

RESEARCH PAPER

Discovery of a troglomorphic trechine beetle from the Ryukyu Archipelago, Southwestern Japan (Coleoptera: Carabidae: Trechinae)

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Abstract. A new genus and new species of Trechina (Coleoptera: Carabidae: Trechinae: Trechini), *Ryukyuaphaenops pulcherrimus* gen. & sp. nov. is described from the Ryukyu Archipelago, Japan. This is the first troglomorphic Carabidae in the Ryukyus and probably the most cave-adapted beetle in Japan. Interestingly, our observations suggest that this new trechine beetle is not similar to any genera of Trechina distributed in Kyushu and Taiwan, which are geographically close to the Ryukyu Archipelago, but is more similar to several aphaenopsian genera distributed in the inland of China. The genetic diversity of the new species is discussed based on mitochondrial DNA (*COI-5'*, *COI-3'*, 16S).

Key words. Coleoptera, Carabidae, Trechini, Trechina, biospeleology, DNA barcoding, haplotype network, new genus, new species, subterranean fauna, taxonomy, troglobiont, Okinawa-jima Island, Ryukyus, Oriental Region

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Introduction

The subtribe Trechina is one of the most species-rich groups of carabid beetles of the tribe Trechini which contains more than 2,500 species worldwide (BOUSQUET 2012). Many species inhabit subterranean habitats and have troglomorphic features which are specialized morphologies adapted to the underground life (CASALE et al. 1998, MADDISON et al. 2019). In particular, the species traditionally called “aphaenopsian” type (UÉNO 1971, CASALE et al. 1998) are highly specialized to subterranean environment, and show strongly developed troglomorphism, mainly characterized by effaced frontal furrows, elongate body, and exceedingly long appendages.

In East Asia (Fig. 1), troglomorphic Trechina have been found in the Russian Far East (e.g., JEANNEL 1962), the Korean Peninsula (UÉNO 1987, UÉNO & NAMKUNG 1968), China (e.g., TIAN et al. 2023), Taiwan (e.g., UÉNO 1989, 1991), the Japanese archipelago (e.g., UÉNO 1995),

the Himalayas (UÉNO 1980), Myanmar (DEUVE 2017), and Laos (DEUVE 2000, 2017).

In Japan, more than 450 species and subspecies in 30 genera of Trechina have been recorded (UÉNO 1995; BELOUSOV 2017; NAITÔ 2017, 2018, 2020, 2022a, b; SUGAYA & HARA 2020, 2021; MORITA 2021; MORITA et al. 2021; OKUDA 2021; SUNDUKOV & MAKAROV 2021; WADA & ASHIDA 2021). Most of them dwell in caves and the upper hypogean zone (UÉNO 1995) and have troglomorphic features. These troglomorphic Trechina are distributed in broad area of Japan – the four main islands (Hokkaido, Honshu, Shikoku, and Kyushu) as well as some of the isolated islands: Sado Island, Tsushima Island, and Goto Islands. Therefore, Japan is considered one of the hotspots for subterranean Trechina (DEHARVENG & BEDOS 2018).

However, the Ryukyu Archipelago (= the Ryukyus: Figs 1, 2), in the southwestern part of the Japanese archipelago, is an exception in the hotspot of Japanese subterranean



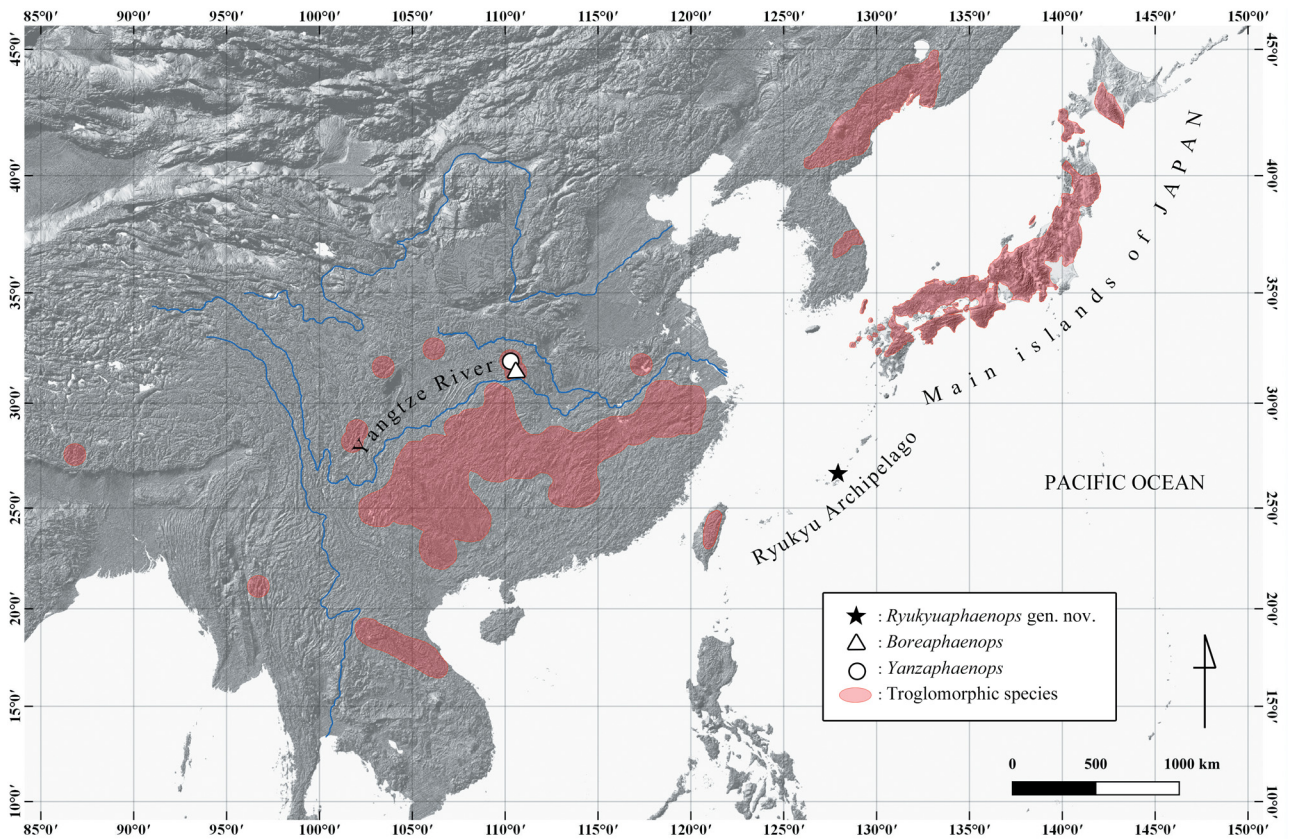


Fig. 1. Location of the Ryukyu archipelago and distribution of troglomorphic Trechina in East Asia.

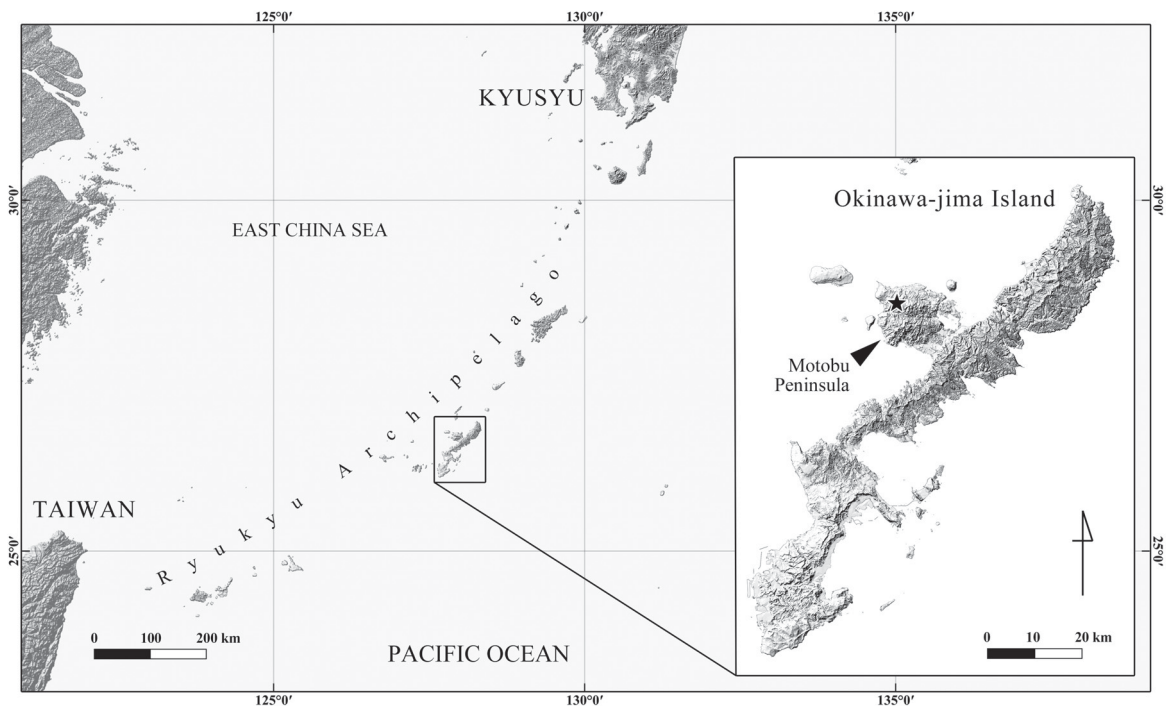


Fig. 2. Map showing present study area, Motobu Peninsula (black arrow), and locality of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov. (black star).

