



## ***Pachyprotasis kojimai* sp. nov.—the “*Pachyprotasis nigronotata*” of Japanese authors (Hymenoptera: Tenthredinidae)**

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### **Abstract**

The species formerly known as *Pachyprotasis nigronotata* Kriechbaumer, 1874 in Japan is described as *P. kojimai* Taeger & Shinohara, **sp. nov.** Both taxa are not very closely related, but were hitherto mixed up because of their similar coloration. The new species is endemic to the mountains of central Honshu and *P. nigronotata* is to be deleted from the fauna of Japan.

**Key words:** Symphyta, sawflies, Japan, COI barcoding, relationships

### **Introduction**

During an expedition to the Nagano area at Honshu, Japan, that was carried out by the first two authors together with Mr. Haruyoshi Kojima, several specimens of a very conspicuous bright green *Pachyprotasis* species were collected. In the field these specimens were named *Pachyprotasis nigronotata*, but already there it was noted that the latter species in Europe has a rather different appearance in coloration. Subsequently we noted that the species previously named *nigronotata* in Japan is hitherto undescribed. Thanking H. Kojima for his valuable support of our expedition, we describe this species here as *Pachyprotasis kojimai* Taeger & Shinohara, **sp. nov.**

*Pachyprotasis* Hartig, 1837 is a large sawfly genus within the Tenthredininae. Currently about 215 taxa are considered valid, including some subspecies (Taeger *et al.* 2010; Zhong & Wei 2010a, b, 2012, 2013; Haris 2014; Zhong *et al.* 2015). All known species occur in Asia, and only five of these are also recorded from Europe. The widely distributed type species *P. rapae* (Linné, 1767) additionally occurs in North America including Mexico (Smith 2003) (lectotype see <http://linnean-online.org/16655/>, designated by Malaise & Benson 1934). The relationships within the genus are unsolved. Zhong & Wei (2010a) provided a grouping based on color and later (Zhong *et al.* 2015) modified this by dividing of one group in two.

Yoshida (2014) recorded 38 *Pachyprotasis* species for Japan. However, Inomata (1984) postulated based on his studies about 70 species for Japan. Unfortunately, many of these still remain undescribed. Only five of the already named Japanese taxa were not originally described from Japan: *P. antennata* (Klug, 1817) (type locality Austria: Carinthia), *P. lineicoxis* Malaise, 1931 (Russia: Vladivostok), *P. longicornis* Jakovlev, 1891 (China: Gansu), *P. rapae* (Linné, 1767) ([Sweden?] no type locality given), *P. nigronotata* Kriechbaumer, 1874 (Germany: Baierbrunn). The remaining Japanese taxa [numbers in brackets] were described by Smith (1871 [2]) Marlatt (1898 [1]), Malaise (1931a [2], 1931b [1]), Okutani (1961 [2]), Togashi (1963 [4]), Inomata (1970 [16], 1984 [4]), and Inomata & Naito (in: Naito & Inomata 2006 [1]).

## Material and methods

The following abbreviations are used:

DEI-GISHym[number]—ID numbers of specimens with additional genetic data or figures;

NHRS—Naturhistoriska riksmuseet, Stockholm, Sweden;

NSMT—National Museum of Nature and Science, Tsukuba, Japan;

SDEI—Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany;

USNM—National Museum of Natural History, Department of Entomology, Washington D.C., USA;

Primers:

SymF1—5'-TTTCAACAAATCATAAARAYATTGG-3' (Prous et al. 2016)

SymC1-J1751—5'-GGAGCNCCTGATATAGCWTTYCC-3' (Prous et al. 2016)

SymR1—5'-TAAACTTCWGGRTGICCAAARAATC-3' (Prous et al. 2016)

A2590—5'-GCTCCTATTGATARWACATARTGRAAATG-3' (Normark et al. 1999)

Photos were taken by the first author with a Leica DFC 495 digital camera and a Leica M405 C stereomicroscope. Composite images with an extended depth of field were created from stacks of images using the software CombineZP, and finally arranged and partly enhanced with Ulead PhotoImpact X3. Additional high resolution images of the new species are given on figshare.com:

