. 神奈川県弘法山公園において自動撮影と夜間観察で得られた哺乳類の記録. 神奈川自然誌資料, (28): 59–65

古内昭五郎・野田光昭・沼田美幸, 1988. 神奈川県における中型哺乳類（タヌキ・キツネ）の生息状況について（２）. 神奈川県立自然保護センター調査研究報告, (5): 37–48.

葉山久世・浅見順一・石渡恭之・北林輝夫・桑原尚史・田畑真悠・根上泰子・李　謙一・山本美和・吉之元喜科, 2005. かながわ野生化アライグマの分布調査と普及啓発パンフレットの作成. プロ・ナトゥーラ・ファンド第14期助成成果報告書, pp.145–150. 財団法人自然保護助成基金･財団法人日本自然保護協会.

広谷浩子, 2006. 哺乳類. 高桑正敏･勝山輝男･木場英久編, 神奈川県レッドデータ生物調査報告書, pp. 225–232.

神奈川県立生命の星・地球博物館 , 小田原 .

広谷浩子 , 2008. 箱根の「けもの」. 自然科学のとびら , 14: 20.

岩崎貴也・奥田真未・渡部凌我・斎藤昌幸・土田彩加・志村映実・泉　進 , 2021. カメラトラップで確認された神奈川大学湘南ひらつかキャンパスにおける哺乳類相とキャンパス建設前後の変化 . 神奈川自然誌資料 , (42): 71–75.

香川正行・田渕千鶴子・北川博正・鈴木　聡 , 2020. 越前町立福井総合植物園プラントピアに生息する哺乳類 . Ciconia（福井県自然保護センター研究報告）, 23: 23–30.

神奈川県県西地域県政総合センター , 2021. 県西地域のニホンザルについて . 神奈川県 . Online. Available from internet: http://www.pref.kanagawa.jp/docs/m2g/cnt/f417344/p6096.html (last modified on 2021-06-29 by the author).

神奈川県公園協会・丹沢大山自然環境総合調査団企画委員会編 , 1997. 丹沢大山自然環境総合調査報告書 1997. 神奈川県環境部 , 横浜 ,.635 pp.

金子弥生 . 2018. ニホンアナグマ—群れ生活も行うイタチ科大型種 . 増田隆一編 , 日本の食肉類—生態系の頂点に立つ哺乳類 , pp. 175–199. 東京大学出版会 , 東京 .

金子弥生・塚田英晴・奥村忠誠・藤井　猛・佐々木　浩・村上隆広 , 2009. 食肉目のフィールドサイン，自動撮影技術と解析−分布調査を例にして . 哺乳類科学 , 49: 65–88.

川田伸一郎・岩佐真宏・福井　大・新宅勇太・天野雅男・下稲葉さやか・樽　創・姉崎智子・横畑泰志 , 2018. 世界哺乳類標準和名目録 . 哺乳類科学 , 58（別冊）: 1–53.

幸田良介・辻野智之・三輪由佳・上森真広 , 2016. 自動撮影カメラで確認された大阪府立環境農林水産総合研究所内の哺乳類相 . 大阪府立環境農林水産総合研究所研究報告 , 3: 9–11.

国立公園協会編 , 1964. 丹沢大山学術調査報告書 , 神奈川県 , 横浜 , 477 pp.

永田幸志 , 2019. 神奈川県におけるシカによる森林被害 . 日本緑化工学会誌 , 44: 475–478.

中村一恵 , 2003. 神奈川の哺乳類相 . 神奈川県立生命の星・地球博物館編 , かながわの自然図鑑 3 哺乳類 , pp. 112–123. 有隣堂 , 横浜 .

野崎英吉・古林賢恒・丸山直樹・常田邦彦・遠竹行俊 , 1979. 関東地方におけるツキノワグマの分布—アンケート･聞き取り調査による—. 哺乳動物学雑誌 , 8: 14–32.

O'Brien, T. G., M. F. Kinnaird & H. T. Wibisono, 2003. Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. Animal Conservation, 6: 131–139.

Ohdachi, S. D., Y. Ishibashi, M. A. Iwasa, T. Fukui & T. Saitoh, 2015. The wild mammals of Japan. 2nd. ed.xxvi+511 pp. Shoukado Book Sellers, Kyoto.

岡田昌也・黒田貴綱・勝野武彦 , 2007. 神奈川県の複数流域におけるイタチの分布と生息環境に関する研究 . 神奈川自然誌資料 , (28): 55–58.

坂田宏志・鮫島弘光・横山真弓 , 2008. 目撃効率からみたイノシシの生息状況と積雪，植生，ニホンジカ，狩猟，農業被害との関係 . 哺乳類科学 , 48: 245–253.

關　義和 , 2019. 箱根自然観察林に生息する野生動物—特にツキノワグマについて—. 玉川大学農学部研究教育紀要 , (3): 39–42.

關　義和・鈴木貴大 , 2018. 神奈川県箱根町南部におけるツキノワグマの生息確認 . 哺乳類科学 , 58: 247–252.

塩沢徳夫・坂本堅五・伊藤正宏 , 1984. 神奈川県における中型哺乳類 3 種（タヌキ・キツネ・ハクビシン）の生息状況について . 神奈川県立自然保護センター調査研究報告 , (1): 21–32.

Sollmann, R., A. Mohamed, H. Samejima & A. Wilting, 2013. Risky business or simple solution – Relative abundance indices from camera-trapping. Biological Conservation, 159: 405–412.

鈴木　聡 , 2018. ニホンイタチ—在来種と国内外来種 . 増田隆一編 , 日本の食肉類—生態系の頂点に立つ哺乳類 , pp. 135–153. 東京大学出版会 , 東京 .

鈴木　聡 , 2020. 早雲寺林の哺乳類 . 早雲寺ヒメハルゼミの会編 . ヒメハルゼミハンドブック—早雲寺林の自然—, pp. 11–12. 早雲寺ヒメハルゼミの会 , 箱根 .

田畑真悠・河内紀浩・村田浩一 , 2006. 神奈川県西部域における外来種アライグマの分布 —2004 年—. 神奈川自然誌資料 , (27): 21–26.

丹沢大山総合調査団 , 2007. 丹沢大山総合調査学術報告書 . 794 pp.（財）平岡環境科学研究所 , 相模原 .

山口尚子・山口喜盛 , 2005. 神奈川県西部におけるコキクガシラコウモリの分布状況 . 神奈川自然誌資料 , (26): 45–48.

山口喜盛 , 2015. 酒匂川水系曽我丘陵の哺乳類 . 酒匂川 , (50): 55–67.

摘　要

鈴木　聡 , 2022. 自動撮影カメラで確認された神奈川県小田原市入生田地区の哺乳類相 , (51): 81–88. [Suzuki, S., 2021. Mammal Fauna Survey Using Camera Traps in Iryuda, Odawara City, Kanagawa Prefecture. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 81–88.]

2020 年 4 月から 2021 年 5 月までの期間、神奈川県小田原市の入生田地区において自動撮影カメラを用いた哺乳類相調査を行った。10 定点に設置したカメラにより、中大型哺乳類 8 種および種同定の困難な齧歯目ネズミ科および翼手目の動物が合計で 2,500 回撮影された。撮影数と撮影頻度指数はともにイノシシ、ハクビシン、タヌキ、ニホンジカの順に大きい値を示した。7 定点以上で撮影された 7 種およびネズミ科を対象とした、定点ごとの撮影頻度指数を用いた種間の相関分析では、イノシシとニホンジカの間で特に高い相関を示し、ニホンイタチとネズミ科の間およびニホンアナグマのネズミ科の間でも相関が見られた。一方でニホンイタチとハクビシンの間などで弱い負の相関が見られた。撮影頻度指数の種間での相関は、生息環境の類似性や種間競争を反映している可能性がある。

Bull. Kanagawa Pref. Mus. (Nat. Sci.), no. 51, pp. 89-94, Mar. 2022

Original article

First Records from Japan of European Vermi-composter *Dendrobaena veneta* (Rosa, 1886) and of "Classical" *Lumbricus terrestris* Linnaeus, 1758 (Annelida, Oligochaeta, Megadrilacea, Lumbricidae)

Robert J. Blakemore[1], Takehiro Sato[1], Chelsea Vasnick[2], Shu Yong Lim[3]

Abstract. European earthworms *Dendrobaena veneta veneta* (Rosa, 1886) and *Lumbricus terrestris* Linnaeus, 1758 are new records for Japan found in vermi-compost and fishing bait supplies. Both were likely introduced via the USA some years earlier when much stock was shipped to meet Japanese composting needs.

Key words: Annelid, exotic alien invertebrates, megadrile earthworm, vermi-compost

Introduction

In 2019 a local Zama school teacher (C. Vasnick) found some school project compost worms that looked interesting and different to the usual *Eisenia fetida* (Savigny, 1836). Preliminary identification as *Dendrobaena veneta* (Rosa, 1886) was confirmed in 2020 from specimens obtained from bait worm growers (Fig. 1). Online bait catalogue images from at least 2018, and the primary author's collection records from 2001 (Blakemore, 2002) of worms "*sold throughout Japan as fishing bait worms from Kyushu (pers. obs. 11.IX.2001)*", now indicate this new exotic species has likely been introduced to Japan several years earlier. Possibly from China (e.g. www.japanfs.org/ja/news/archives/news_id024010.html) or more likely the USA as, from 1985-1987 alone, Japan reportedly imported 3,000 "mt" (= metric tonnes) of earthworms from the USA for cellulose waste composting (Kale, 1998). It is further likely that the similar and common vermicomposting species, *Dendrobaena hortensis* (Michaelsen, 1890), with which *D. veneta* is often confused, is also present in some Japanese cultures, but this has yet to be demonstrated. The current report provides formal identification and distribution records for *Dendrobaena veneta veneta* with unambiguous support sought from mtDNA barcode analysis.

The research continued in 2021 with *Lumbricus terrestris* Linnaeus, 1758 also found in "Ozeki" ("大関") bait worm boxes that are routinely sent around Japan (Fig. 1).