Trechina. Only four oculate hygrophilous and saprophilous Trechina (*Epaphiopsis janoi* (Jeannel, 1937), *E. wataberorum* Uéno, 1978, *Nesiotrechus convexiusculus* (Uéno, 1975), *Trechoblemus microphthalmus* Uéno, 1955) have been known from the Ryukyus so far (UÉNO 1974, 1975, 1985). No troglomorphic species has been found yet in spite of enthusiastic investigations by many biospeleologists. Thus, there is a large, ca. 1,200 km wide, distributional gap

of the subterranean fauna of Trechina between Kyushu and Taiwan.

In this paper, we report the first troglomorphic Trechina from the Ryukyus and describe it under the name *Ryukyuaphaenops pulcherrimus* gen. & sp. nov. We also analyze the genetic diversity of the new species by examining its mitochondrial DNA, albeit our analysis is based on a small number of individuals.

Material and methods

Study area. The Motobu Peninsula (Fig. 2), an approximately 13 km square peninsula protruding into the East China Sea from the west coast of northern Okinawa-jima Island, is located in the central Ryukyus. This peninsula is more than 500 km distant from Kyushu and Taiwan, respectively, and belongs to the humid subtropical climatic zone. The northwestern area of the peninsula including the localities of the present new species is covered by limestone of the Triassic origin (Nakijin Formation). This region is known as one of the few unique areas in Japan where the conical karst landforms have developed, characterized by hot and humid climatic conditions in the subtropical to tropical zones (MEZAKI 1984).

Field survey. Field surveys were conducted from December 2021 to April 2023. All the living specimens including DNA vouchers were collected by direct collecting. In addition, the bait trap method was conducted. The baits were mixed as follows: 80% powder from dried silk moth pupae, 10% blue cheese, and 10% potassium sorbate aqueous solution. Beetles were collected into propylene glycol as bait fixation fluid. The data of the traps were not used for quantitative assessment in this study because their condition varied greatly depending on the degree of fermentation of the baits and the microenvironment and water level fluctuations in the caves.

Specimen preparation. The living specimens of the new species were fixed by ethyl acetate, except for the specimens for DNA studies, which were fixed by 99.5% ethanol. All the specimens including DNA vouchers after DNA extraction were dried and glued onto paper entomological cards using polyvinyl acetate resin; genitalia were preserved in micro tubes with glycerin pinned under each specimen. Label data of the holotype were cited verbatim, with a slash (/) indicating line breaks. All specimens of the new species were labeled with a unique ID (“KSES” + five number digits). The GPS data for the localities of the new species are kept undisclosed to prevent unexpected problems and accidents.

Depository of the specimens. The specimens examined in this study are preserved in the following collections:

DSSJ	Daisuke Sumikawa's private collection, Sapporo, Japan;
EUMJ	Ehime University Museum, Matsuyama, Japan (Hiroyuki Yoshitomi);
HUM	Hokkaido University Museum, Sapporo, Japan (Masahiro Ôhara);
KSTJ	Kazuki Sugaya's private collection, Tokyo, Japan;
KUGJ	Koji Uchida's private collection, Ginowan, Japan;
KUM	Kyushu University Museum, Fukuoka, Japan (Munetoshi Maruyama);
NMPC	National Museum, Prague, Czech Republic (Jiří Hájek);
NSMT	National Museum of Nature and Sciences, Tsukuba, Japan (Shûhei Nomura);
RUMJ	Ryukyu University Museum (Fujukan), Okinawa, Japan (Takeshi Sasaki);
SHSJ	Shigehisa Hori's private collection, Sapporo, Japan;
SKIJ	Showtaro Kakizoe's private collection, Ibaraki, Japan;
SKTJ	Shinya Kawai's private collection, Tokyo, Japan.

Morphological observation. The morphological observations were conducted under a Leica S8 APO stereoscopic microscope and an Olympus BX43 optical microscope. The

methods of SUGAYA & YAMASAKO (2014) and YAMASAKO (2015) were used for the observation of the internal sac of male genitalia and the membranous structures of female genitalia, respectively.

The photographs of habitus were taken using an Olympus OM-D E-M1 Mark II digital camera with an Olympus M. ZUIKO DIGITAL ED 60mm F2.8 Macro macro lens and an Olympus STF-8 macro flash. Photographs of the chaetotaxy, mouth parts, legs and genitalia were taken using an Olympus BX43 optical microscope. Combine ZP (Alan Hadley, UK) was used for image stacking. SEM photos were taken with KEYENCE VHX-D500 in NSMT. The distributional maps were created with “hill shade map” files downloaded from the GSI Tiles website of Geospatial Information Authority of Japan (<https://maps.gsi.go.jp/development/ichiran.html>) using QGIS 3.4.14. All figures were edited using Adobe Photoshop® CS6.

The terminology used for the description of the present new genus and species mainly follows SUGAYA & HARA (2020) and UÉNO (1999), and QUÉINNEC et al. (2021) for the mandible. All measurements are given as the minimum length–maximum length range in mm (mean ± SD). The abbreviations for the measurements are detailed in the caption of Table 1.

The videos of the living specimens of the present new species in the cave were shot using an iPhone 12 mini (Apple Inc.) and stabilized using the Warp Stabilizer Effect in Adobe Premiere Pro 2023 (ver. 23.6.0).

Comparative specimens examined. We examined the following specimens for comparative purposes:

Boreaphaenops angustus Uéno, 2002: 1 ♂ (holotype, NSMT), Lengre Dong, 1640 m, Caiqi, Muyu Zhen, Shennongjia, Hubei, C. China, 13.v.2002, T. Kishimoto leg.; 1 ♀ (allotype, NSMT), same locality as the holotype, S. Uéno leg.; 1 ♂ 2 ♀♀ (paratypes, NSMT), same data as the holotype.

Yanzaphaenops hirundinis (Uéno, 2005): 1 ♂ (holotype, NSMT), Jin'yanhu Dong, 2110 m alt., Yanzi Ya, Hongping Zhen, Shennongjia, W. Hubei, 9.vi.2004. S. Uéno leg.; 1 ♀ (allotype, NSMT), same data as the holotype; 2 ♂♂ 2 ♀♀ (paratypes, NSMT), same data as the holotype.

DNA extraction and sequencing. DNA of the new species was extracted from several paratype specimens using DNeasy Blood & Tissue Kit (Qiagen, Germany). Subsequently, each mitochondrial gene region was amplified using KOD One ® PCR Master Mix -Blue (TOYOBO, Japan) according to the standard protocol (Toyobo Co., Ltd. 2023) under an annealing temperature of 48°C. The following primer pairs were used: LCO1490 and HCO2198 (FOLMER et al. 1994) for the *COI-5'* region (648 bp), C1-J-2183 (Jerry) and TL2-N-3014 (Pat) (SIMON et al. 1994) for the *COI-3'* region (753 bp), and 16SA and 16SB (HOSOYA et al. 2001) for the 16S region (525 bp). The amplified DNA fragments were purified and sequenced using an Applied Biosystems 3500xL Genetic Analyzer (Thermo Fisher Scientific, USA) according to the standard protocol of a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). The resulting sequences were assembled using MEGA7 (KUMAR et al. 2016). All sequences of the new species obtained in this study were submitted to GenBank / EMBL / DDBJ (accession numbers: LC777251–LC777256

(16S); LC777257–LC777262 (*COI-5'*); LC777263–LC777268 (*COI-3'*). All DNA extracts from this study have been deposited at NSMT.

Population genetic analyses. Population genetic analyses were conducted to examine genetic differentiation of the new species among all the known localities (three pit caves: type locality, Cave A, and Cave B; see the section of the type specimens of the present new species). We examined the diversity of the *COI* and *16S* regions of the mitochondrial DNA using a total of six specimens.

For the *COI* region, a TCS haplotype network (CLEMENT et al. 2000) was constructed using PopART (LEIGH & BRYANT 2015) using the sequence combining the 648 bp *COI-5'* and 753 bp *COI-3'* sequences obtained by the method described above. Genetic distances were calculated using MEGA7 (KUMAR et al. 2016). Since there were no single nucleotide variations in the *16S* region among the six individuals used in this study, we did not construct a haplotype network and calculate the genetic distance.

Results

Taxonomy

Ryukyuaephaenops gen. nov.

Japanese name: リュウキュウアシナガメクラチビゴムシ属

Type species. *Ryukyuaephaenops pulcherrimus* sp. nov., here designated.

Diagnosis. *Ryukyuaephaenops* gen. nov. is distinguished from all known genera of Trechina in the world by the following combination of characteristics: 1) aphaenopsian facies; 2) both dorsal and ventral body surfaces sparsely covered with microscopic hairs (Fig. 24); 3) right mandible seemingly bidentate though actually tridentate, due to middle tooth frequently absent (Figs 4, 5b); 4) mentum fused with submentum; 5) submentum multisetose; 6) labial palpomere 2 quadrisetose; 7) maxillary palpi glabrous though palpomere 2 unisetose subapically, palpomere 3 longer than palpomere 4; 8) prothorax barrel-shaped, with notopleural suture completely invisible from above (Fig. 19b), front and hind angles effaced; 9) scutellum distinct; 10) elytral basal transverse furrow absent; 11) elytral lateral margins ciliated (Fig. 20a); 12) elytral bases distinctly depressed; 13) four pores of humeral set not ranged equidistantly and adjoining marginal gutter except for 3rd and 4th pores, 1st pore isolated anteriorly from posterior pores, 2nd and 3rd pores close to each other, and 4th pore widely isolated posteriorly from first three pores (Figs 4, 20a, 20b); each two pores of middle and apical sets distant from marginal gutter and isolated from other sets, close to each other in the former, while well distant from each other in the latter (Figs 4, 21); 14) abdominal sternites 3 and 4 completely fused together; 15) protibiae wholly pubescent and not externally grooved; 16) protarsomere 1 not longer than protarsomeres 2–4 combined; 17) protarsomeres 1 and 2 of male dilated, inwardly denticulated at apices, and furnished beneath with adhesive appendages (Fig. 10); 18) median lobe of male genitalia with simple apical lobe neither reflexed dorsad nor bent ventrad; 19) internal sac without sclerotized teeth patches but with anisotropic

copulatory piece; 20) styles elongate, with long setae at apices; and 21) female genitalia with distinct spermathecal complex not on common oviduct but on bursa copulatrix (Fig. 15), gonocoxite 1 almost glabrous.