DEI-GISHym85024 <https://dx.doi.org/10.6084/m9.figshare.4216200>

DEI-GISHym85027 <https://dx.doi.org/10.6084/m9.figshare.4216203>

DEI-GISHym85028 <https://dx.doi.org/10.6084/m9.figshare.4216206>

DEI-GISHym85029 <https://dx.doi.org/10.6084/m9.figshare.4216212>

DEI-GISHym85030 <https://dx.doi.org/10.6084/m9.figshare.4216209>

Complete set: <https://dx.doi.org/10.6084/m9.figshare.4010628>.

Additional figures are also given for

*Pachyprotasis nigronotata*: <https://dx.doi.org/10.6084/m9.figshare.4216269> and

*P. simulans*: <https://dx.doi.org/10.6084/m9.figshare.4233194>

Morphological terms follow Viitasaari [2002].

The distribution map was made with Map data © 2016 Google, ZENRIN.

DNA extraction from three specimens of *Pachyprotasis nigronotata* (DEI-GISHym31223, 31244 and 84936), four specimens of *P. kojimai* (DEI-GISHym86989–86992) and two additional *Pachyprotasis* specimens from the Russian Far East (*P. pedatoria*, DEI-GISHym86260 and *Pachyprotasis* sp. prope *simulans*, DEI-GISHym86218) was performed using a single leg. Total genomic DNA was extracted using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek Inc., Norcross, USA) according to the manufacturer protocol for tissue DNA, except some smaller modifications. Elution was performed twice with 100 µl Elution buffer each. A partial fragment (1078bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by PCR using the primers SymF1 and A2590. Amplifications were performed in 20 µl reactions containing 10 µl 2x Qiagen Multiplex PCR Plus Master Mix (Qiagen, Hilden, Germany), 0.3 µM of each primer, RNase-free water and 1 µl template DNA. Amplification conditions were: initial PCR activation step at 95°C 5 min, 38 cycles of 30 s denaturing at 95°C, 90 s annealing at 47°C, 90 s extension at 72°C, followed by a final extension of 30 min at 68°C. PCR products were visualized on a 1.4% agarose gel stained with Gel Red (0.1, Biotium, Hayward, USA). PCR products were purified with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Life Technologies, Darmstadt, Germany) and sequenced on an ABI3730XL sequencer using Big Dye v. 3.1 Terminator Kit (Thermo Fisher Scientific, Darmstadt, Germany) by Macrogen, Netherlands. Sequencing was performed with SymF1 and the internal primers symC1-J1751 and SymR1. Sequences were assembled and manually checked using Geneious 9.1.6 (Kearse et al. 2012) and aligned using BioEdit 7.2.5 (Hall 1999).

DNA extraction and sequencing of specimens of other *Pachyprotasis* species with DEI-GISHym and BC ZSM HYM numbers included in the calculation of the cladogram were performed by the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Canada (see Schmidt et al. 2016 for details). The *Pachyprotasis* sequences used for

the tree (Fig 5) are available in GenBank, except for the two specimens from Taiwan which are accessible through the website of Barcode of Life Data Systems ([www.barcodinglife.com](http://www.barcodinglife.com)). A specimen of *Macrophya montana* (Scopoli, 1763) (GenBank accession number KC976115.1) was used for the rooting of the cladogram. Maximum Likelihood analyses were performed in MEGA 7.0.18 (Kumar *et al.* 2016). Best fitting model for the data set, the Tamura 3-parameter model (Tamura 1992) including gamma-distributed rates, was revealed by jModelTest 2.1.7 (Darriba *et al.* 2012) and run with 1000 bootstrap replications.

## Results

### *Pachyprotasis kojimai* Taeger & Shinohara sp. nov.

(Figs 1, 2, 4)

*Pachyprotasis nigronotata* [not Kriechbaumer 1874]: Takeuchi 1952: 20; Togashi 1961: 32; 1965: 246, plate 125, 32; 1970: 63; Naito 1982: 574.