Materials and methods

Representative specimens (in 70 % EtOH), identified under low magnification using keys and descriptions in Blakemore (2000, 2002, 2020), are deposited in Kanagawa Prefectural Museum: *D. veneta*, is Accession No. KPM-NJL 78 (from compost in garden in Zama, collected November, 2020 by RJB) with small tissue samples sent to Monash University genomics laboratory for mtDNA barcode sequencing (detailed in the Supplementary file); *L. terrestris* specimens deposited in 2021 are KPM-NJL 79–80. Both species are briefly described and compared to similar taxa in the following account.

Results

The current specimen of concern (KPM-NJL 78; Fig. 2) matches with other *D. veneta* reports with a DNA analysis confirmation in the Supplementary file.

Lumbricus terrestris (Accession Nos. KPM-NJL 79–80)

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Fig. 1. Fishing baits as supplied throughout Japan contain a variety of worm specieas including *Eisenia fetida*, *Dendrobaena veneta* & *Lumbricus terrestris* (author's photo).

Dendrobaena veneta (Rosa, 1886). Zama, Japan. Nov. 2020. Id: R.J.Blakemore

Fig. 2. *Dendrobaena veneta* (Rosa, 1886) dorsal and zoomed ventral habitus of mature specimen from Zama, Nov. 2020; fixed in 70 % EtOH (KPM-NJL 78 that provided small tissue samples for DNA analysis) (author's photo).

was further identified (Fig. 3).

Discussion

First records from Japan of *Eisenia fetida* were by Michaelsen (1892: 320, 1900: 476). (The primary author's copy of Michaelsen's 1900 monograph is now donated to Kanagawa Prefectural Museum of Natural History library). Its occurrence in Japan extends to before 1876 and likely introduction along with medicinal plants to Koishikawa Gardens, Tokyo with specimens collected there by Dr Franz Hilgendorf.

Michaelsen (1900) described *Dendrobaena veneta* as widespread in Europe and the Middle East (cf. https://www.gbif.org/species/5739805), it is now known from

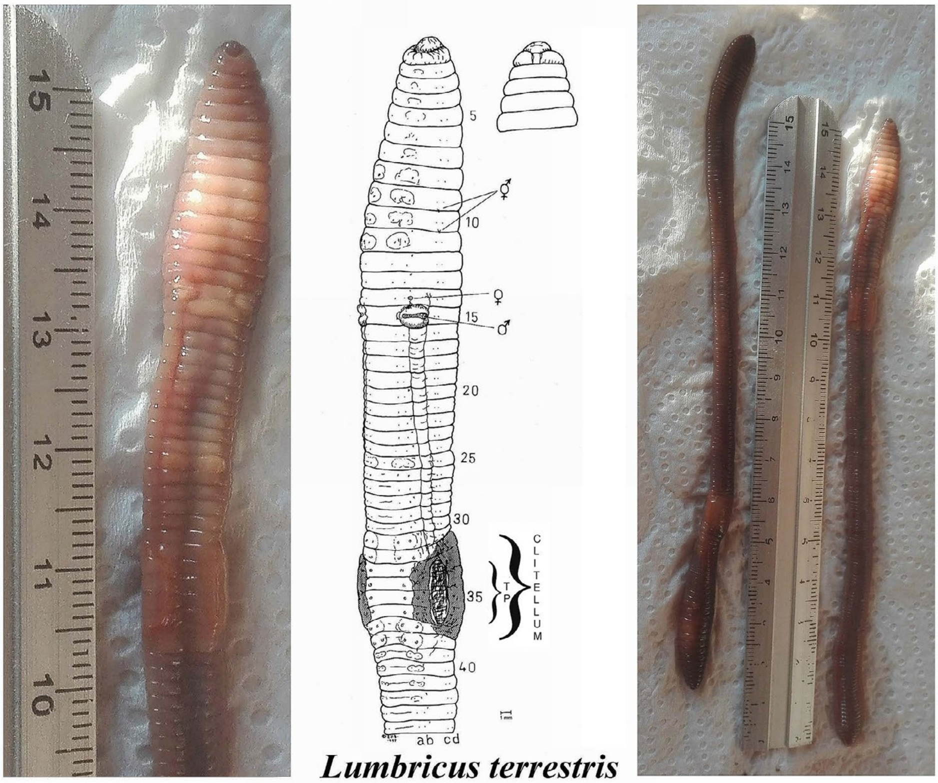


Fig. 3. *Lumbricus terrestris* Linnaeus, 1758 diagram (modified from Blakemore 1997, 2000, 2020, 2021) compared to 2021 KPM specimens (KPM-NJL 79–80).

introduction to the USA, South America (e.g. Argentina, Brazil, Chile), Iran (although its identification there may now be questioned – see Supplementary file), South Africa, China, South Australia (J. Buckerfield, pers. com. 1991) and New Zealand (Blakemore *et al.*, 2007) – the latter both new records for those countries. [In an update to this, an unpublished Australian Museum record online is of *D. veneta* from Lake Taupo, N.Z. from as early as 1935 – https://biocache.ala.org.au/occurrences/8cf893c7-2de7-43cd-9451-372348aba463]. Full eco-taxonomy, synonymy, distribution and DNA data are provided in Blakemore (2020 a copy of which is also donated to KPM library – accession No. KPM-XYZ 99) with a July 2021 update of a claim from Guatamala (https://invertebase.org/stri/collections/list.php?db=23&reset=1&country=Guatemala) and of its compost use in Ethiopia (https://academicjournals.org/journal/AJB/article-full-text-pdf/D3A468E58527).

Dendrobaena hortensis (Michaelsen, 1890) – also reported from South Australia (J. Buckerfield, pers. com. 1991) and Tasmania (Blakemore, 2000) both as new records – is similar in distribution and appearance to *D. veneta* and is differentiated by Briones *et al.* (2009: tab. 3). The taxonomy, morphology and genetic identification of these and related taxa are revised in Szederjesi *et al.* (2019) and Blakemore (2020). In summary:

Length mature > 50mm; pigment always banded …...
............................*Dendrobaena veneta* (Rosa, 1886)

Length < 50mm; pigment more uniform ....
...............................*D. hortensis* (Michaelsen, 1890)

Although the epithet "*veneta*" in Latin can mean "sea blue" or "blue-green", the name derives from its type locality of Campo di Marte, Venice (and Rosa noted its colour as being the same as *Eisenia fetida*, i.e., ruddy with yellow intersegmental stripes) hence these two species are easily and often confused. A simple distinction is that the setae are wider in *Dendrobaena veneta* (readily observable in the dark under blue light) also the clitellum tends to be whitish in *D. veneta* but more ruddy in *E. fetida* (Figs. 4–6).

Photo images are also available for comparison on Ben Crabb's website (https://bjc792.wixsite.com/earthworm-images). Note that *E. fetida* often has a yellow caudal tip (rarely reported for *D. veneta*) and exudes fetid-smelling yellow coelomic fluid when distressed or handled, unlike the more placid *D. veneta* (RJB pers. obs.).

Incidentally, confusion by non-specialist growers in a UK report (Katsiamides & Stürzenbaum, 2021) found only one of 25 specimens from five suppliers of "*Eisenia fetida*" was correct (conflation with *E. andrei* Bouché, 1972 or other sub-species notwithstanding), most were *D. veneta.* A similar situation may pertain in Japan as, despite having a lower reproductive rate, *D. veneta* is larger than *E. fetida* and may come to dominate in cultures under certain conditions (RJB pers. obs.).

As well as for fishing bait markets, beneficial human

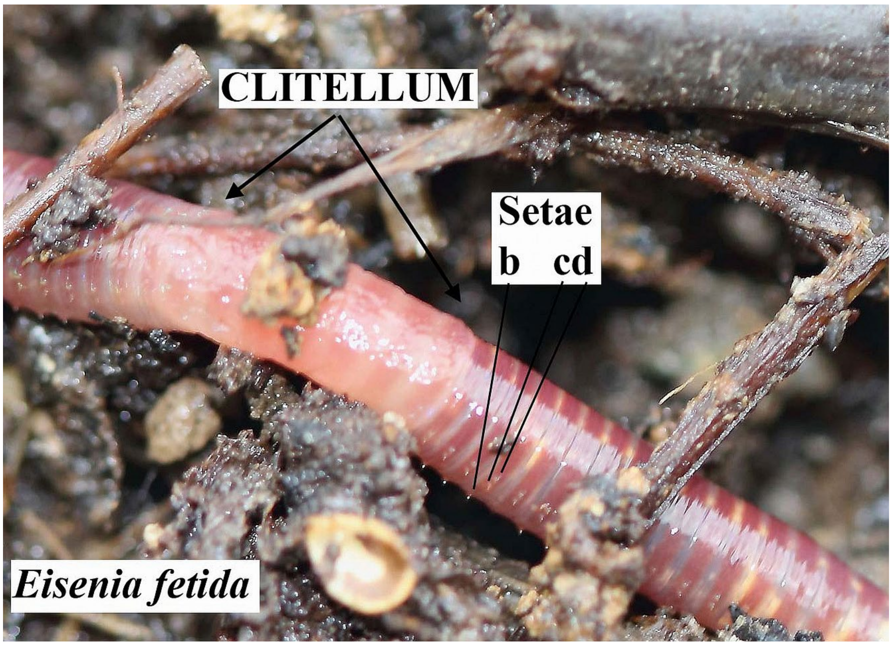


Fig. 4. Striped body and pale or pinkish matured clitellum are shared characteristics, but closely paired setae are distinctive for *Eisenia fetida* as indicated (after Wiki image "*close-up of red wiggler worm [sic] with visible bristles [sic]*" Eisenia_fetida_HC1.jpg).