Among all known genera of Trechina, *Ryukyuaephaenops* gen. nov. shows a close resemblance to some Chinese aphaenopsian genera, in particularly *Boreaphaenops* Uéno, 2002 and *Yanzaphaenops* Uéno, 2008 by sharing the following characteristics: 1) body surface sparsely covered with microscopic hairs; 2) elongated head with wide neck; 3) mentum fused with submentum; 4) pronotum barrel-shaped with incomplete lateral borders, basal area pre-pedunculated, front and hind angles degenerated; 5) elytral lateral margins ciliated; 6) humeral set of marginal umbilicate pores not aggregated; 7) protibiae wholly pubescent and not externally grooved; 8) protarsomere 1 not longer than protarsomeres 2–4 combined; 9) protarsomeres 1 and 2 modified in male; and 10) male genitalia with elongated aedeagus and styles, and anisotropic copulatory piece. However, *Ryukyuaephaenops* gen. nov. can be clearly distinguished from the above two genera as follows: 1) labial palpomere 2 quadrisetose [bisetose in the two genera]; 2) maxillary palpomere 2 unisetose, palpomere 3 longer than palpomere 4 [palpomere 2 completely glabrous in the two genera; palpomere 3 shorter than palpomere 4 in *Boreaphaenops*, almost as long as palpomere 4 in *Yanzaphaenops*]; 3) elytral setiferous dorsal pores on stria 3 composed of two pores [three pores in *Boreaphaenops*, four pores in *Yanzaphaenops*]; 4) abdominal sternites 3 and 4 completely fused together [not fused in the two genera]; and 5) female genitalia with distinct spermathecal complex on bursa copulatrix [spermathecal complex unrecognizable in the two genera]. In addition to the above, *Ryukyuaephaenops* gen. nov. also differs from *Boreaphaenops* in the first three pores of the humeral set of marginal umbilicate pores not ranged equidistantly and the aedeagus not reflexed dorsad, and from *Yanzaphaenops* in the 1st pore of the humeral set of marginal umbilicate pores adjoining marginal gutter. Incidentally, in *Yanzaphaenops*, it was found that the first three pores of the humeral umbilicate set are not ranged equidistantly, and the 1st pore is removed inward from marginal gutter, based on our re-examination of the type series, although it was originally described with the first three pores ranged equidistantly and adjoining marginal gutter (UÉNO 2005, 2010). In addition, *Boreaphaenops liyuani* Tian & He, 2020 significantly differs from the type species, *B. angustus* Uéno, 2002, in the generically important characteristics, including the four pores of the humeral set of marginal umbilicate pores ranged equidistantly, and elytral lateral borders not ciliated (TIAN & HE 2020). Therefore, *Ryukyuaephaenops* gen. nov. is easily distinguished from *B. liyuani* as well.

Description. Medium-sized Trechina with aphaenopsian facies; apterous and depigmented; body surface sparsely covered with very minute microscopical hairs.

Head elongated subquadrate, gradually narrowing posteriorly with wide neck; dorsum feebly convex; frontal furrows absent posteriorly; two pairs of supraorbital pores present; ventral surface of genae (Figs 18b, 18c) bearing pair of long



Fig. 3. Habitus of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov., holotype male (KSES00004), dorsal (a) and lateral (b) views.

hairs before neck constriction; eyes completely missing; clypeus quadrisetose. Labrum sextisetose. Mandibles long and slender, falcate. Right mandible visually bidentate to tridentate, composed of well-developed premolar and wide retinaculum; retinaculum bidentate, though proximal tooth frequently missing while distal one always developed. Left mandible bidentate, with two partially overlapping undeveloped teeth on dorsal and ventral sides. Mentum bisetose and fused with multisetose submentum; ligula trapezoidal in apical part, with two long setae in middle of truncated portion, and three lateral setae on each oblique part; paraglossae elongate, moderately arcuate inwardly, extending much beyond ligula, with minute hairs along inner sides; palps elongate and glabrous, but with labial palpomere 2 quadrisetose, two on inner and outer margins, respectively, and with maxillary palpomere 2 unisetose on inner margin subapically; penultimate palpomere longer than apical one in labium and maxillae, respectively. Antennae exceedingly elongated filiform, longer in male than female, wholly pubescent except on basal half of segment 1.

Prothorax hardly tumid laterad. Pronotum barrel-shaped; dorsum moderately convex; median line distinct, not reaching apical nor basal margins; apical transverse impression distinct though superficial, with longitudinal fine wrinkles; basal transverse impression (Figs 4, 19a, 19b) distinct, not continuous and merging on each side into basal fovea; sides (Figs 19a, 19b) not ciliated, incompletely and very finely bordered, and deflexed ventrad before anterior latero-marginal pore; marginal gutters vestigial; both apical and basal margins not bordered entirely; basal margin (Figs 3a, 4, 19a, 19b) almost straight or feebly emarginated in middle, obliquely rounded at just inside of hind angles; front angles effaced; hind angles (Figs 19a, 19b) never produced, subrectangular or entirely rounded. Propleura (Figs 4, 19a, 19b) almost invisible from above; notopleural sutures (Figs 4, 19a, 19b) completely invisible from above. Scutellum distinct.

Elytra fused together, elongated pyriform; basal peduncle without transverse furrow; dorsum convex though basal area longitudinally and obliquely depressed except for scutellar area; sides narrowly bordered throughout and very finely ciliated; marginal gutters distinct; stria 1 (Figs 4, 20b) adjoining elytral suture except for basal area; basal pore present; two setiferous dorsal pores present on stria 3; preapical pore located at apical anastomosis of striae 2 and 3; two apical pores present; marginal umbilicate pores not aggregated; four pores of humeral set of marginal umbilicate pores not ranged equidistantly and adjoining marginal gutter except for 3rd and 4th pores, 1st pore isolated anteriorly from posterior two pores, 2nd and 3rd pores close to each other, and 4th pore widely isolated posteriorly from first three pores; two pores of middle set of marginal umbilicate pores close to each other, both isolated from humeral set and widely distant from marginal gutter, 1st pore more removed inward from marginal gutter than the 2nd pore; two pores of apical set of marginal umbilicate pores well distant from each other, isolated from middle set, 1st pore widely distant from marginal gutter while 2nd pore not so distant.

Abdominal sternites 3 and 4 completely fused together, suture between them completely absent; sternites 4–6 each bearing pair of paramedian setae near posterior margin; sternite 7 bisetose in male, quadrisetose in female.

Legs exceedingly long; protibiae wholly pubescent and not externally grooved; tarsomeres 4 with long hyaline ventral apophysis in pro- and mesotarsi; in male, protarsomeres 1 and 2 dilated, inwardly denticulate at apices, and furnished beneath with adhesive appendages.

Male genital organ small and slightly sclerotized; median lobe elongated tubular, gradually tapered toward apex; apical lobe almost straight, with simple extremity; sagittal aileron distinct. Internal sac without sclerotized teeth patches; copulatory piece anisotropic and spatulate. Styles elongate; left style obviously more elongate than right one, each usually bearing four long setae at apex. Genital segment devoid of distinct basal peduncle.

Female genital organ with distinct spermathecal complex on dorsal surface of bursa copulatrix near its junction with vagina. Gonocoxite 1 short, pick-shaped and almost glabrous, with a few short apical fringe setae (afs) along apical margin ventrally, two elongated apico-medial setae (ams) on apico-internal corner dorsally; gonocoxite 2 subconical, with two long dorsal ensiform setae (des)



Fig. 4. Chaetotaxy of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov., holotype (KSES00004), dorsal (a) and dorso-lateral (b) views.

proximally, two apical nematiform setae (ans) preapico-ventrally.

Etymology. A combination of “Ryukyū” and “*Aphaenops*”. “Ryukyū [琉球]” refers to the range of the new trechine beetle. *Aphaenops* Bonvouloir, 1862 is a generic name of the famous Pyrenean trechine beetle with a strongly developed troglomorphy and is also traditionally used as root of the generic name of other strongly troglomorphic (aphaenopsian) trechines. Gender is masculine.

Remarks. Though the direction of apical striole has been traditionally considered important to the trechine taxonomy, we do not use this character in generic diagnosis of *Ryukyūaphaenops* gen. nov., because the character has been recently reported to be unstable in several genera and/or species (UÉNO 1999, BELOUSOV & KABAK 2003). In fact, the character state of the type species of *Ryukyūaphaenops* gen. nov. is unstable, and in many cases, the direction of apical striole is undetermined, due to its degeneration.