**Description.** FEMALE (Figs 1A–F, 2A): Body length 8.5 mm, fore wing 10.0 mm, antenna about 5.5 mm (holotype). Antenna filiform, almost not narrowed toward the apex, last antennomere apically blunt. 3rd antennomere about  $1.2 \times$  as long as 4th and  $2.0 \times$  as long as 8th. 8th antennomere about  $4.0 \times$  as long as broad. Head in dorsal view somewhat narrowed behind the eyes. Postocellar area about  $1.8 \times$  as broad as long, almost flat and not distinctly higher than the remaining upper head, lateral postocellar furrows deep. Occipital carina prominent and complete. Distance between the lateral ocelli (postocellar line, POL) about  $1.5 \times$  as long as diameter of the median ocellus (OD), ocular ocellar line (OOL)  $4 \times$  OD, ocellar-occipital carina line (OOCL) nearly  $3 \times$  OD. Frontal area with only slightly elevated ridges with shallow furrow between them, antennal furrows shallow. Clypeus apically roundly emarginated to about 0.4 of its length. Labrum apically almost straight, somewhat reflexed. Malar space slightly longer than OD, distance between the toruli about  $2 \times$  OD. Head silky shiny, with very shallow microsculpture and few small distant pits. Microsculpture on the remaining body clearly stronger, less shiny, pits more distinct with distances of about  $2\text{--}5 \times$  their own diameter. Hairs usually clearly shorter than OD. Apical margin of sternum 7 (hypopygium) slightly emarginate. Lancet with rather flat serrulae.

MALE (Figs 1G–L, 2B–C): similar to the female; antennae more slender, 8th antennomere almost  $6 \times$  as long as broad. Inner tooth of the claw larger than in female.

**Color:** alive almost completely pale green, dead specimens fading to straw. Antenna dorsally black (scape may be completely pale), pro- and mesotibiae and their tarsi posteriorly black lined, metatibia dorsally black lined and apically black, metatarsus mainly black, metafemur subapically posteriorly black marked. Area between the ocelli, apices of the mandibles, parts of the down bent areas of the lateral mesoscutal lobes, and middle parts of mesopostnotum black marked. Wings hyaline, pterostigma green, remaining veins predominantly dark.

**Variability.** Body size 8–10 mm. First antennomere (scape) dorsally more or less black lined or completely pale (e.g., holotype). The median mesoscutal groove and the anterior margin of the median mesoscutal lobe may be narrowly black marked. Metatibia either completely black lined, or subapically with green ring (e.g., holotype).

**Haploid chromosome number:** 10 (Naito 1982, given for “*P. nigronotata*”); spermatheca: fig. 246 in Togashi (1970), given for “*P. nigronotata*”. COI barcodes see below (paratypes).