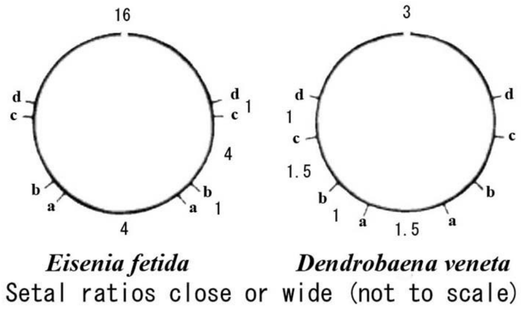
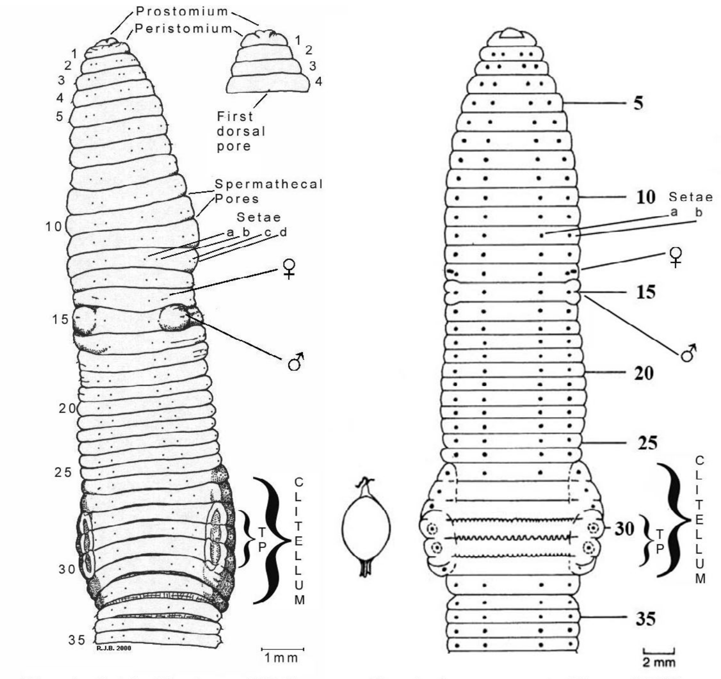


Fig. 5. Setal ratios provide simple differentiation.

uses of *D. veneta* are as an important species for vermicomposting of "wastes" used in organic farming for healthy, pesticide-free foods. A recent publication (Szederjesi *et al.*, 2019) lists its application for "*several cell-biological (Siekierska 2003; Adamowicz 2005), immunological (Molnár* et al. *2015; Swiderska* et al. *2017), soil biological and environmental studies (Marinussen* et al. *1997; Natal-da-Luz* et al. *2011).*" In addition, another 2019 study (Fiolka *et al.*, 2019) found anti-tumor effect from *D. veneta* coelomic fluid on human lung cancer cells as "*an interesting and promising preparation for further biological, chemical, and biomedical research.*" [Cf. medical uses of *Eudrilus eugeniae* (Kinberg, 1867) as summarized in Blakemore (2015)].

The likely origin of the Japanese specimens was via the USA (as noted in Introduction) and the US Government Aphis website (https://www.aphis.usda.gov/) says:- "*Eisenia* (=*Dendrobaena*) *veneta*" and *D. hortensis* are currently the only earthworm species that are permitted for importation into the U.S. under strict conditions from countries other than Canada for release into the environment. Japan has also imported compost worms from Asia or India as other possible sources (e.g. for *E. fetida*, *D. hortensis* or other species?), although the Japanese Ministry of Agriculture Forestry & Fisheries (MAFF – https://www.maff.go.jp/e/) at this time does not restrict *Eisesnia* spp., *Dendrobaena* spp. nor *Lumbricus* spp, import and redistribution into and within Japan. [Email enquiries to MAFF by the primary author in 2021 on any restrictions, on these or other worm species, are unanswered].

Initial preliminary report of *Lumbricus terrestris* from Japan by Blakemore (2021 – https://vermecology.wordpress.com/2021/12/31/lterrestris-in-japan/) is now supported with specimens deposited in KPM. Its full taxonomic description based upon the restored British Museum Neotype plus many other specimens, its ecology and global distribution (with an earlier new report from Tasmania and thus for Australia) are provided in Blakemore (1997, 2000, 2013, 2020). Confirmation of this "classical" species as a new exotic record from Japan is thus also a new record for East Asia (see CABI map in Blakemore, 2021 as also in Supplementary file appended below).



Eisenia fetida (Savigny, 1826) *Dendrobaena veneta* (Rosa, 1886)

Fig. 6. *Eisenia fetida* (author's camera lucida image) compared to *D. veneta* [after Sims & Gerard (1999: fig. 28) of "*Eisenia veneta*" with its cocoon]. TP = tubercula pubertatis.

This report brings the number of known exotics to more than 42 taxa in Japan (and Okinawa/Ryukyus) which, plus ~80 natives, gives a total tally of approximately 122 earthworm species (Easton, 1981; Blakemore, 2003, 2019, 2021 cf. Minamiya, 2021).

Acknowledgements

Thanks are extended to Martha Hellander of Illinois and Olga Kuchukov from NY/Vermont for motivational discussion of the introduction of Japanese earthworms to the USA and vice versa. A fisherman friend, Mr Shingo Okuyama from Ebina, alerted me to the “Ozeki” mimizu bait specimens. Mr Kohei Shibata from Kamakura provided confirmation of the lack of prior records of *D. veneta* in Japan (http://ss490886.stars.ne.jp/g/mimizu/mimizu.htm). Staff and managers at Monash University Malaysia ably conducted DNA sequencing. This study was unfunded (or, rather, self-funded by the primary author) and has no known conflicts of interest.

References

Blakemore, R. J., 1997. First ‘common earthworm’ found in Tasmania. Invertebrata, 9: 1–5.

Blakemore, R. J., 2000. Tasmanian earthworms. CD-ROM Monograph with review of world families. VermEcology, Kippax ACT 2615. Australia, 800 pp.

Blakemore, R. J., 2002. Cosmopolitan earthworms – an eco-taxonomic guide to the peregrine species of the world, VermEcology, Kippax, ACT, Australia, 506 pp.

Blakemore, R. J., 2003. Japanese earthworms (Annelida : Oligochaeta): a review and checklist of species. Organisms, Diversity and Evolution, 3(3): 1-43; 241–244.

Blakemore, R. J., 2013. Restoration of London type of first earthworm – *Lumbricus terrestris* Linnaeus, 1758 (Annelida: Oligochaeta: Lumbricidae), and setting aside of a ‘neo-neotype’. Opuscula Zoologica, 44(2): 211–212.

Blakemore, R. J., 2015. Eco-taxonomic profile of iconic vermicomposter – the ‘African Nightcrawler’ earthworm, *Eudrilus eugeniae* (Kinberg, 1867). African Invertebrates, 56(3): 527–548.

Blakemore, R. J., 2020. Cosmopolitan earthworms. (6th Amended Edition). VermEcology, Yokohama, Japan, 1,250 pp. + 150 figs.

Blakemore, R. J., 2021. *Lumbricus terrestris* L. 1758 newly found in Japan. Online. Available from internet: https://vermecology.wordpress.com/2021/12/31/lterrestris-in-japan/.

Blakemore, R. J. & N. Kaneko, 2007. Alien earthworms in the Asia/Pacific region with a checklist of species and the first records of *Eukerria saltensis* (Oligochaeta: Ocnerodrilidae) and E*iseniella tetraedra* (Lumbricidae) from Japan, and *Pontoscolex corethrurus* (Glossoscolecidae) from Okinawa. In: Koike, F., M. N. Clout, M. Kawamichi, M. De Poorter & K. Iwatsuki (eds.), Assessment and control of biological invasion risks, pp. 173–181. IUCN, Gland, Switzerland; Cambridge, UK; and Shoukadoh, Kyoto.

Briones, M. J. I., P. Moran & D. Posada, 2009. Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy? Soil Biology & Biochemistry, 41: 2257–2271.

Easton, E. G., 1981. Japanese earthworms: a synopsis of the megadrile species (Oligochaeta). Bulletin of the British Museum (Natural History, Zoology), 40(2): 33–65.

Fiołka, M. J., J. Rzymowska, S. Bilska, K. Lewtak, M. Dmoszyńska-Graniczka, K. Grzywnowicz, W. Kaźmierski & T. Urbanik-Sypniewska, 2019. Antitumor activity and apoptotic action of coelomic fluid from the earthworm *Dendrobaena veneta* against A549 human lung cancer cells. APMIS, 127(6): 435–448.

Kale, R.D., 1998. Earthworms: Nature's gift for utilization of organic wastes. In: Edwards, C. (ed.), Earthworm ecology, pp. 355–376. St Lucie Press, New York.

Katsiamides, A., Stürzenbaum, S. R, 2021. Cryptic speciation and blurred species boundaries of the earthworm: a challenge for soil-based toxicological risk assessments. Comparative Biochemistry and Physiology - Part C, 239: 108880.

Linnæus, C., 1758. Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Holmiæ. (Salvius), pp. [1–4], 1–824.

Michaelsen, W., 1892. Terricolen der Berliner Zoologischen Sammlung. II. Archiv für Naturgeschichte, 58: 209–261.

Michaelsen, W., 1900. 10. Lieferung. Oligochaeta. In: Spengel, J.W. (ed.), Vermes. In: Schulze, F.E. (ed.), Das Tierreich. Verlag von R. Friedländer und Sohn, Berlin, pp. [I]-XXIX, 1–575.

Minamiya, Y., 2021. Guidebook to Japanese earthworms. Online. Available from internet: https://japanese-mimizu.jimdo.com/; web.archive.org/web/20160404065026/http://japanese-mimizu.jimdo.com:80/.

Rosa, D., 1886. Note sui lombrici del Veneto. Atti Reale Ist Veneto Science, 4: 673–687.

Rosa, D., 1886. Note sui Lombrici del Veneto (Riassunto). Bollettino dei musei di zoologia ed anatomia comparata della Torino, 1(3): 1.

Sims, R. W. & B. M Gerard. 1999. Earthworms: notes for the identification of British species. 4th ed. Field Studies Council (31), Montford Bridge, Shrewsbury, Shropshire, UK, 169 pp.

Szederjesi T., T. Pavlíček, O. Márton,V. Krízsik & C. Csuzdi, 2019. Integrative taxonomic revision of *Dendrobaena veneta* (Rosa, 1886) sensu lato with description of a new species and resurrection of *Dendrobaena succinta* (Rosa, 1905) (Megadrili: Lumbricidae). Journal of Natural History, 53: 5–6 + 301–314.

摘　要

Blakemore, R. J., 佐藤武宏 , C. Vasnick & S. Y. Lim, 2022.　ヨーロッパ産ミミズの一種 *Dendrobaena veneta* (Rosa, 1886) と *Lumbricus terrestris* Linnaeus, 1758（環形動物門，貧毛綱，Megadrilacea, ツリミミズ科）の日本からの初記録について . 神奈川県立博物館研究報告（自然科学）, (51): 89–94. [Blakemore, R. J., T. Sato, C. Vasnick & S. Y. Lim, 2022. First Records from Japan of European Vermi-composter Dendrobaena veneta (Rosa, 1886) and of "Classical" *Lumbricus terrestris* Linnaeus, 1758 (Annelida, Oligochaeta, Megadrilacea, Lumbricidae). *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 89–94.]