***Ryukyūaphaenops pulcherrimus* sp. nov.**

Japanese name: オキナワアシナガメクラチビゴミムシ
(Figs 3–24, 28)

Type material. HOLOTYPE: ♂, KSES00004 (NSMT), “JAPAN: Okinawa-ken, Okinawa-jima Is., / Motobu-chō, Furujiima, Ufugushikumui- / abu-dai-ichi Cave [上城森アブ第 / 一], 26.6744°N, 127.9056°E, (el. 220m), / 28 II 2022, H. TAMURA” (printed on white label), “HOLOTYPE / *Ryukyūaphaenops pulcherrimus* / des. Sugaya, Kakizoe, Ooka, / Tamura & Sone, 2023” (printed on red label). PARATYPES (35 ♂♂ 40 ♀♀): 1 ♀, KSES00001 (NSMT), same locality as the holotype, 10.xii.2021, S. Ooka leg.; 2 ♂♂, KSES00002, 00003 (NSMT), same locality and date as above, H. Tamura leg. [KSES00003: DNA voucher (SK185)]; 1 ♂ 1 ♀, KSES00024, 00025 (KSTJ), same locality as above, 7.vi.2022, S. Ooka leg.; 4 ♂♂, KSES00033–00036 (NSMT), same locality as above, 8.vi.2022, H. Tamura leg. [KSES00033: subdivided into micro parts for the detailed examination; KSES00036: DNA voucher (SK193), teneral]; 1 ♂ 1 ♀, KSES00005, 00018 (NSMT), Cave A, Ufudō (大堂), Motobu-chō, Okinawa-ken, 10.iii.2022, H. Tamura leg. [KSES00018: subdivided into micro parts for the detailed examination; found teneral but reared and died in 12.vii.2022]; 1 ♀, KSES00006 (KSTJ), same locality and date as above; 1 ♀, KSES00007 (NSMT), same locality and date as above, S. Ooka leg.; 1 ♂ 1 ♀, KSES00008, 00009 (KSTJ), same locality as above, 3.iv.2022, K. Uchida leg.; 1 ♀, KSES00017 (SKIJ), same locality and date as above, S. Kakizoe leg.; 1 ♀, KSES00028 (NSMT), same locality and date as above, D. Sumikawa leg. [DNA voucher (SK197); teneral]; 1 ♀, KSES00026 (NSMT), same locality and date as above, K. Sugaya leg. [DNA voucher (SK199); found teneral but reared and died in 5.vi.2022]; 1 ♂, KSES00010 (KSTJ), same locality as above, 2.iv.2022, K. Sugaya leg.; 1 ♂ 2 ♀♀, KSES00012–00014 (NSMT), same locality and date as above, K. Nishigaki leg.; 1 ♂ 1 ♀, KSES00016, 00027 (NSMT), same locality and date as above, S. Kakizoe leg. [KSES00027: DNA voucher (SK195)]; 1 ♂, KSES00015 (SKIJ), same locality and date as above, S. Kakizoe leg.; 1 ♂, KSES00019 (NSMT), same locality and date as above, S. Sone leg. [found teneral but reared and died in 21.vii.2022]; 1 ♂, KSES00032 (NSMT), same locality as above, 20.iv.2022, H. Tamura leg.; 2 ♀♀, KSES00021, 00022 (NSMT), same locality and date as above, Ooka & H. Tamura leg. [in a baited trap set by S. Kakizoe on 3.iv.2022]; 1 ♀, KSES00023 (NSMT), same locality as above, 25.v.2022, S. Ooka leg.; 2 ♀♀, KSES00030, 00031 (NSMT), same locality and date as above, H. Tamura leg. [in a baited trap set by S. Kakizoe on 3.iv.2022]; 1 ♀, KSES00121 (NSMT), same locality as above, 1.x.2022, S. Kakizoe leg. [preserved in 99.5% ethanol]; 1 ♀ KSES00037 (NSMT), same locality and date as above, S. Kakizoe leg. [subdivided into micro parts for the detailed examination; in a baited trap set by S. Kakizoe on 3.iv.2022]; 1 ♂ 1 ♀, KSES00038, 00039 (EUMJ), same data as above [complete condition]; 1 ♂ 1 ♀, KSES00056, 00066 (SHSJ), same data as above; 1 ♂ 1 ♀, KSES00040, 00041 (HUM), same data as above; 5 ♂♂ 5 ♀♀,

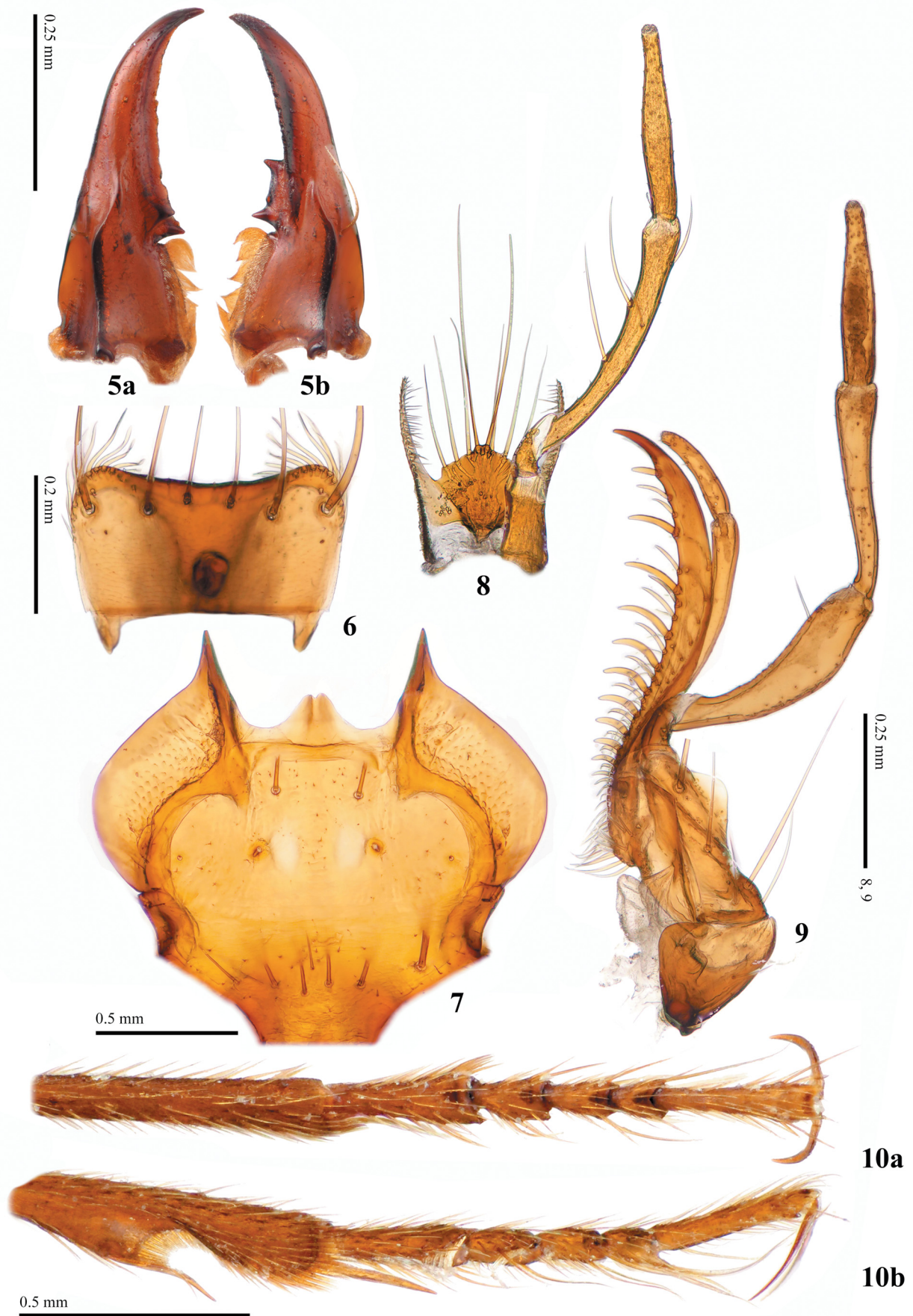
KSES00042–00051 (KSTJ), same data as above; 1 ♂ 1 ♀, KSES00052, 00053 (KUM), same data as above; 1 ♂ 1 ♀, KSES00057, 00067 (NMPC), same data as above; 1 ♂ 1 ♀, KSES00054, 00055 (RUMJ: RUMF-ZI-53351, 53352), same data as above; 8 ♂♂ 8 ♀♀, KSES00058–00065, 00068–00075 (SKIJ), same data as above; 1 ♂, KSES00120 (NSMT), same locality and date as above, S. Ooka leg. [used for Videos S1, S2; preserved in 99.5% ethanol]; 1 ♀, KSES00114 (KSTJ), same locality and date as above, S. Kawai leg.; 1 ♀, KSES00115 (NSMT), Cave B, Ufudō, Motobu-chō, Okinawa-ken, 8.iv.2023, K. Sugaya leg. [DNA voucher (SK202)].

Additional material examined (incomplete specimens mainly from traps). 12 ♂♂ 29 ♀♀ 4 spec.: 1 ♀, KSES00011 (DSSJ), Cave A, Ufudō (大堂), Motobu-chō, Okinawa-ken, 3.iv.2022, D. Sumikawa leg.; 1 ♀, KSES00029 (KSTJ), same locality as above, 25.v.2022, H. Tamura leg. [teneral; in a baited trap set by S. Kakizoe on 3.iv.2022]; 6 ♀♀, KSES00076–00081 (KSTJ), same locality as above, 1.x.2022, S. Kakizoe leg. [in a baited trap set by S. Kakizoe on 3.iv.2022]; 1 ♂ 1 ♀, KSES00082, 00083 (KUGJ), same data as above; 5 ♂♂ 5 ♀♀, KSES00084–00093 (SKIJ), same data as above; 5 ♂♂ 15 ♀♀ KSES00094–00113 (KSTJ), same data as above [incomplete condition]; 1 spec., KSES00020 (KSTJ), Cave B, Ufudō, Motobu-chō, Okinawa-ken, 3.iv.2022, K. Sugaya leg. [found dead, only elytra]; 1 ♂ 3 spec., KSES00116–00119 (KSTJ), same locality as above, 8.iv.2023, K. Sugaya leg. [found dead with some body parts].