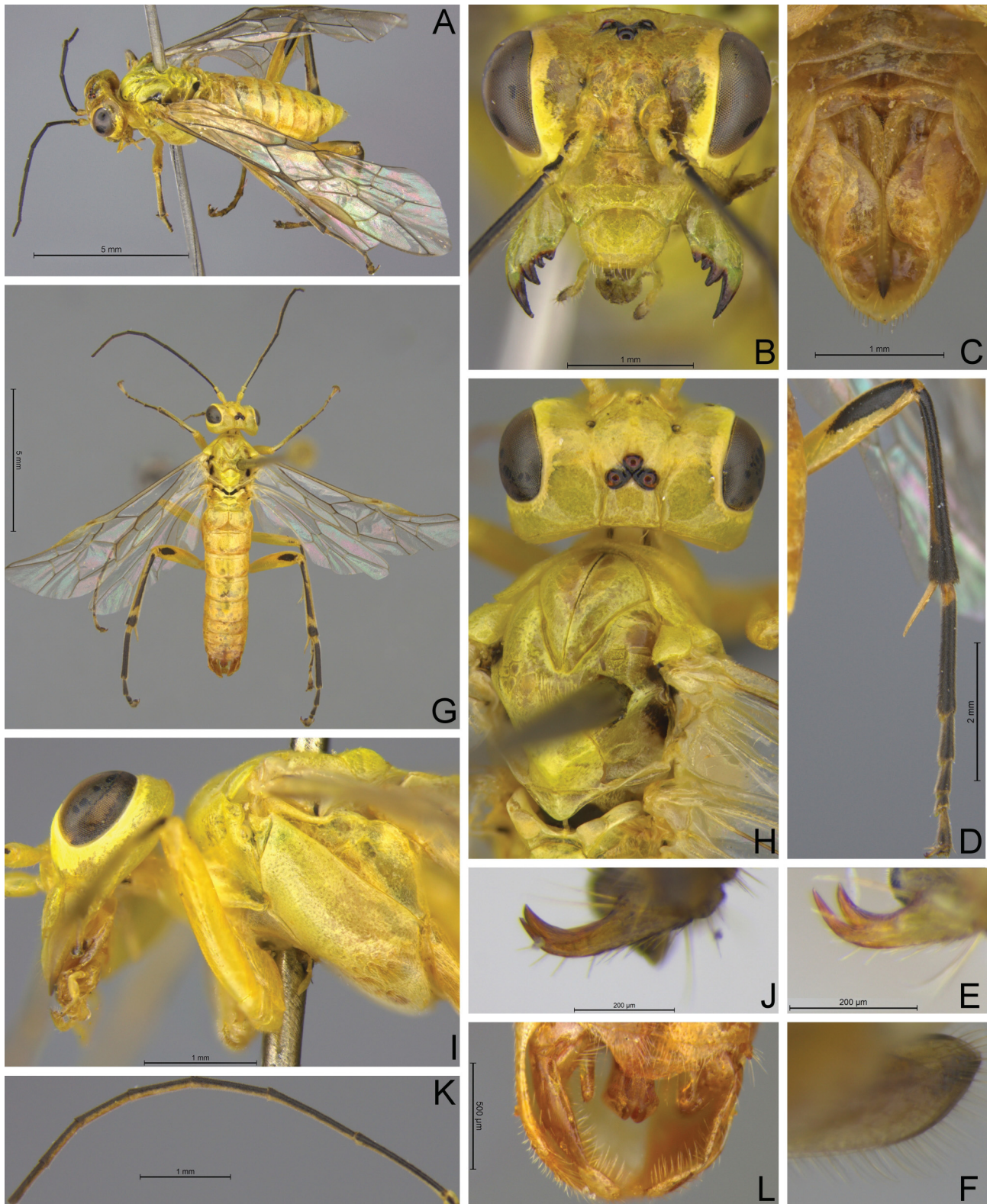
**Host plant(s)** unknown.

**Type material.** *Holotype* ♀, **Niigata Pref.:** N of Lake Otomi, 36.873°N 138.063°E, 1275 m, 30.07.2016, H. Kojima, NSMT (DEI-GISHym85030).

*Paratypes* 10 ♂ 46 ♀ (NSMT, SDEI, USNM, NHRS). Specimens collected by A. Shinohara if not mentioned otherwise. Altitudes in brackets [ ] are not taken from labels but estimated according to the locality information. **Gifu Pref.:** 1♀ Shinhotaka-onsen, 36.285°N 137.574°E, [1100 m], 23.07.1980; 1♀ Kuriyadani (Kita Alps) 36.278°N 137.556°E [1200–1900m], 13.08.1977, H. Kumamoto; **Gunma Pref.:** 1♀ Sugenuma–Marunuma, 36.800°N 139.300°E, [1450–1750 m], 08.07.1979, T. Niisato; 1♂ Marunuma, Gunma, 36.834°N 139.347°E, [1450 m], 27.06.1980, T. Shimomura; **Nagano Pref.:** 2♀ Kamikochi, 36.250°N 137.633°E, [1550 m], 17.07.1927, K. Sato; 1♂ Yokoodani, Kamikochi, 36.249°N 137.639°E, 1600–2000 m, 21.07.1989–23.07.1989 (DEI-GISHym85024); 1♂ Yarisawa, Kamikochi, Nagano, 36.256°N 137.653°E, 1600–1900 m, 18.07.1989–22.07.1989 (DEI-GISHym85027); 1♂ 6♀, 1800–2100 m, 18.07.1989–22.07.1989; 1♂ 31.07.1990; 1♀ Tokusawa, Kamikochi, 36.264°N 137.690°E, [1600 m], 17.07.1989; 1♀ 04.08.1989–06.08.1989; 1♀ 01.08.1989, S. Shinonaga; 1♀