日本初記録となるヨーロッパ原産のミミズ *Dendrobaena veneta* (Rosa, 1886) と *Lumbricus terrestris* Linnaeus, 1758 が確認された。本種はミミズコンポスト構成種や釣餌として利用されており、日本のコンポストの需要増加を満たすために、数年前より米国を経由して導入されたと考えられる。

Supplementary Materials

Supplementary file.

Appendix with Figs. and Reference online links – https://archive.org/details/appendix_202202.

Bull. Kanagawa Pref. Mus. (Nat. Sci.), no. 51, pp. 95-104, Mar. 2022

Original article

Two New Species of Japanese Earthworms (Annelida, Oligochaeta, Megadrilacea, Megascolecidae) Update Biodiversity on Okinawa and at Lake Biwa to ca. 30 Species

Robert J. Blakemore1), Shawn Miller2), Shu Yong Lim3)

Abstract. Two large native worms are an *Amynthas* from Kumejima, Okinawa and a *Metaphire* from Lake Biwa, Shiga-ken both supported by mtDNA COI barcodes, and two new species are described. New 2016 Okinawa exotic reports are of small American lumbricid *Bimastos parvus* (Eisen, 1874) and confirming of Asian megascolecid *Pithemera bicincta* (Perrier, 1875).

Key words: exotics, megadrile earthworm, mtDNA barcoding, natives, new species

Introduction

In 2015 an interesting image of a luminescent earthworm from Kumejima Island, Okinawa was uploaded to a natural history photo website (by Nature photographer Shawn Miller – Fig. 1). The next year, in 2016, an opportunity arose for the survey of earthworms on Kumejima to try to find and identify this possibly novel species.

Kumejima "久米島" is a volcanic, sub-tropical island shaped like the silhouette head of an English cocker spaniel (Fig. 2). No earthworms were previously reported from this island whereas other Okinawan Islands have a limited history of reports since Kobayashi (1941) described ten species and Ohfuchi (1956, 1957a,b) reported ~24 with current totals of ~30 Ryukyu megadrile taxa (Easton, 1981; Blakemore, 2003, 2020a, b).

Furthermore, another unknown earthworm from Kinki Region, Honshu, Japan was found. Both the earthworms from Kumejima and from the Kinki Region are described as new species herein.

Materials and methods

Representative Kumejima specimens collected in February, 2016 were deposited in Lake Biwa Museum (LBM, Accession Nos. FY2015-11) with small tissue samples taken for mtDNA barcoding at molecular genetics laboratories using standard routines for DNA extraction, PCR amplification and COI gene Sanger sequencing (e.g., Blakemore *et al.* 2010). Other earthworms donated to LBM's collection were simultaneously inspected with a summary of the current Lake Biwa worm biodiversity provided in an Appendix.

Results

Specimens deposited in Lake Biwa Museum (LBM) and Kanagawa Prefectural Museum (KPM) are described below.

Amynthas kume Blakemore, sp. nov.

Distribution: Mt Aratake, Kumejima Island, Okinawa, Japan (26°18'38.3"N 126°47'15.0"E); also reported from Mt Daruma by S. Miller (RJB pers. comm.).

Etymology: The species name is derived from its island location (noun in apposition).

Material examined (see Figs. 3–4): Holotype (H), LBM FY2015-11-1 mature, dissected (DNA tissue

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Fig. 1. Mystery luminescent Kumejima Island earthworm (photo: Shawn Miller).

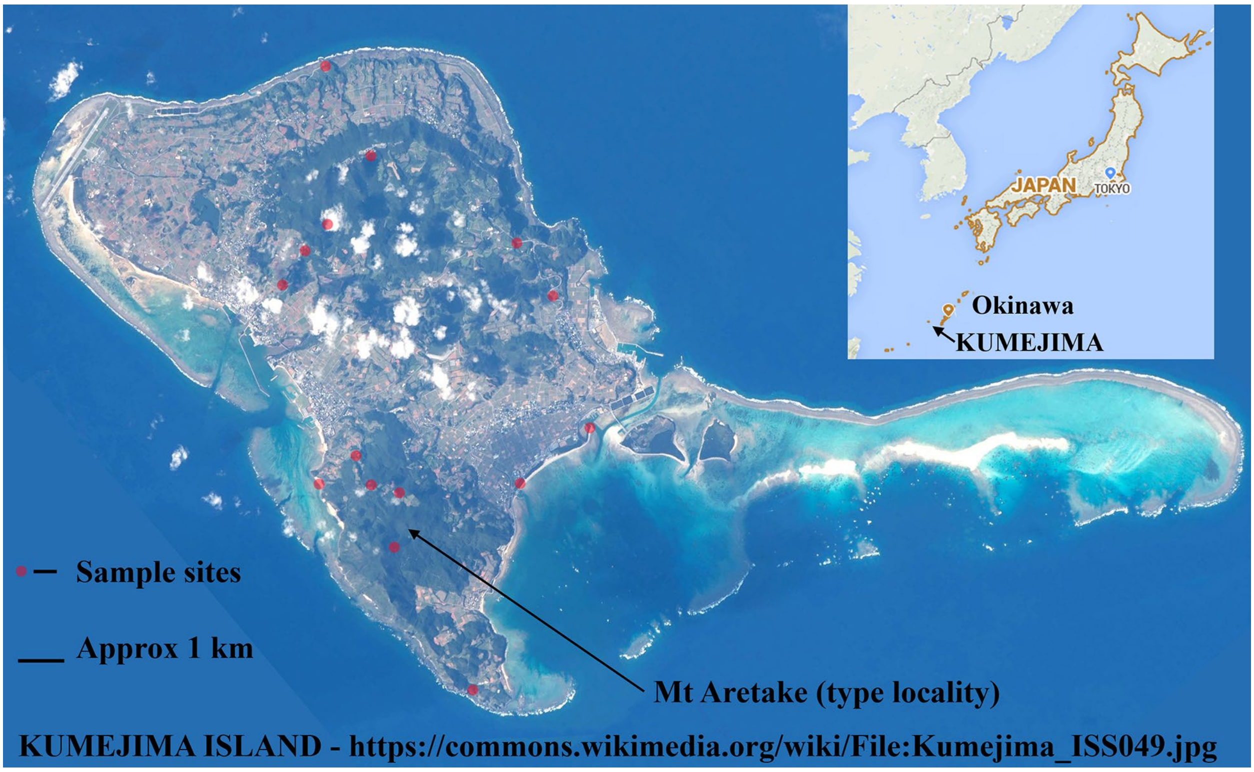

Fig. 2. Satellite image of Kumejima with collection sites (Wiki Commons: ISS049.jpg).

Fig. 3. *Amynthas kume* sp. nov. from Kumejima Island (photo courtesy of Shawn Miller).

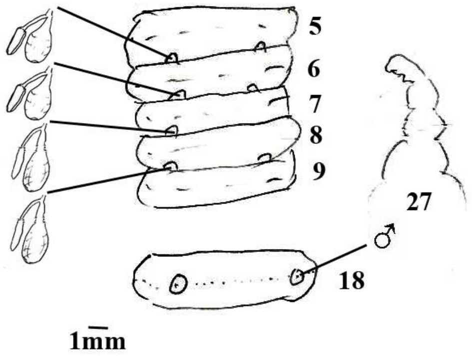

Fig. 4. *Amynthas kume* sp. nov. Holotype sketch with spermathecae and caecum.

samples #K3-5.1, #K5.2 re-sent to DNA lab via KPMNH); Paratype 1 (P1) FY2015-11-2 (DNA #K1); Paratypes 2–3 (P2–4) FY20015-11-3–4; all collected 15th Feb., 2016 (by RJB) from under forest litter or logs beside mountain road.

Description: Large and brown with yellow/buff intersegmental bands (i.e., striped). Lengths, H 190+20 mm (posterior amputee = 210 mm); P1, 220 mm; P2, 205 mm; P3, 190 mm. Prostomium epi-lobous. First dorsal pore 11/12. Setae about 60 on segment 12. Spermathecal pores in line with male pores four pairs in 5/6/7/8/9 in semi-circular pits. Clitellum 14–16. Female pore on 14. Male pores superficial in small circular patches on 18 with about 14 (H) or 12 (P1) setae intervening between pores. No genital markings (GMs) found. Septa 8/9/10 absent around muscular gizzard in 8. Spermathecae four pairs in 6–9 each with single diverticulum about as long as ampulla. Seminal vesicles in 11 and 12. Ovaries and last hearts in 13. Small ovisacs in 14. Intestine from 15. Racemose prostates in 18. Intestinal caeca smooth and simple in 27–24. No glands found near spermathecal pores.

Diagnosis: Other megascolecid species currently reported from Okinawan Islands with four pairs of spermathecae in segments 5/6/7/8/9 are exotic *Amythas corticis* (Kinberg, 1867) species-complex and *A. micronarius* (Goto & Hatai, 1898) parthenogenetic species-complex with *A. obtusus* (Ohfuchi, 1957) currently in its synonymy (see Blakemore, 2012a, b, 2020). All these taxa have genital markings (GMs). A recent taxon on Okinawa Island is *A. cucurbita* Azama & Ishizuka, 2018 (emend.) that, however, is smaller at <75 mm and also has markings around its male pores. Quadrithecate *Metaphire riukiuensis* (Ohfuchi, 1957) has seminal grooves in 17–19 amongst other differences while the *M. formosae* (Michaelsen, 1922) group is mainly from Taiwan and adjacent islands. The present specimens thus appear unique to Kumejima representing a new species. It is unusual for an *Amynthas* to lack GMs, a trait more common to *Metaphire* that typically has fewer spermathecae and eversible penes to compensate.