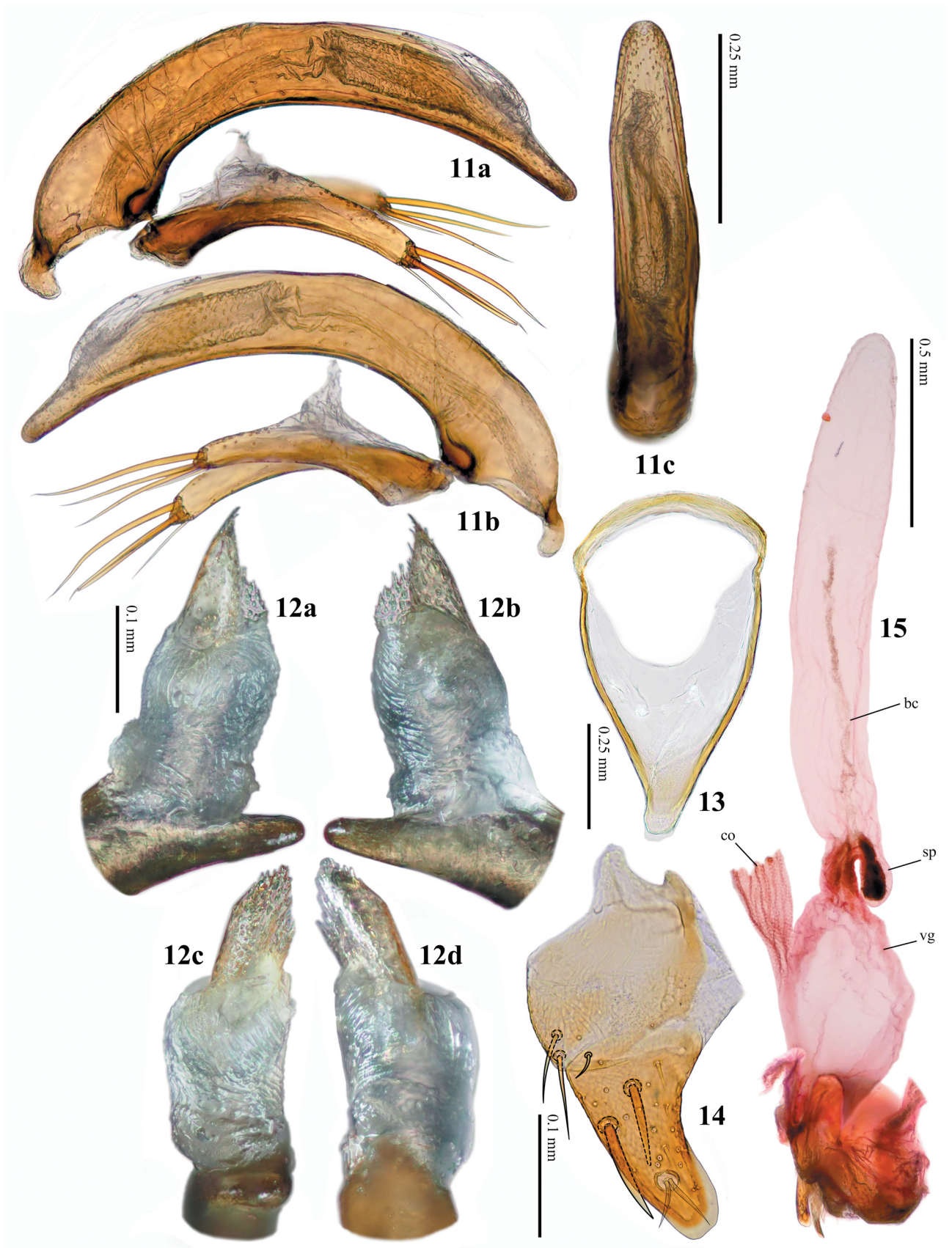
Diagnosis. This species is readily distinguished from all other Trechina by the generic diagnostic characteristics mentioned above.

Description. Males and females. Color (Figs 3, 4) light to dark reddish brown; palpi, apical half of antennae, tibiae and tarsi a little lighter. Body surface polished and not iridescent, sparsely covered with very minute microscopical hairs except for clypeus, neck, gula, scutellum, mesoventrite, metaventrite, and lateral parts of each sternite.

Head (Figs 3, 4, 18) much longer than wide, widest at about level of anterior supraorbital pore; neck as wide as 3/4 of HW; dorsum transversely rugose on frons and supraorbital areas, and usually with pair of supplemental hairs on posterior inner sides of anterior and posterior supraorbital pores, respectively; vertex without suprafrontal seta; frontal furrows distinct though absent behind level of anterior supraorbital pore; supraorbital pores located in about apical 1/2 and 1/5 of HL, respectively; microsculpture distinct, deeply impressed anteriorly, almost consisting of isodiametric polygonal meshes, but transverse meshes laterally; genae hardly convex laterad, and sparsely covered with a few hairs both on latero-ventral and ventral surface, former is usually longer than latter: ventral surface of genae (Figs 18b, 18c) transversely rugose. Labrum (Figs 6, 16) with apical margin shallowly trisinate. Right mandible (Figs 4, 5b) usually bidentate; proximal tooth of retinaculum usually missing. Labial suture (Figs 7, 17) unrecognizable though with very shallow trace in just middle portion; mental tooth bifid; submentum usually octosetose. Antennae (Fig. 3) less than twice as long as EL, slightly longer in male than female; segment 1 shortest and segment 5 longest, of all segments; segments 2–5 each gradually longer than preceding segment; segments 6–10 each gradually shorter than preceding segment; segment 11 as long as segment 9; approximate ratios of left antennal segments 1–11 as follows: holotype: 1.0 : 1.4 : 2.3 : 2.6 : 2.7 : 2.6 : 2.4 : 2.1 : 1.9 : 1.7 : 1.9; male (n = 15, including



Figs 5–10. Body parts of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov., paratypes (5 – KSES00018; 6–10 – KSES00033). 5 – left (a) and right (b) mandibles, dorsal view; 6 – labrum, dorsal view; 7 – labium, ventral view; 8 – ligula and labial palpus, ventral view; 9 – left maxilla, ventral view; 10 – left protarsi of male, dorsal (a) and internal (b) views.

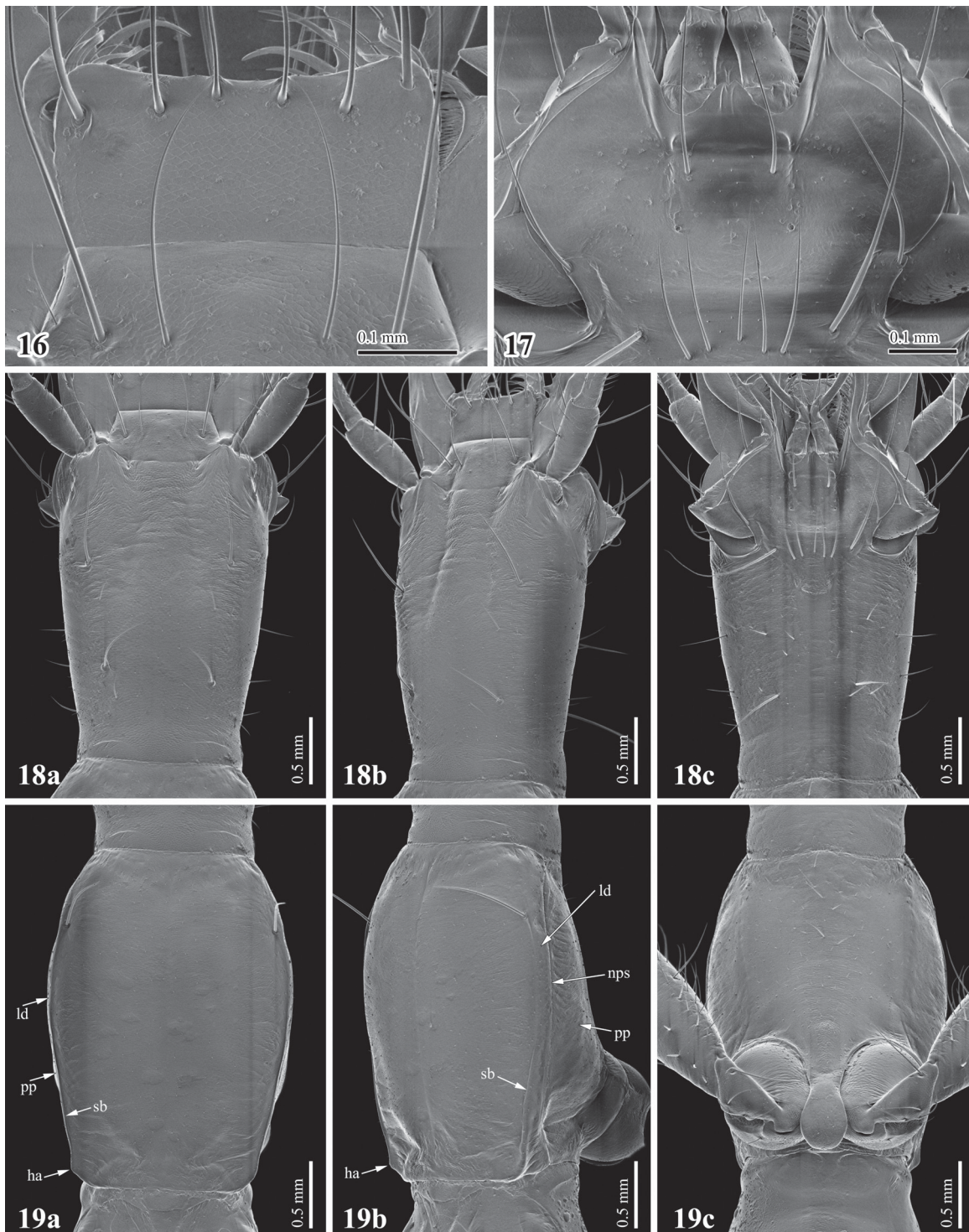


Figs 11–15. Male (11–13) and female (14, 15) genital organs of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov. 11, 13 – holotype (KSES00004); 12, 14, 15 – paratypes (12 – KSES00005; 14 – KSES00001; 15 – KSES00037). 11 – median lobe, left lateral (a), right lateral (b) and dorsal (c) views; 12 – internal sac, left lateral (a), right lateral (b), front (c) and behind (d) views; 13 – genital segment, reversed side view; 14 – left gonocoxite, ventral view; 15 – reproductive tract, left lateral view. Abbreviations: bc – bursa copulatrix; co – common oviduct; sp – spermathecal complex; vg – vagina.

holotype): 1.0 : 1.3 : 2.1 : 2.6 : 2.8 : 2.5 : 2.2 : 2.0 : 1.9 : 1.7 : 2.0; female (n = 15): 1.0 : 1.3 : 2.1 : 2.4 : 2.6 : 2.4 : 2.2 : 2.0 : 1.9 : 1.7 : 1.8.