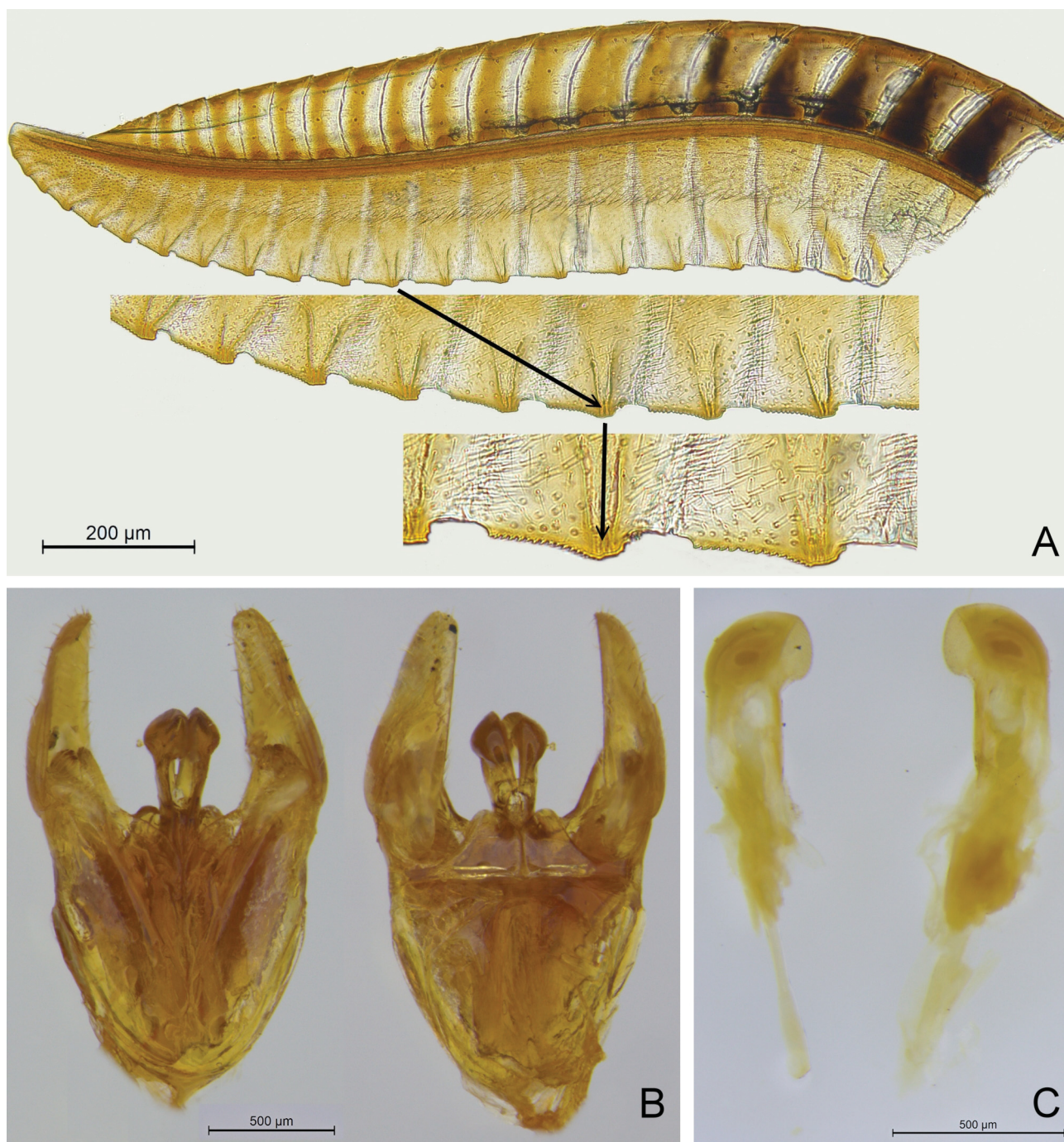


Shimashimadani, 36.170°N 137.778°E, [750–2000 m], 25.07.1980–28.07.1980, E. Nishida; 1 ♂ Ichinosawa (Nagano), 36.334°N 137.772°E, [1250–2000 m], 01.08.1933, Y. Nakajima; 1 ♀ Mt. Kyogatake, Chuo-Alps, 35.912°N 137.862°E, [2250 m], 28.07.1985, K. Mizuno; 1 ♀ Asama ski area, road, 36.412°N 138.440°E, 2000 m,



**FIGURE 1.** *Pachyprotasis kojimai* Taeger & Shinohara **sp. nov.** A—holotype, ♀, dorsolaterally; B–F (paratype, ♀) B—face, C—apex of abdomen ventrally, D—hind leg dorsolaterally, E—claw of metatarsus, F—sawsheath laterally; G–L (paratype, ♂) G—dorsally, H—head and thorax dorsally, I—head and thorax laterally, J—claw of metatarsus, K—antenna laterally, L—apex of abdomen dorsally.





**FIGURE 2.** *Pachyprotasis kojimai* Taeger & Shinohara **sp. nov.** (paratypes). A—ovipositor: lance and lancet, details of serrulae; B—genital capsule ventrally and dorsally; C—penis valves.