Other earthworms found on the island in this study were the exotic lumbricid of American origin: *Bimastos parvus* (Eisen, 1874) (LBM FY2015-11-5) from Gushikawa Castle, Kumejima collected 16th Feb., 2016 from under rocks as a new record for Okinawa (published in Blakemore, 2016a), and Asiatic exotic *Pithemera bicincta* (Perrier, 1875) (LBM FY2015-11–6 providing tissue samples #K2 & K6 – KPM-NJL 82) that had been previously reported from Okinawa, not least by Ohfuchi (1957a: 254), Easton (1981) and Blakemore (2002, 2003, 2020a,b; Blakemore *et al.*, 2007). Despite Ito *et al.*'s (2019) claim, Ohfuchi's description precisely matches *P. bicincta* and he clearly states: "*Intestine begins in XIV, and a pair of intestinal caeca which are very short, are less developed compared with many other species; broad, apparently rudimentary, or may be altogether absent.*." but he has then inadvertently added a transcription error with: "*..commences (sic) in XXVI or XXVII.*." (in actuality its small caeca are often paired in 22). As for *B. parvus*, it is relatively common on the main islands of Japan and Kobayashi (1941) reports it from " 海士村 " that is translated as Ama mura, possibly on Nakanoshima in Oki Islands, Shimane-ken, but it has not been previously found on Okinawan Islands.

Metaphire kinki Blakemore, sp. nov.

Distribution: Kinki Region, Shiga-ken, Otsu-shi, Katsuragawa, Boumura-cho (35.247719, 135.868247) and stream at Sakamoto just below the Mt. Hiei cable-car station (35.069342, 135.865797); Kyoto-fu, around Mt Kumotori/Kibune River (35°10'25.7"N 135°46'05.2"E) (Wes Lang pers. comm.: found "coiled up in the middle of the hiking path" from the bus stop at Hanase Kogen). Searches by RJB assisted by Dr Grygier in rivers and on hills at Sakamoto in Feb., 2016 failed to find further specimens.

Etymology: The name is derived from its regional location (noun in apposition).

Material examined (see Figs. 5–7): Holotype (H), LBM FY2015-8-1 mature, dissected (DNA tissue samples #K7–9, sample #K8 now KPM-NJL 83) collected by Kazuhiro Masunaga "*2015/8/1*"; "*On road where water runs across. Captured alive but died overnight. 80–85 % EtOH*"; Paratype 1 (P1) FY2015-8-2 large, macerated specimen (no DNA) ditto "*2015-8-2*"; Paratype 2 (P2) FY2010-24 "*Sakamoto coll. Masunaga*" March, 2011; Paratype 3 (P3) FY2011-19 ditto, "25.III.2012".

Description: Large and dark blue with light grey intersegmental bands (i.e., striped). Lengths, H 260 mm

Fig. 5. *Metaphire kinki* sp. nov. P2 specimen (photo courtesy Kazuhiro Masunaga March, 2011 with LBM permission via M.J. Grygier pers. comm. 22nd July, 2015).

Fig. 6. "Kumotori/Kibune" worm, likely *M. kinki* in Nature (image permissions courtesy Wes Lang https://tozantales.wordpress.com). Compare with uniformly-coloured, bright electric-blue *M. sieboldi* from Fukuoka and Kumamoto, Kyushu www.flickr.com/photos/pokoroto/2950708988; www.flickr.com/photos/anaguma/7540289876.

x 15 mm wide; P2, 210 mm; P3, 195 mm. Segments (H) ~140. Prostomium epi-lobous, open. First dorsal pore 12/13. Setae about 80 on segment 12. Spermathecal pores in line with male pores three pairs in 6/7/8/9. Clitellum 14-16. Female pore on 14. Male pores slits on 18 with about 16 (H) setae intervening. No genital markings (GMs) found. Septa 8/9/10 absent around muscular gizzard in 8. Spermathecae three pairs in 7-9 with single, coiled diverticulum longer than ampulla when unravelled. Seminal vesicles in 11 and 12. Ovaries and last hearts in 13. Racemose prostates in 18. Intestinal caeca from 27, deeply incised on one side at the base (not strictly manicate, more like intermediate or multiple, as found in *M. megascolidioides* – see Blakemore (2016a: Fig. 3 and Supplementary Materials, Appendix 1: Fig. 12).

Diagnosis: The only known native *Metaphire* species currently from Shiga-ken with three pairs of spermathecae in segments 6/7/8/9 is reported as "*M. sieboldi*" *sensu lato* by Minamiya *et al.* (2009). Major differences from the original and most subsequent species diagnoses of proper *M. sieboldi* (Horst, 1883) – the first species described from Japan – are lack of bright and uniform blue iridescent colouration and less defined manicate caecae; also the spermathecal diverticula may be longer than the ampullae even when they are coiled. The most definitive difference is the mtDNA COI barcode (in Appendix 1) that differs by 4.13 % from its closes match of AB482078 "*Metaphire sieboldi*" from Nara-ken that was part of Minamiya *et al.*'s (2009) "Group I" mostly confined to the Kinki region and also most diverged of their study samples. Only their AB482080, also Group I, was from Shiga-ken and it differs by 4.57 %. These authors' found: "*Among the* M. sieboldi *samples, the infraspecific sequence divergences of the COI gene ranged from 0.1 % to 18.1 %, and the mean infraspecific sequence divergences between phylogenetic groups of our study ranged 5.7 % to 15.9 %* (Table 2)."

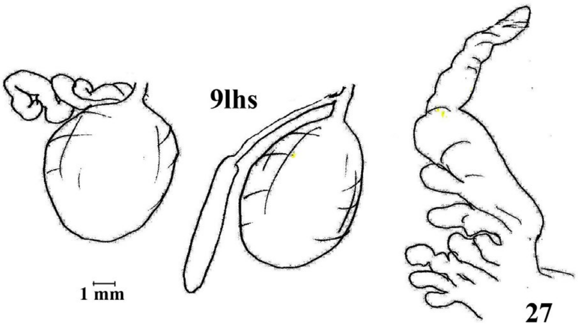

Fig. 7. *Metaphire kinki* sp. nov. holotype spermatheca 9lhs and caecum.

However, an accepted COI barcode range for inter-specific differentiation is as low as 2–3 % (e.g. Hebert *et al.* 2003, 2004; Smith *et al.* 2005), also by Zhang & Zhang (2014) and by Wang *et al.* (2018) who found: "*almost 98 % of the species can be correctly distinguished for both COI and 16S when a threshold of 3 % nucleotide divergence was used for species discrimination*". As the current specimen divergence is >4 % from other known samples it would qualify, on the information currently available, for specific status. Its furthest BLASTn result differs from other sampled concepts of "*M. sieboldi*" by up to 14 % (see Appendix 1).

Other examples in the Appendix support an inter-specific COI gene division at >3–4 %.

Metaphire sieboldi proper is defined by its Leiden type (RNHL 1825) and any substantial derivation or deviation from this (e.g. DNA >3–4 %) warrants separate specific, or at least sub-specific status, seemingly as do most of Minamiya *et al.*'s (2009) group taxa.

Three LBM collection worms from Hokkaido dated "2014 09 11" sent by Y. Minamiya were identified (by RJB in 2016) as common exotic *Aporrectodea rosea* (Savigny, 1826).

About 35 species occur on the Ryukyu (Okinawan) Islands and all 30+ earthworm species reports from Lake Biwako's satoyama habitats are summarized in Appendix 1, several supported by mtDNA. Along with ~50 aquatic worm taxa, Biwako is Japan's most megadrile & microdrile biodiverse site due to intensity of eco-taxonomic survey.

Discussion

Application of DNA to nomenclatural typification helps reduce taxonomic impediments. The first genetic analysis of worms (megadriles and microdriles) was by Siddall *et al.* (2001) and explicit linking of a genetic barcode to a worm's type was by Blakemore *et al.* (2010). Henceforth, molecular confirmations are advised for any eco-taxonomic surveys, albeit determining specific cut-offs between intraspecific and interspecific series, within *Amynthas* and *Metaphire*, as with other genera, is often difficult when a morphological continuum occurs. Yet DNA data are reliable only if initial identifications are good. Appendix 1 has several examples of misidentifications/misnomers relating to DNA data.

A suggestion for Genbank's DNA database is thus for a colour confidence system (or a kind of taxonomic triage) that, as with taxonomy, requires regular review, e.g.:-

RED – definitive, based upon taxon's primary types.

ORANGE – reliable, based upon taxon's secondary types.

GREEN – verified, on authoritative taxonomic identification.

BLACK – speculative or non-specialist identification of specimen.

[GREY or in braces – obvious misidentifications or highly unlikely taxon].

An unfortunate autocorrect feature of GenBank results in uploaded species names being changed according to some "official" list, not infrequently wrong, and also a modification of some uploaded sequences to comply with their models. Both these kinds of "corrections" detract from the validity of the unadulterated original data.

Returning to a supposed lack of molecular monophyly of both *Amynthas* and *Metaphire* (e.g., Zhang *et al.*, 2016), this is irrelevant to ICZN (1999) nomenclatural taxonomy where the genus is defined by characteristics of its type-species, as was clearly and simply explained by Blakemore (2002, 2008a, 2012c, 2020b). Thus male pores (when present!) are superficial in species defaulting to *Amynthas* and non-superficial in *Metaphire* species as, indeed, are found in its type *M. javanica* (Kinberg, 1867) [and its possible synonym, *M. californica* (Kinberg, 1867)]. Whereas the male pores in proper *Pheretima* type (*P. montana* Kinberg, 1867) are in deep invaginations, these are most developed as doubled and eversible penes in *Duplodicodrilus* [type *D. schmardae* (Horst, 1883) itself often misidentified as *Metaphire javanica* or *M. californica* in overseas reports (RJB pers. obs.)]. There is no implication that form of male pores is the same in these latter genera, merely that they are all "non-superficial".

Consequently, the recently described *Metaphire glauca* (Azama & Ishizuka, 2018) n. comb. (as mooted in Blakemore 2020a) from northern Okinawa complies with a *Metaphire houlleti* (Perrier, 1872) species-complex as in Blakemore (2016b, 2020b) that has >50 member taxa including many synonyms or parthenogenetic morphs that now need comparison with it. Several other taxonomic names proposed by these authors are possibly invalidated by non-compliance with ICZN (1999: Arts. 11.4 and/ or 11.5) by synonymy and homonymy (as noted by Blakemore, 2003, 2010, 2020a) (see Appendix 2).