Pronotum (Figs 3, 4, 19) slightly wider than head, as long as head, much longer than wide, widest before middle, and more gradually narrowed toward base than apex; dorsum almost smooth but with fine confused transverse rugosity along pronotal sides; microscopic hairs sparser and finer than those of head, almost absent around basal foveae; lateral margins moderately arcuate, though

shallowly concave around anterior marginal pore and feebly emarginated before hind angles; microsculpture distinct, almost consisting of transverse meshes, gradually deepened and narrowed laterad; side borders (Figs 19a, 19b) incomplete, very fine and hardly reflexed, vestigial near middle and completely absent near apices; lateral declivities of pronotum (Figs 19a, 19b) feebly visible from above in about middle; anterior latero-marginal pores present, located in apical 1/4 of PL, and adjoining marginal gutter; posterior latero-marginal pores absent; each basal



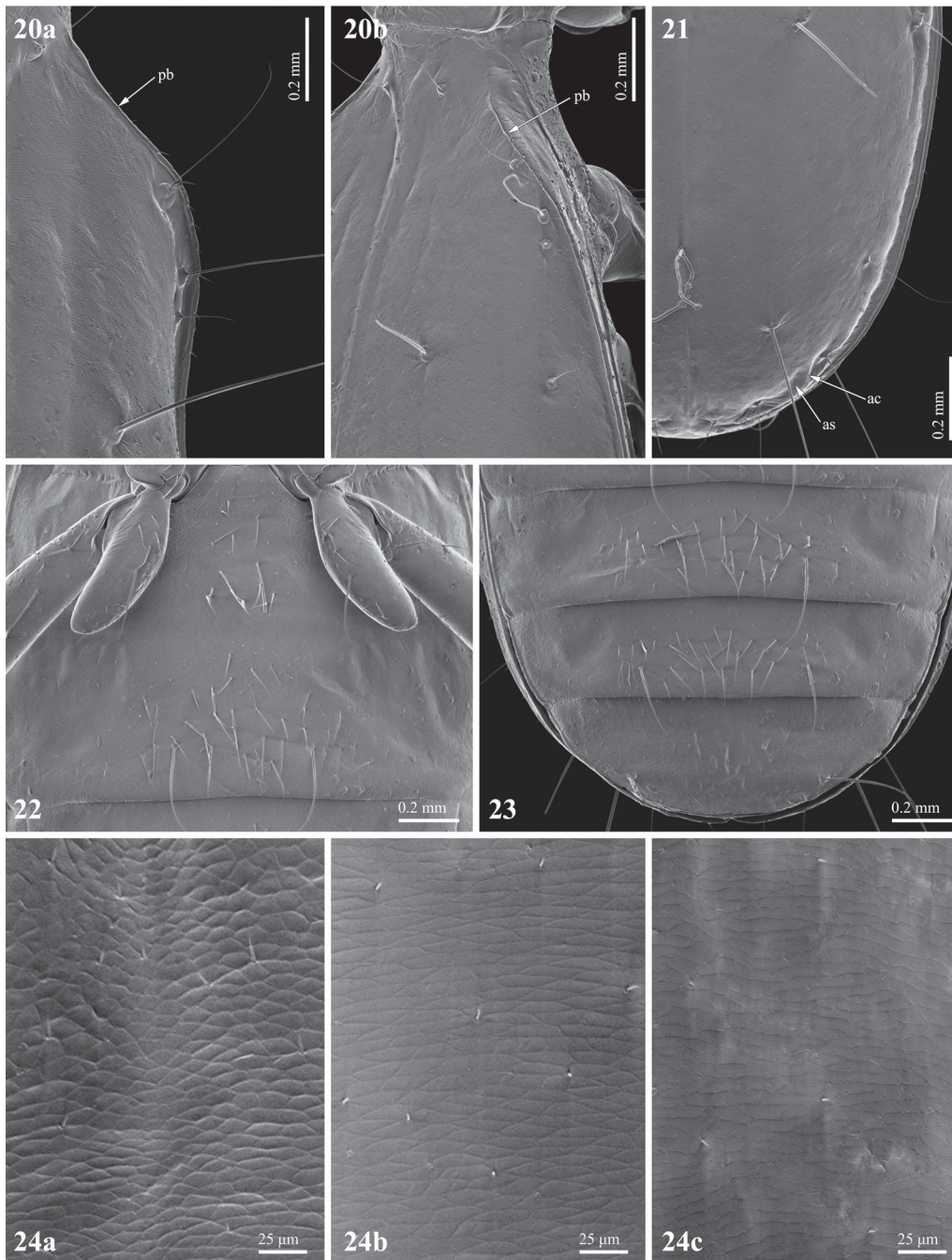
Figs 16–19. SEM photos of body parts of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov., holotype (KSES00004). 16 – labrum; 17 – labium; 18 – head; 19 – prothorax. a – dorsal view; b – dorsolateral view; c – ventral view. Abbreviations: ha – hind angle; ld – lateral declivity; nps – notopleural suture; pp – propleura; sb – side border.

fovea with bottom almost smooth but usually forming a few oblique ridges parallel to oblique side of basal margin and terraced; postangular carinae absent; basal area uneven and longitudinally strigose. Propleura (Figs 4, 19a, 19b) very narrowly visible from above behind middle. Scutellum elongated triangle.

Prosternum (Fig. 19c) sparsely covered with short hairs on anterior area near middle; metaventrite with pair of long hairs along midline near middle, and sparsely covered with short hairs except for lateral sides and posterior area.

Elytra (Figs 3, 4, 20, 21) slightly wider and obviously longer than pronotum, much longer than wide, widest behind middle; dorsum well convex, distinctly higher than pronotum; basal depression extending on more than

basal 1/5 of EL; microscopic hairs extremely sparse and fine, hardly recognizable under optical microscope except for marginal area; microsculpture distinct but very fine, almost consisting of long transverse meshes; prehumeral margins strongly oblique and moderately emarginated; prehumeral borders entirely (Fig. 20) visible from above even in basal portion, though gradually vestigial anteriorly and completely absent at basal peduncle; shoulders distinct, slightly produced antero-laterad and reflexed intero-dorsad though widely rounded, placed in about basal 1/5 of EL, about twice as wide as elytral base, about 3/4 as wide as EW; lateral margins feebly emarginated behind shoulders, then moderately arcuate and convergent to apices; preapical emarginations very obtuse; marginal cilia and serrations



Figs 20–24. SEM photos of body parts of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov., holotype (KSES00004). 20 – basal part of right elytron, left latero-dorsal (a) and right latero-dorsal (b) views; 21 – apical part of right elytron, right latero-dorsal view; 22 – sternites 3 and 4; 23 – sternites 5–7; 24 – microscopic hairs and microsculpture in head (a), pronotum (b) and elytra (c). Abbreviations: ac – apical carina; as – apical striole; pb – prehumeral border.

Table 1. Measurements (mm) of *Ryukyuaaphaenops pulcherrimus* gen. & sp. nov. Abbreviations: ABL – approximate body length (apical margin of clypeus to elytral apex); AL – antennal length (total value of antennal segments 1–11); ATL – approximate total length (apex of mandible to elytral apex); EL – elytral length (from pronotal basal margin to elytral apex); EW – maximum width of elytra; EWL – length from pronotal basal margin to widest part of elytra; HL – head length (apical margin of clypeus to neck constriction of head); HnW – width of neck; HW – maximum width of head; HWL – length from apical margin of clypeus to widest part of head; PaW – width of pronotal apical margin (between intersection of sides of neck and pronotum); PbW – width of pronotal basal margin (between tips of pronotal hind angles); PL – pronotal length; PW – maximum width of pronotum; PWL – length from apical margin to widest part of pronotum.

Measured portion	Holo-type	Males (n = 15)	Females (n = 15)	Total (n = 30)
ATL	7.02	6.15–7.25 (6.73±0.36)	6.62–7.60 (7.05±0.31)	6.15–7.60 (6.89±0.37)
ABL	6.40	5.55–6.54 (6.10±0.33)	5.96–6.86 (6.37±0.27)	5.55–6.86 (6.24±0.33)
HW	0.80	0.71–0.82 (0.77±0.03)	0.75–0.86 (0.81±0.03)	0.71–0.86 (0.79±0.04)
HL	1.21	1.03–1.25 (1.15±0.07)	1.12–1.28 (1.19±0.04)	1.03–1.28 (1.17±0.06)
HWL	0.48	0.44–0.55 (0.49±0.03)	0.49–0.59 (0.53±0.03)	0.44–0.59 (0.51±0.03)
HnW	0.59	0.53–0.64 (0.58±0.03)	0.56–0.65 (0.61±0.02)	0.53–0.65 (0.60±0.03)
AL	7.44	6.30–7.50 (7.00±0.40)	6.40–7.50 (6.90±0.30)	6.30–7.50 (7.00±0.40)
PW	0.94	0.85–1.01 (0.92±0.04)	0.88–1.06 (0.99±0.04)	0.85–1.06 (0.95±0.05)
PL	1.29	1.09–1.33 (1.23±0.07)	1.19–1.39 (1.27±0.06)	1.09–1.39 (1.25±0.07)
PWL	0.49	0.45–0.60 (0.53±0.05)	0.53–0.63 (0.57±0.03)	0.45–0.63 (0.55±0.04)
PaW	0.61	0.54–0.64 (0.59±0.03)	0.58–0.67 (0.63±0.03)	0.54–0.67 (0.61±0.03)
PbW	0.70	0.60–0.76 (0.67±0.04)	0.66–0.78 (0.71±0.04)	0.60–0.78 (0.69±0.04)
EW	1.90	1.60–1.95 (1.76±0.12)	1.78–2.07 (1.88±0.09)	1.60–2.07 (1.82±0.12)
EL	3.79	3.29–3.89 (3.61±0.19)	3.45–4.07 (3.77±0.17)	3.29–4.07 (3.69±0.19)
EWL	2.11	1.87–2.21 (2.04±0.10)	1.96–2.33 (2.15±0.10)	1.87–2.33 (2.10±0.11)