29.07.2016, A. Taeger (DEI-GISHym86991, KY124264) 2 ♀ same data, H. Kojima; 1 ♀ Omeshidake W, Road 112 (= Mt. Omeshidake, 36-37-30N 138-27-17E, Takayama), 36.624°N 138.454°E, 1850–1900 m, 22.07.2016; 1 ♀ 1900 m, 22.07.2016, A. Taeger (DEI-GISHym86990, KY124263); 1 ♀ same data; 1 ♀ 27.07.2016; 1 ♀ 27.07.2016, A. Taeger (DEI-GISHym85029); 1 ♀ Makuiwa, Shiga-kogen, 36.704°N 138.485°E, [1550 m], 02.06.1985; 1 ♂ Minoto, Mts. Yatsugatake, 35.982°N 138.336°E, [1850 m], 18.07.1980; 1 ♂ 1 ♀ 29.07.1986–03.08.1986; 2 ♀ 04.08.1987–08.08.1987; 1 ♀ 04.08.1988–08.08.1988; 2 ♀ 23.07.1996–26.07.1996; 2 ♀ 27.07.1999–31.07.1999; 1 ♂ 1 ♀ 25.07.2000–29.07.2000; 1 ♀ Ichinose, Yamanouchi, 36.744°N 138.524°E, 1600 m, 20.07.2016; **Niigata Pref.:** 1 ♀ N of Lake Otomi (= Sasagamine, 36-52-28N 138-03-38E, Myoko), 36.873°N 138.063°E, 1240 m, 21.07.2016; 1 ♀ 1275 m, 21.07.2016, A. Taeger (DEI-GISHym86989, KY124262); 1 ♀ 30.07.2016, A. Taeger (DEI-GISHym86992, KY124265); 1 ♀ 30.07.2016, H. Kojima; 1 ♀ same data (DEI-GISHym85028); **Tochigi Pref.:** 1 ♀