Aside from classic ICZN nomenclatural taxonomy based upon morphology, now molecular DNA information plus behaviour and ecology are obviously needed to fully define a species. The new species *M. kinki* seemingly occurs often in streams or wet situations that may indicate an habitual characteristic separate from its sibling taxa.

Blakemore (2002, 2020b) speculated that bright blue colouration (only manifest in adults) of *Metaphire sieboldi*

Fig. 8. Large unknown worm from North Vietnam on the Phan Si Pan Mountain in Lao Cai district with striking blue-striped colouration (photo taken in 2016 courtesy of Dr Richard Barnes of Logan Botanic Gardens, Scotland, UK; pers. comm. April, 2018).

[as with *Didymogaster sylvatica* Fletcher, 1886 in NSW and *Diporochaeta terraereginae* (Fletcher, 1890) in tropical forests in Qld., Australia], may serve to distract predators, especially birds. Similarly for banding as in the two new taxa herein seen also in *A. yambaruensis* (Ishizuka & Azama, 2000) in Okinawa; *Fletcherodrilus fasciatus* (Fletcher, 1890) in Australia and *Eophila tellinii* (Rosa, 1888) or *E. crodabepis* Paoletti 2016 in Italy. Both characteristics are manifest in an unidentified megadrile found wandering on the soil surface in North Vietnam (Fig. 8).

During the Kumejima survey an abundance of several other native and exotic earthworms (plus enchytraids and planarians) were found in the woodlands and public areas, most not collected, but, remarkably, no earthworms were detected in the adjacent sugar cane fields. Possibly the earthworm fauna has been eradicated by intensive agrichemical use, a situation recently reported as a global issue of concern (Blakemore, 2018). This latter publication determined that organic sugar production offered substantial benefits in terms of yield, soil health (e.g. earthworm counts) and soil carbon sequestration compared to detriments of conventional agrichemical agriculture.

Other Kinki/Shiga species in LBM collection were described in Blakemore & Kupriyanova (2010), Blakemore (2010, 2016a) and Blakemore & Grygier (2011) that, with the current new species, now bring known Lake Biwa region taxa to about 30 megadriles, the most biodiverse habitat currently in Japan (see summary and DNA data in Appendix 1). This earthworm biodiversity compares to ~21 megadrile taxa from Lake Pedder in Tasmania (Blakemore, 2010: tab. 2) and other examples from Blakemore (2018) such as: "*a mixed farm in Queensland, Australia had 24 earthworm species whilst an organic farm studied in the Philippines had 23 [17,47,48]. In one instance, 13 species were unearthed from gardens at Y Plas House at Machynlleth, Wales in half-a-day's collection in 2013 [49] matching the earthworm diversity reported at another UK site sampled for an entire 10-year period (1990–2000) [50].*" This Y Plas site report (Blakemore, 2013) was the most biodiversity in UK or Ireland until surveys found 19 lumbricid earthworm species at Isabella Tree's and Charlie Burrell's Knepp Castle rewilding farm in England (Cole, 2013). Such high counts from proper eco-taxonomic surveys contradict hackneyed claims that earthworms have but few "common" species per site.

The present report brings the number of known natives to about 82 plus more than 42 exotics in Japan (including new records of *Dendrobaena veneta* and *Lumbricus terrestris* as described in this Bulletin issue) to give a total tally of ~124 earthworm species (Easton, 1981; Blakemore, 2003, 2012d, 2019a, 2020a cf. Minamiya, 2021). However, full taxonomic review is yet required as classical Japanese earthworm types are unearthed after their removal and hiding, as discovered and reported by Blakemore & Ueshima (2011) and Blakemore (2012b, 2016a: Figs. 1–2) (see Appendix 2).

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References

Blakemore, R. J., 1994. Earthworms of south east Queensland and their agronomic potential in Brigalow Soils. PhD Thesis, University of Queensland, Australia. Online. Available from internet: https://espace.library.uq.edu.au/view/UQ:366290/THE8652.pdf.

Blakemore, R. J., 2002. Cosmopolitan earthworms – an eco-taxonomic guide to the peregrine species of the world, VermEcology, Kippax, ACT, Australia, pp. 506.

Blakemore, R. J., 2003. Japanese earthworms (Annelida : Oligochaeta): a review and checklist of species. Organisms, Diversity and Evolution, 3(3): 1–43; 241–244.

Blakemore, R. J., 2008a. Cosmopolitan earthworms – an eco-taxonomic guide to the species (3rd Edition). VermEcology, Yokohama, Japan, pp. 757.

Blakemore, R. J., 2008b. Review of Criodrilidae (Annelida: Oligochaeta) including *Biwadrilus* from Japan. Opuscula Zoologica Budapest, 37: 11–22.

Blakemore, R. J., 2010. Unravelling some Kinki earthworms (Annelida: Oligochaeta: Megadrili: Megascolecidae) - Part II. Opuscula Zoologica Budapest, 41: 191–206.

Blakemore, R. J., 2012a. New earthworms from NIBR Jeju-do biosphere compared to historical and new Japanese types (Oligochaeta: Megadrilacea: Megascolecidae). Journal of Species Research, 1(2): 133–115.

Blakemore, R. J., 2012b. On opening a box of worms (Oligochaeta, Megascolecidae). Bulletin of the National Science Museum, Tokyo, Series A, 38(3): 95–124.

Blakemore, R. J., 2012c. Restating scope of genus *Metaphire* Sims & Easton, 1972: 40 years on (Oligochaeta: Megadrilacea: Megascolecidae). Zoology in the Middle East, 58(4): 5–14.

Blakemore, R. J., 2012d. Japanese earthworms revisited a decade on (Oligochaeta: Megadrilacea). Zoology in the Middle East, 58(4): 15–22.

Blakemore, R. J., 2012e. *Amynthas carnosu*s (Goto & Hatai, 1899) redescribed on its neotype (Oligochaeta : Megadrilacea: Megascolecidae). Journal of Species Research, 1(1): 35-43.

Blakemore R. J, 2013. *Megascolex* (Perichaeta) diffringens Baird, 1869 and P*heretima pingi* Stephenson, 1925 types compared to the *Amynthas corticis* (Kinberg, 1867) and *A. carnosus* (Goto and Hatai, 1899) species-groups (Oligochaeta: Megadrilacea: Megascolecidae). Journal of Species Research, 2: 99–126.

Blakemore, R. J., 2016a. Eco-taxonomic profile and mtDNA barcode of *Metaphire megascolidioides* (Goto & Hatai, 1899) – a megadrile earthworm from Japan, plus taxonomic miscellany. VermEcology Occasional Papers (Veop), 1(1): 1–16.

Blakemore, R. J., 2016b. Darwin's earthworms (Annelida, Oligochaeta, Megadrilacea) with review of cosmopolitan *Metaphire peguana*–species group from Philippines. Opuscula Zoologica, 47(1): 09–30.

Blakemore, R. J., 2018. Critical decline of earthworms from organic origins under intensive, humic SOM-depleting agriculture. Soil Systems, 2(2): 33.

Blakemore, R. J., 2019a. Extinction of Japan's first formally described earthworm *Amynthas japonicus* (Horst, 1883). Bulletin of Kanagawa Prefectural Museum (Natural Sciences), 48: 55–60.

Blakemore, R. J., 2019b. Redescription of extinct New Zealand earthworm: *Tokea orthostichon* (Schmarda, 1861). Bulletin of Kanagawa Prefectural Museum (Natural Sciences), 48: 61–68.

Blakemore, R. J., 2020a. Definitive checklist & IUCN Redlist of Japanese earthworms. Online. Available from internet: https://vermecology.files.wordpress.com/2020/12/rjb-japan-iucn-redlist-definitive-checklist-1.pdf.

Blakemore, R. J., 2020b. Cosmopolitan earthworms. (6th Amended Edition). VermEcology, Yokohama, Japan, pp. 1250 + 150 figs.

Blakemore, R. J. & M. J. Grygier, 2011. Unravelling some Kinki worms (Annelida, Oligochaeta, Megadrili, Lumbricidae) Part III. Soil Organisms, 83(2): 231–244.

Blakemore, R. J. & N. Kaneko, 2007. Alien earthworms in the Asia/Pacific region with a checklist of species and the first records of *Eukerria saltensis* (Oligochaeta : Ocnerodrilidae) and *Eiseniella tetraedra* (Lumbricidae) from Japan, and

Pontoscolex corethrurus (Glossoscolecidae) from Okinawa.

Blakemore, R. J. & E. K. Kupriyanova, 2010. Unravelling some Kinki worms (Annelida:Oligochaeta:Megadrili:Moniligastridae) Part I. Opuscula Zoologica, 40: 3–18.

Blakemore, R. J., E. K. Kupriyanova & M. J. Grygier, 2010. Neotypification of *Drawida hattamimizu* Hatai, 1930 (Annelida, Oligochaeta, Megadrili, Moniligastridae) as a model linking mtDNA (COI) sequences to the ʻCan of Wormsʼ theory of cryptic species. ZooKeys, 41: 1–29.

Blakemore, R. J. & S. Lee, 2013. Survey of Busan Oligochaeta earthworms supported by DNA barcodes. Journal of Species Research, 2(2): 127–144.

Blakemore, R. J., S. Lee & H.-Y. Seo, 2014. Reports of *Drawida* (Oligochaeta: Moniligastridae) from far East Asia. Journal of Species Research, 3(2): 127–166.

Blakemore, R. J., S. Lee & H.-Y. Seo, 2015. Account of montane and insular speciation in some Korean megadriles (Annelida: Oligochaeta). Journal of Species Research, 4(1): 1–22.

Blakemore, R. J. & R. Ueshima, 2011. Catalogue of Annelida: Oligochaeta in University of Tokyo Zoology Museum (ZMUT). In R. Ushima (ed.). Catalogue of invertebrate collection deposited in the Department of Zoology, the University Museum, the University of Tokyo (3), Phylum Annelida (Class Polychaeta, Oligochaeta, and Hirudinida). Material Reports, 90: 59–83.

Cole, J., 2013. The effect of pig rooting on earthworm abundance and species diversity in West Sussex, UK. MSc thesis, Centre for Environmental Policy, Faculty of Natural Sciences, Imperial College London. Online. Available from internet: https://charlie-burrell-54h9.squarespace.com/s/Pig-Rooting-and-Earthworm-Abundance-2013-Joseph-Cole.pdf.