(Fig. 20a) present from base of prehumeral margins to preapical emarginations, both very sparse and very fine though slightly more dense and prominent around shoulders; striae very shallow and indistinct, gradually evanescent laterad, and very sparsely and very faintly punctate; stria 1 almost disappearing, though deeply impressed at basal part; striae 2 and 3 distinct and slightly deeper than outer striae, though vestigial near both ends; striae 4 and 5 very fine and very superficial, and vestigial near both ends; striae 6 and 7 barely traceable; stria 8 almost disappearing, though deeply impressed around 4th pore and 5th to 6th pores of marginal umbilicate pores; intervals feebly convex except for basal depression and posthumeral lateral area; interval 8 swelled around middle set of marginal umbilicate pores; scutellar striole indistinct; apical striole (Fig. 21) vestigial and usually not connected with any striae; apical carina

Table 2. Body ratios of *Ryukyuaaphaenops pulcherrimus* gen. & sp. nov. For abbreviations, see Table 1.

Ratio	Holo-type	Males (n = 15)	Females (n = 15)	Total (n = 30)
HL/HW	1.51	1.43–1.56 (1.49±0.04)	1.40–1.53 (1.47±0.04)	1.40–1.56 (1.48±0.04)
HWL/HL	0.40	0.38–0.47 (0.43±0.03)	0.41–0.48 (0.45±0.02)	0.38–0.48 (0.44±0.02)
AL/EL	1.86	1.90–2.20 (1.90±0.10)	1.80–1.90 (1.80±0.00)	1.80–2.20 (1.90±0.10)
PW/HW	1.18	1.16–1.23 (1.20±0.02)	1.17–1.25 (1.22±0.02)	1.16–1.25 (1.21±0.02)
PL/HL	1.07	1.04–1.11 (1.07±0.02)	1.03–1.11 (1.07±0.02)	1.03–1.11 (1.07±0.02)
PL/PW	1.37	1.27–1.38 (1.33±0.04)	1.22–1.38 (1.28±0.04)	1.22–1.38 (1.31±0.05)
PWL/PL	0.38	0.38–0.47 (0.43±0.02)	0.40–0.51 (0.45±0.03)	0.38–0.51 (0.44±0.03)
PaW/PbW	0.87	0.84–0.92 (0.88±0.02)	0.85–0.90 (0.88±0.01)	0.84–0.92 (0.88±0.02)
EW/PW	2.02	1.84–2.02 (1.92±0.06)	1.83–2.02 (1.90±0.06)	1.83–2.02 (1.91±0.06)
EL/PL	2.94	2.86–3.02 (2.95±0.05)	2.88–3.06 (2.98±0.06)	2.86–3.06 (2.96±0.06)
EL/EW	1.99	1.96–2.13 (2.05±0.05)	1.91–2.07 (2.00±0.05)	1.91–2.13 (2.03±0.06)
EWL/EL	0.56	0.54–0.59 (0.57±0.01)	0.55–0.59 (0.57±0.01)	0.54–0.59 (0.57±0.01)

(Fig. 21) barely recognizable; basal pore located at about level of a little anterior portion of apex of scutellum; two setiferous dorsal pores on stria 3 each located in about basal 3/10 and 3/5 of EL, anterior pore lying before level of 4th pore of marginal umbilicate series, and posterior one at same or just behind level of 5th pore of marginal umbilicate pores; preapical pore located in about apical 1/7 to 1/9 of EL, lying at about before level of 8th pore of marginal umbilicate series, closer to elytral suture than to elytral apices; anterior apical pore slightly closer to elytral suture than to preapical pore, much closer to elytral apices than to elytral suture; posterior apical pore equally very close to both elytral suture and elytral apices.

Sternites 3–7 (Figs 22, 23) each densely covered with short to long hairs near middle along posterior margin; sternites 4–6 each bearing with pair of paramedian setae.

Metafemur and metatibia about 4/5 and 3/4 as long as elytra, respectively; metatarsus about 3/4 as long as metatibia; tarsomere 1 not longer than tarsomeres 2–4 together in fore legs, but evidently longer in middle and hind legs.

Male genital organ as in Figs 11–13 (n = 13). Median lobe (Fig. 11) less than 1/5 as long as EL, moderately arcuated and almost symmetrical; ventral margin hardly concave in just distal portion of junction with styles; basal part less than 1/5 as long as median lobe; basal orifice broadly opening on basal end of basal part; sagittal aileron hyaline, baso-ventrally protruded; apical lobe about 1/2 as long as median lobe, almost straight and gradually tapering toward rounded apex; in dorsal view, apical lobe symmetrical elongated triangle, widely rounded at apex. Internal sac (Figs 11, 12) simply invaginated in median



Figs 25–28. Habitat of type locality (25–27) and living individual (28) of *Ryukyaphaenops pulcherrimus* gen. & sp. nov. 25 – Mt. Ufugushikumui; 26 – entrance of Ufugushikumui-abu-dai-ichi Cave; 27 – inside of Ufugushikumui-abu-dai-ichi Cave; 28 – living individual at the wall face of Ufugushikumui-abu-dai-ichi Cave.

lobe; internal sac in fully inflated condition with eversion ($n = 3$) projected dorso-apically from apical orifice, about 2/9 as long as median lobe, oblong bulbous, sparsely covered with fine hyaline scales, and devoid of sclerotized teeth patches; copulatory piece attached on apical end of internal sac, asymmetrical spatulate, about 1/2 as long as internal sac and 1/5 as long as median lobe, moderately sclerotized, sparsely covered with very fine spicules, with apical margin irregularly serrate; basal part of copulatory piece covered by internal sac and never exposed. Styles (Figs 11a, 11b) without ventral apophysis.

Female genital organ as in Figs 14, 15 ($n = 14$). Bursa copulatrix elongated tube-shaped, clearly distinguishable from bulbous vagina, distinctly longer than vagina, and widely rounded at extremity; spermathecal complex elongated bulbous, extending toward vagina; common oviduct branching from middle part of vagina ventrally.

Larva and pupa. Unknown.

Measurements and ratios. See Table 1 and 2.

Variation. Character states are stable between the three populations. On the other hand, some individual variations not related to the geographic origin of the specimen were recognized: presence of supplemental hairs on supraorbital area of head; degree of development of right mandibular proximal tooth of retinaculum (usually missing: Fig. 4, but sometimes perceptible and very slightly prominent: Fig. 5b); number of submental setae (six to nine setae); strength and state of engraving of pronotal basal foveae; degree of development of pronotal hind angles, serration on elytral shoulders, elytral striae, and apical striole of elytra (usually vestigial, but if perceptible, barely directed to termination of stria 5); position of elytral setiferous dorsal pores (frequently unstable between each elytron even in a single specimen; in holotype, position of anterior pore of left elytron exceptionally behind level of 4th pore of marginal umbilicate series: Fig. 4); presence of elytral scutellar striole, and posterior apical pore of elytra; presence of supplemental fairly long hairs on sternites 4–6 (sometimes present at inner part of paramedian setae on both or one side of some sternites, more frequently on sternites 4 and 5); number of paramedian setae on sternite 7 in female (usually two pairs but sometimes three pairs); 14) degree of curvature of median lobe, and size of sagittal aileron.

Etymology. The specific name is derived from the superlative of the Latin adjective *pulcher*, meaning the most beautiful; adjective.

Habitat information. Ufugushikumui-abu-dai-ichi Cave, the type locality of the present new species is located in the northwestern part of the Motobu Peninsula (Figs 2, 25–27), and included in the special protection zone of the Okinawa Kaigan Quasi-National Park. This cave opens near the peak of Mt. Ufugushikumui (237 m above sea level) and is composed of two pitches. The first and second pitch are about 35 m and 13 m deep, respectively, and are connected by a constriction. The floors of both caves are covered with rubble. The individuals of *Ryukyuaephaenops pulcherrimus* gen. nov. & sp. nov. were found only in the second pitch, with most individuals walking on moist stalagmitic walls. The air temperature and the relative humidity of the bottom

were about 21°C and 100%, respectively (measured on 1st November 2022). Although biological surveys were conducted in this cave in the past, no trechine beetles were found before (SHIMOJANA 1979).

Cave A, the second known locality, is about 1.3 km to the east from the type locality. This cave opens on a small conical hill and is about 15 m deep. The floor is connected to a small room (about 4 × 7 m wide) via a narrow horizontal passage. The floor of the room is extensively covered with thickly deposited clay and rubble. The individuals of *Ryukyuaephaenops pulcherrimus* gen. nov. & sp. nov. were collected in the room and most individuals were walking on moist stalagmitic walls (see Videos S1–S2). The air temperature and the relative humidity of the room were about 22.1°C and 100%, respectively (measured on 8th June 2022).

Cave B, the third known locality, is about 150 m to the east from Cave A. This cave has a large entrance and is dry on a wide area and composed of two pitches. The depth of the first and second pitch are about 5 m and 7 m, respectively. The individual of *Ryukyuaephaenops pulcherrimus* gen. nov. & sp. nov. was found in the narrowest part of the second pitch, which is highly humid. Despite multiple intensive surveys, only single female specimen and some body parts have been found in this cave. The air temperature and the relative humidity of the second pitch were about 17.4 °C and 93%, respectively (measured on 8th April 2023).