Easton, E. G., 1981. Japanese earthworms: a synopsis of the megadrile species (Oligochaeta). Bulletin of the British Museum (Natural History, Zoology), 40(2): 33–65.

Hebert, P. D. N., S. Ratnasingham & J. R. DeWaard, 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceding of the Royaol Society. B Biol. Sci., 270(S1): S96–S99.

Hebert P. D. N., M. Y. Stoeckle, T. S. Zemlak & C. M. Francis, 2004. Identification of birds through DNA barcodes. PLoS Biology, 2(10): e312.

ICZN (International Commission on Zoological Nomenclature) 1999. Online. Available from internet: http://www.nhm.ac.uk/hosted-sites/iczn/code/index.jsp,

Ito T. M., S. Satoh, M. Hasegawa, K. Kawakami & Y. Minamiya, 2019. A new record of the exotic earthworm species *Pithemera bicincta* (Perrier, 1875) (Annelida: Clitellata: Megascolecidae) from Japan. Biogeography, 21: 12–16.

Kobayashi, S., 1941. Summary of the terrestrial earthworm fauna of Kyusyu. Botany and Zoology, Tokyo, 9: 511–518. (in Japanese).

Koike, F., M. N. Clout, M. Kawamichi, M. De Poorter & K. Iwatsuki (eds.), 2006. Assessment and control of biological invasion risks. IUCN, Gland, Switzerland; Cambridge, UK; Shoukadoh Books, Kyoto, pp. 173–181.

Minamiya, Y., 2021. Guidebook to Japanese earthworms. Online. Available from internet: web.archive.org/web/20160404065026/http://japanese-mimizu.jimdo.com:80/.

Minamiya,Y., J. Yokoyama & T. A. Fukuda, 2009. Phylogeographic study of the Japanese earthworm, *Metaphire sieboldi* (Horst, 1883) (Oligochaeta: Megascolecidae): Inferences from mitochondrial DNA sequences. European Journal of Soil Biology, 45: 423–430.

Ohfuchi, S., 1956. On a collection of the terrestrial Oligochaeta obtained from the various localities in Riu-kiu Islands, together with the consideration of their geographical distribution. Part I. Journal of Agriculture science. Tokyo University of Agriculture, 3: 131–176.

Ohfuchi, S., 1957a. On a collection of the terrestrial Oligochaeta obtained from the various localities in Riu-kiu Islands, together with the consideration of their geographical distribution. Part II. Journal of Agriculture science. Tokyo University of Agriculture, 3: 243–261.

Ohfuchi, S., 1957b. On a collection of the terrestrial Oligochaeta obtained from the various localities in Riu-kiu Islands, together with the consideration of their geographical distribution. Part III Journal of Agriculture science. Tokyo University of Agriculture, 3: 357–378.

Ohtaka, A. & M. Nishino, 2006. Studies on the aquatic oligochaete fauna in Lake Biwa, central Japan. IV. Faunal characteristics in the attached lakes (naiko). Limnology, 7: 129–142.

Schneider, C. W. & D. C. McDevit., 2002. Are earthworms a possible mechanism for airborne dispersal of the alga *Vaucheria*? Northeastern Naturalist, 9(2): 225–234.

Shekhovtsov S. V., Y. N. Sundukov, R. J. Blakemore, K. B. Gongalsky & S. E. Peltek, 2018. Identifying earthworms (Oligochaeta, Megadrili) of the southern Kuril Islands using DNA barcodes. Animal Biodiversity and Conservation, 41(1): 9–17.

Siddall, M. E., K. Apakupakul, E. M. Burreson, K. A. Coates, C. Erséus, S R. Gelder, M. Källersjö & H. Trapido-Rosenthal, 2001. Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of Oligochaetes. Molecular Phylogeny & Evolution, 21: 346–351.

Smith M. A., B L. Fisher & P. D. N. Hebert, 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Philosophical Transactions of Royal Society, B Biological Sciences, 360(1462): 1825–1834.

Wang, Z.-L., X.-Q. Yang, T. Z. Wang & X. Yu, 2018. Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. Mitochondrial DNA Part A, 29(5): 695–702.

Zhang L, P. Sechi, M. Yuan, J. Jiang, Y. Dong & J. Qiu, 2016. Fifteen new earthworm mitogenomes shed new light on phylogeny within the *Pheretima* complex. Scientific

Reports, 6: 20096.

Zhang R.L. & B. Zhang, 2014. Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida). Ticks Tick Borne Diseases, 5(3): 352–358.

摘 要

Blakemore, R. J., S. Miller & S. Y. Lim, 2022. 日本産フトミミズ科の2新種（環形動物門, 貧毛綱, Megadrilacea）. 神奈川県立博物館研究報告（自然科学）, (51): 95–104. [Blakemore, R. J., S. Miller & S. Y. Lim, 2022. Two New Species of Japanese Earthworms (Annelida, Oligochaeta, Megadrilacea, Megascolecidae) Update Biodiversity on Okinawa and at Lake Biwa to ca. 30 Species. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci*), (51): 95–104.]

形態学的特徴およびmtDNAのCOIバーコーディングに基づき、沖縄県久米島産のアズマフトミミズ属の新種と、滋賀県琵琶湖産のフクロフトミミズ属の新種を記載した。また、2016年に採集された標本に基づき、アメリカ原産のフクロナシツリミミズ *Bimastos parvus* (Eisen, 1874) と東南アジア原産のフィリピンミミズ *Pithemera bicincta* (Perrier, 1875) を沖縄県からの初記録として報告した。

Supplementary Materials

Appendix 1. DNA analyses support IDs with earthworm biodiversity table at Lake Biwa compared to Samford, Qld. (plus some items of taxonomic "housekeeping").

Supplementary file Online. Available from internet: https://archive.org/details/appendix-1

Appendix 2. On rediscovering some of Japan's historical & heritage earthworm types (with confirmed *Eisenia japonica* record in Japan from 1876; also an earlier *Amynthas vittatus* or "*Metaphire levis*" USA record here corrected as *A. tokioensis*). Thus the Odawara earthworm, *Amynthas vittatus* (Goto & Hatai, 1898) (Appendix 2: Fig. 16), is not proven to occur in USA and its tentative report from there should be scratched in favour of *A. tokioensis* (Beddard,1892) that already has many USA records (Appendix 2: Fig. 17).

Supplementary file Online. Available from internet: https://archive.org/details/appendix-2

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報　告

日本産トガリヒメバチ亜科（ハチ目、ヒメバチ科）6 種の新分布記録

New Distribution Records of Six Species of Japanese Cryptinae (Hymenoptera, Ichneumonidae)

渡辺恭平 [1)]

Kyohei WATANABE[1)]

Key words: Aptesini, Cryptini, fauna, new record, parasitoid wasps

トガリヒメバチ亜科 Cryptinae Kirby, 1837 はヒメバチ科の中では 2 番目に大きな亜科で、日本からは 62 属 148 種が報告されている（Watanabe, 2020）。チョウ目やコウチュウ目など、様々な昆虫に寄生するほか、クモ類にも寄生する分類群もおり、生態的に多様な一群である。また、中には農林業害虫の天敵となる種も含まれるなど、総合的病害虫管理等 の観点からも重要な分類群である。Watanabe (2019, 2020) は知見が断片的な日本産の本亜科について分類学的検討を加えるとともに、多くの分布記録を報告したが、依然として国内のファウナの解明度は低いままである。

筆者は現在も日本各地から収集した本亜科の標本の整理を進めているが、その過程で 6 種において新しい分布記録がいくつか見いだされたので、以下に目録形式で報告する。

本報告で用いた標本はすべて神奈川県立生命の星・地球博物館に収蔵されている。標本は Nikon SMZ800 実体顕微鏡により観察し、同定した。図に用いた画像は OLYMPUS TG-4 デジタルカメラを顕微鏡の接眼レンズに近接させて撮影した。画像の編集は Adobe Photoshop® CC により行った。各標本のデータは KPM-NK で始まる神奈川県立生命の星・地球博物館の資料番号、個体数（報告する個体はすべてメスのため F と表記）、採集地名、採集年月日（月はローマ数字で表記）、採集者名で示した。また、採集地名と採集者名についてはラベルに準じてローマ字で表記した。

目　録

トガリヒメバチ亜科

Subfamily **Cryptinae** Kirby, 1837

Tribe **Aptesini** Smith & Shenefelt, 1955

テラニシキモントガリヒメバチ

Javra teranishii (Uchida, 1952)

標本. JAPAN: KPM-NK 81397, F, Hokkaido, Horokanai Town, Moshiri, 16. VII 2012, M. Ito leg.

分布. 日本（北海道、本州）.

備考. 本稿での記録は本種の北海道初記録である。

マルヤマコガタフトトガリヒメバチ

Schenkia sylvatica Townes, Momoi & Townes, 1965

標本. JAPAN: KPM-NK 81398, F, Toyama Pref., Toyama City, Arimine, Kamedani, 14–21. VII. 2009, M. Watanabe leg. (Malaise trap); KPM-NK 81399, F, Toyama Pref., Toyama City, Arimine, Inonedani, 11–16. VIII. 2009, M. Watanabe leg. (Malaise trap); KPM-NK 81400, F, Toyama Pref., Toyama City, Arimine, Juroudani, 1–8. IX. 2009, M. Watanabe leg.

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(Malaise trap).

分布．日本（国後島、北海道、本州）.

備考．本種は原記載（Uchida, 1936）で用いられた北海道札幌市円山と国後島で得られた2個体のメスしか知られていなかった。本稿での記録は本種の本州初記録であるとともに、原記載以降初めての追加記録となる。なお、本種は *Microcryptus maruyamensis* Uchida, 1936 として記載されたが、種小名が先行して別のタクソンで使用されていたため Townes *et al.* (1965) により置換名が提唱された。

Tribe **Cryptini** Kirby, 1837

ハラアカシロテントガリヒメバチ（新称）
Agrothereutes abbreviatus (Fabricius, 1794)
(Figs 1 A, B)

標本．JAPAN: KPM-NK 81401, F, Hokkaido, Otaru City, Zenibako, Shinkawa-kako, 11. VII. 2013, A. Yamamoto leg.

分布．日本（国後島、北海道）; 全北区.

備考．本種は全北区に広く分布し、他種と比べて大きな種内変異をもち、その影響で異名も多い。Horstmann (1993) は本種のメスを4つの型に分類したが、上記北海道産の標本はこのうちの "Forma *abbreviator*" の特徴に合致した。この型は中体節がほとんど、あるいは完全に黒色である点と、前翅の長さが後脚脛節の長さの 0.5–0.8 倍であり、短翅型とならない点で定義される。本種は日本産の同属他種とは以下の形質状態により容易に区別できる : 触角の基部方は白帯の基部方に赤褐色部をもつ : 後体節第1–3背板は赤褐色 ; 脚は後脚腿節の基部と後脚脛節の基部を除き赤褐色（図1 A, B）。

本種は Kasparyan *et al.* (2012) で国後島の記録が扱われているものの、小西・松本（2020）の目録には含まれておらず、和名もない。本報では本種を北海道から初めて記録するとともに標準和名としてハラアカシロテントガリヒメバチを

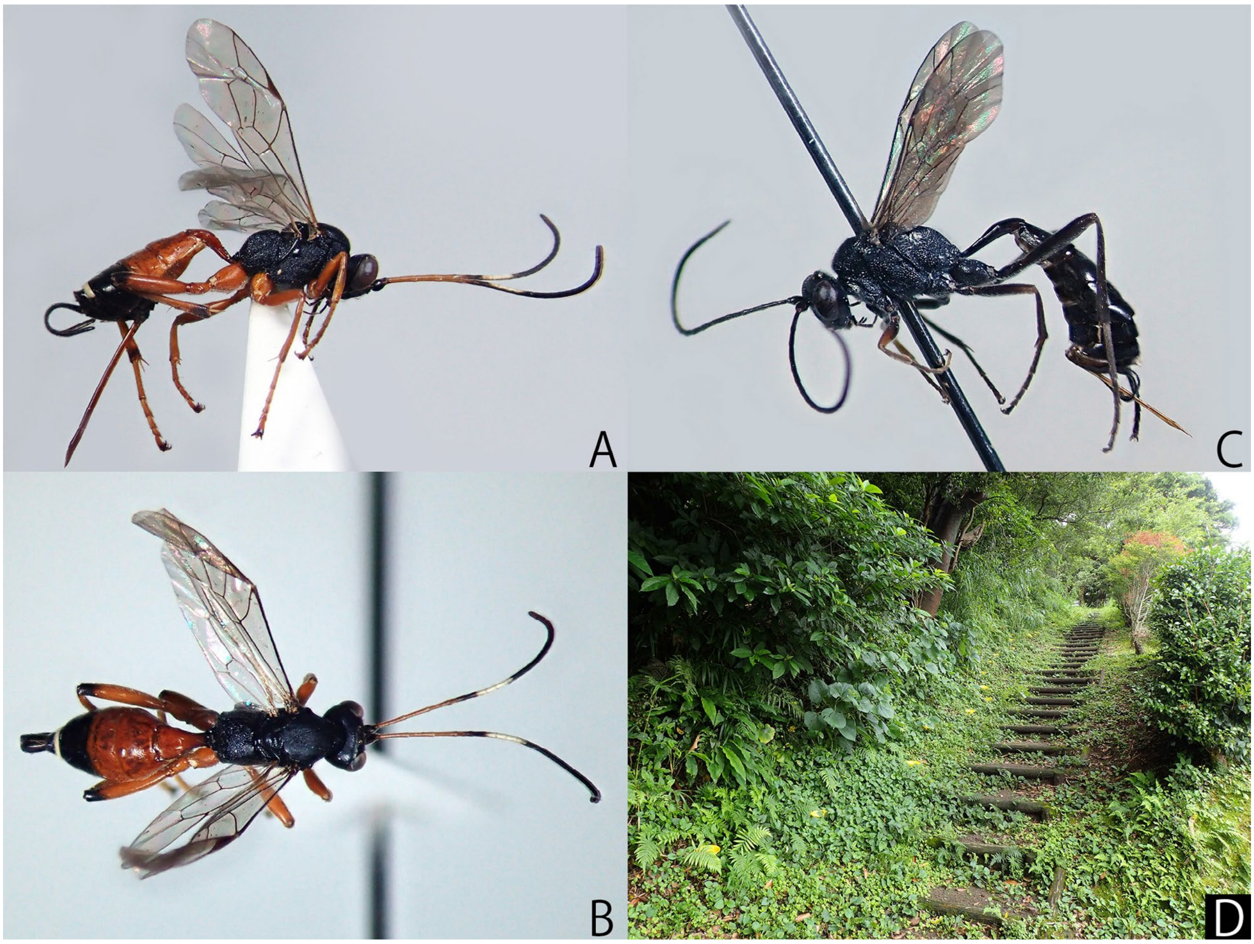

図1. A, B: ハラアカシロテントガリヒメバチ（新称）*Agrothereutes abbreviatus* (Fabricius, 1794) のメス（KPM-NK 81401）; C: トウキョウツヤトガリヒメバチ *Trychosis tokioensis* (Uchida, 1930) のメス（KPM-NK 81408）; D: トウキョウツヤトガリヒメバチの生息環境 . A, C: 側方から見た全形 ; B: 背方から見た全形 .

Fig. 1. A, B: *Agrothereutes abbreviatus* (Fabricius, 1794), female (KPM-NK 81401); C: *Trychosis tokioensis* (Uchida, 1930), female (KPM-NK 81408); D: habitat of T. tokioensis. A, C: lateral habitus; B: dorsal habitus.

提唱する。

なお、本種はユーラシア大陸では比較的普通に得られるヒメバチである（筆者が各国の博物館の収蔵庫で見た標本数より判断）が、日本国内での採集例は極めて少なく、稀な種である。この違いが地史的な理由によるものであるか、寄主をめぐって競合する寄生蜂が日本国内に豊富に生息しているためによるかは、現時点では不明である。

オウシュウトガリヒメバチ
Cryptus spiralis (Fourcroy, 1785)

標本．JAPAN: KPM-NK 81402, F, Hokkaido, Rishiri Is., Rishiri Town, Oshidomari-sakae, 19. VIII. 2019, T. Amano leg.; KPM-NK 81403, F, Hokkaido, Rishiri Is., Rishiri Town, Kutsugata, 21. VIII. 2019, T. Amano leg.

分布．日本（利尻島、北海道、本州）; 旧北区.

備考．本種は全北区に広く分布し、国内では北海道と本州から記録がある。本稿での記録は本種の利尻島初記録である。

ミナミコクロオナガトガリヒメバチ
Picardiella rufa (Uchida, 1932)

標本．JAPAN: KPM-NK 81404, F, Okinawa Pref., Iriomotejima Is., Taketomi Town, Otomi-rindo, 1. VI. 2006, K. Watanabe leg.; KPM-NK 81405, F, Okinawa Pref., Iriomotejima Is., Taketomi Town, Uehara, 19. VII. 2007, H. Kawamoto leg.

分布．日本（奄美大島、沖縄本島、石垣島、西表島）; 台湾.

備考．本稿での記録は本種の西表島初記録である。

トウキョウツヤトガリヒメバチ
Trychosis tokioensis (Uchida, 1930)
(Figs 1 C, D)

標本．JAPAN: KPM-NK 81406, F, Toyama Pref., Toyama City, Arimine, Kamedani, 14–21. VII. 2009, M. Watanabe leg. (Malaise trap); KPM-NK 81407, 81408, 2 F, Kagoshima Pref., Kimotsuki Town, Minamikata, Kanaedake, 18–20. VII. 2019, K. Watanabe & K. Matsumoto leg. (YPT).

分布．日本（本州、九州）.

備考．本種はUchida (1930)により東京で得られた3個体のメスに基づき記載されたが、その後の記録はなかった。本稿での記録は本種の九州初記録であるとともに、原記載以降初めての追加記録となる。九州で得られた個体（図1C）は低木やササ類が茂る森林の林縁（図1D）に沿って設置したイエローパントラップで得られたものである。本種のタイプは4月に得られているが、今回確認した追加個体はいずれも7月に得られており、体に顕著な破損は認められなかった。従って、本種の成虫は年二回出現する可能性がある。

末筆ではあるが、貴重な標本をご提供いただいた天野　匠、伊藤誠人、能瀬晴菜、清水　晃、山本亜生、山内健生の各氏に感謝申し上げる。また、本研究の一部は科研費（17K15185）の助成を受けて行われたものである。

引用文献

Horstmann, K., 1993. Revision der brachypteren Weibchen der westpaläarktischen Cryptinae (Hymenoptera, Ichneumonidae). Entomofauna, 14(7): 85–148

Kasparyan, D. R., A. Hull, A. Tereshkin, A. I. Humala & M. I. Proshchalykin, 2012. 47. Fam. Ichneumonidae. In: Lelej, A. S. (ed.), Annotated Catalogue of the Insects of Russian Far East. Vol. I. Hymenoptera. Dal'nauka, Vladivostok, pp. 210–299. (In Russian)

小西和彦・松本吏樹郎, 2020. Family Ichneumonidae ヒメバチ科. 日本昆虫学会日本昆虫目録編集委員会編, 日本昆虫目録 第9巻 膜翅目 (第2部細腰亜目寄生蜂類). pp. 358–578. 櫂歌書房, 福岡.

Townes, H., S. Momoi & M. Townes, 1965. A catalogue and reclassification of the eastern Palearctic Ichneumonidae. Memoirs of the American Entomological Institute, 5: 1–661.

Uchida, T., 1930. Fuenfter Beitrag zur Ichneumoniden-Fauna Japans. Journal of the Faculty of Agriculture, Hokkaido Imperial University, 25: 299–347.

Uchida, T., 1936. Erster Nachtrag zur Ichneumonidenfauna der Kurilen. (Subfam. Cryptinae und Pimplinae). Insecta matsumurana, 11: 39–55.

Watanabe K., 2019. Taxonomic and zoogeographical notes of Japanese Cryptinae (Hymenoptera, Ichneumonidae), with description of five new species. Bulletin of the Kanagawa Prefectural Museum (Natural Science), (48): 81–113.

Watanabe K., 2020. Taxonomic and zoogeographic notes on Japanese Cryptinae (Hymenoptera, Ichneumonidae), with descriptions of 12 new species. Bulletin of the Kanagawa Prefectural Museum (Natural Science), (49): 29–66.

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