Distribution. Japan: Okinawa-jima Island in the Ryukyus (Figs 1, 2) (known only from three pit caves in the Motobu Peninsula).

Population genetic analyses

Genetic analysis based on the mitochondrial *COI* region (1401 bp) identified four haplotypes of *Ryukyuaephaenops pulcherrimus* gen. & sp. nov. from the type locality, Cave A, and Cave B (Fig. 29). The percentage of sequence differences (*p*-distance) among these haplotypes were 0–0.14% within the same cave and 0.07–0.14% between different caves. The mitochondrial *16S* region (525 bp) was

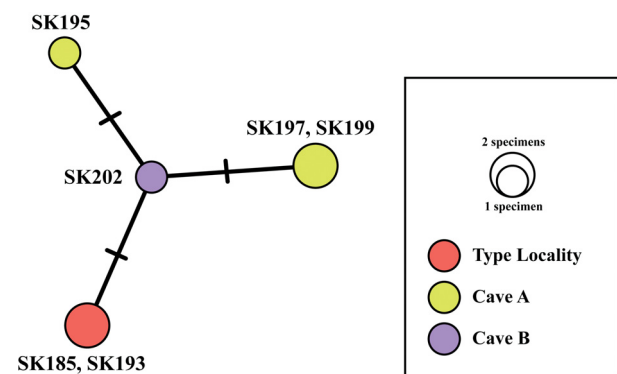


Fig. 29. Haplotype network of *Ryukyuaephaenops pulcherrimus* gen. & sp. nov. based on mitochondrial cytochrome oxidase subunit I (*COI*) gene sequences (1401 bp) consisting of 4 haplotypes ($n = 6$ specimens). The short black bars on the lines connecting haplotypes indicate the number of substitution sites, the size of the circle indicates the sample size for each haplotype, and the color of the circle indicates the collecting locality.

identical in all six individuals from the three caves used in this study. In order to capture a potential phylogeographic structure, more individuals would be necessary.

Discussion

Ryukyuaphaenops pulcherrimus gen. & sp. nov. is undoubtedly one of the most specialized subterranean species of all known troglomorphic beetles in Japan so far. The discovery of this highly specialized troglomorphic species of the aphaenopsian type from the Ryukyus, a mere 1,200 square kilometers in the large distributional gap of subterranean Trechina that stretches between Japan and Taiwan (Figs 1, 2), is biogeographically and biospeleologically quite remarkable.

The Ryukyu Archipelago contains many limestone caves scattered throughout the archipelago, and a number of cave animals have been known so far (SHIMOJANA 1979, MACHIDA et al. 2001, NAKA & MARUYAMA 2018, FUJITA 2018, WORSAAE et al. 2021). However, the fact that highly distinctive lineages as *Ryukyuaphaenops* gen. nov. have not been found so far strongly suggests that the diversity of the subterranean fauna of the Ryukyus may still be underestimated. It is possible that more troglomorphic trechine beetles will be discovered in the Ryukyus through intensive fieldwork in the future.

Unfortunately, it is difficult to discuss the origin and phylogenetic position of *Ryukyuaphaenops* gen. nov. within the subtribe Trechina at present time, due to the lack of comprehensive molecular phylogenetic studies in East Asia. However, morphological observations in the present study suggest that *Ryukyuaphaenops* gen. nov. has no direct relationship with other genera distributed in areas adjacent to the Ryukyus, such as Taiwan and western Japan, but may have some relationship to the aphaenopsian genera distributed in the inland of China. We are currently preparing a paper on the phylogenetic position of *Ryukyuaphaenops* gen. nov. and other major Japanese genera of Trechina within the world Trechina. *Ryukyuaphaenops* gen. nov. may be an important taxon for revealing the origin and phylogenetic position of aphaenopsian Trechina in Asia.

Although the monophyly of the “*Trechoblemus* Phyletic Series” of JEANNEL (1928), CASALE & LANEYRIE (1982) and CASALE et al. (1998) remains to be tested, *Ryukyuaphaenops* gen. nov. may be considered to be placed in the *Trechoblemus* Phyletic Series, based on the following characteristics: 1) body pubescent; 2) mentum fused with submentum; (3) submentum multisetose; 4) protibiae wholly pubescent; and 5) aedeagus with anisotropic copulatory piece. Members of the *Trechoblemus* Phyletic Series, comprising more than 270 species in 30 genera, are distributed in the Euro-Siberian Region, East and Central Asia, and western North America (JEANNEL 1928; UÉNO & PAWLOWSKI 1981; CASALE & LANEYRIE 1982; UÉNO 1987, 2011; BELOUSOV & KABAK 1993, 2003; BOUSQUET 2012; FANG et al. 2016, 2020; BELOUSOV 2017; NAITÔ 2017, 2018, 2020, 2022a,b; TIAN et al. 2023). In addition, MADDISON et al. (2019) recently revealed that the North American *Ameroduvallius* Valentine, 1952 and *Darlingtonia* Valentine, 1952, both originally placed in their

own series, are part of the *Trechoblemus* Phyletic Series based on molecular characters. *Ryukyuaphaenops* gen. nov. is immediately distinguished from all above genera except *Ameroduvallius* and *Darlingtonia* by a different body form, with large body size and elongate appendages. Moreover, *Ryukyuaphaenops* gen. nov. differs strikingly in the peculiar formation of the humeral set of umbilicate pores (compared to the almost equidistant formation in the above genera except *Suzuka* Uéno, 1956 and *Gulaphaenops* Uéno, 1987). *Suzuka* and *Gulaphaenops* are immediately distinguishable from *Ryukyuaphaenops* gen. nov. by the peculiar body shape, small body size, presence of distinct pronotal hind angles and elytral basal transverse furrow, the 1st pore of the humeral set of marginal umbilicate pores is widely distant from marginal gutter, and striae completely vanished (UÉNO 1956, 1987).

On the other hand, as mentioned in the diagnosis, *Ryukyuaphaenops* gen. nov. shares many taxonomically important characters with some Chinese aphaenopsian genera, such as *Boreaphaenops* and *Yanzaphaenops*. Surprisingly, the two genera are distributed in the northern bank of Yangtze River in the Hubei Province (UÉNO 2002, 2005), more than 1,700 km from the locality of *Ryukyuaphaenops* gen. nov. (Fig. 1). Future comprehensive taxonomic studies of Chinese Trechina may clarify the relationship between *Ryukyuaphaenops* gen. nov. and Chinese aphaenopsian genera.

Genetic analysis based on the mitochondrial *COI* region ($n = 6$) revealed only 1–2 base pair substitutions and genetic distances (p -distance) of 0.07–0.14% among the three known localities of *Ryukyuaphaenops pulcherrimus* gen. & sp. nov. Their genetic divergence is very low-level compared to results of previous studies examining intraspecific variation in cave-inhabiting Trechini (FAILLE et al. 2015, BOYD et al. 2020). Although we sequenced only a small number of specimens due to the difficulty of collecting, our results indicate that specimens from all three caves may represent a single largely undifferentiated population with ongoing genetic exchange through subterranean interstices. In the future, a follow-up study with a large number of specimens will need to be conducted for each population to determine haplotype diversity more accurately. In addition, population genetic analyses using RAD-seq (ARNOLD et al. 2013) and/or MIG-seq (SUYAMA & MATSUKI 2015) should be conducted to verify the presence or absence of genetic exchange between habitats in order to understand appropriate conservation units for the populations. The DNA sequence in the *COI*-5' region is also a general region of DNA barcoding in insects (HEBERT et al. 2003, JINBO et al. 2011); the DNA data provided in this study will hence be useful for future studies focused on conservation and environmental DNA.

This study suggested that *Ryukyuaphaenops pulcherrimus* gen. et sp. nov. is widely distributed in the massif of the northwestern area of the Motobu peninsula including all the known localities. Considering that more than one individual was found during almost every time during multiple surveys in the caves and that many specimens (81 in total) were collected using traps, we suppose that the

sampled populations of *R. pulcherrimus* gen. & sp. nov. are stable and the number of individuals of *R. pulcherrimus* gen. & sp. nov. in the habitat area is likely not very small. However, due to trap condition bias, 95% of the individuals obtained in the traps were the result of only two traps (see also materials and methods section). At this point, the survey of subterranean Trechina in the Ryukyu Archipelago has only just begun. Further surveys may resolve this issue; moreover, new taxa may be discovered in the process. In recent years, the central government and local governments in Japan have been increasingly regulating the collection and research of organisms, but considering the risk that such discoveries may be overlooked, we believe that excessive collection restrictions should be imposed with great caution. With regard to this new species, we believe that there is little likelihood that normal collection surveys will have an impact on the conservation of the population. In fact, in 1979, a biological survey conducted in the cave where *R. pulcherrimus* gen. & sp. nov. was later discovered (SHIMOJANA 1979) did not find *R. pulcherrimus* gen. & sp. nov., and it was overlooked until this paper. We hope that further field surveys by professional and amateur entomologists and enthusiasts will help to reveal the diversity of Trechina in the Ryukyu Archipelago.

On the other hand, land development and soil pollution may have extremely serious effects on the conservation of *Ryukyuaephaenops pulcherrimus* gen. & sp. nov. The limestone areas of the Motobu Peninsula have been preserved for many years through the efforts of local residents. While paying respect to their efforts, continued conservation of the habitat is necessary in the future.

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Supplementary data

Videos S1–S2. *Ryukyuaephaenops pulcherrimus* gen. & sp. nov. walking on the surface of cave wall at Cave A. Videos taken in October 2022 by SK. Available at the web page of *Acta Entomologica Musei Nationalis Pragae* as well as in the Zenodo archive (<https://doi.org/10.5281/zenodo.8288469>) and YouTube (<https://youtu.be/OXFaHbzFI18> and <https://youtu.be/Va2cktjbQoc>).

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