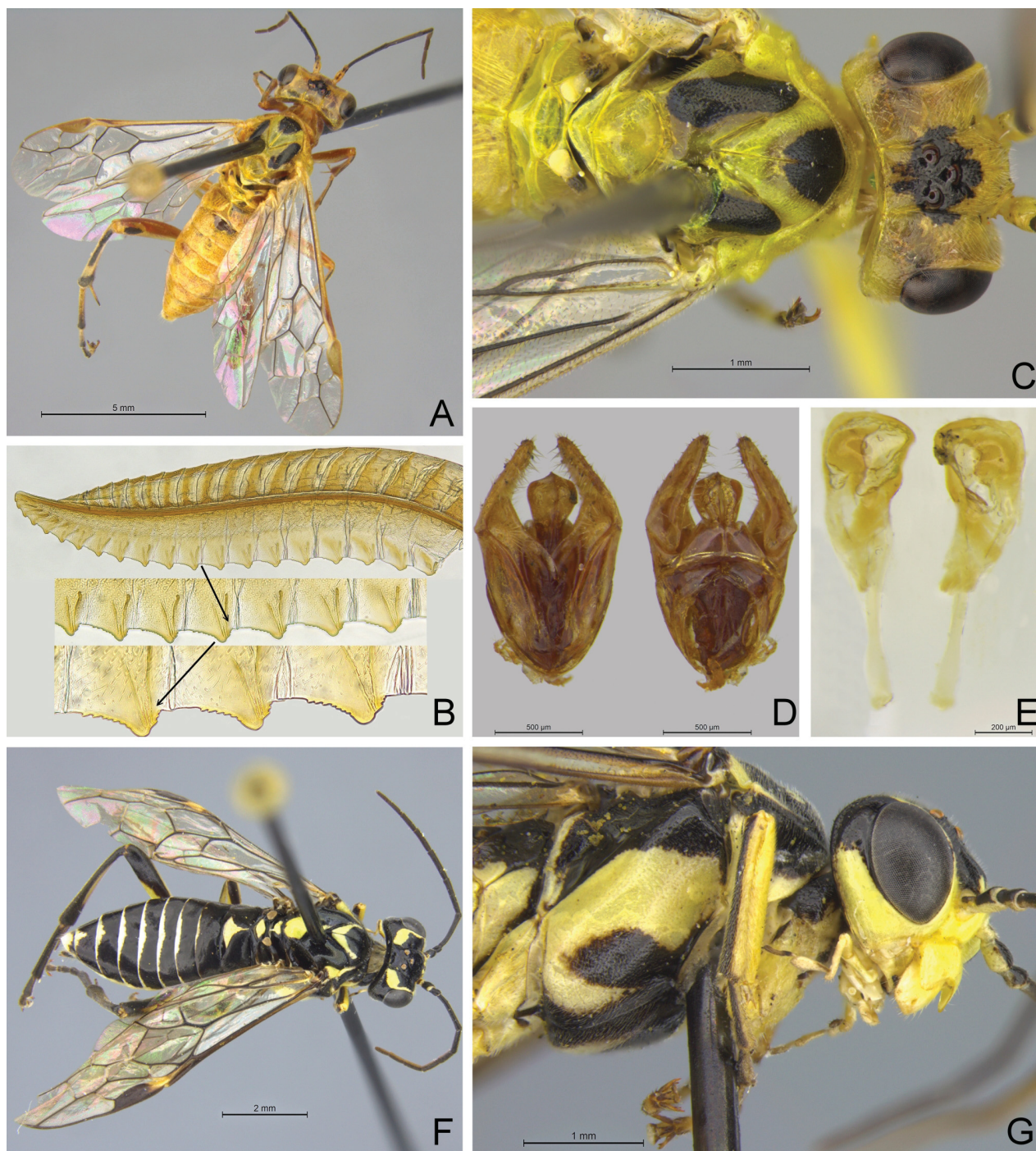


Yumoto, Oku-Nikko, 36.806°N 139.418°E, [1500 m], 26.06.1977; 1♀ 10.06.1978; 1♀ 28.06.1972, T. Saito; 1♂ 1♀ 30.07.1986, T. Saito; **Yamanashi Pref.:** 1♀ Aoki-kosen–Houougoya, 35.700°N 138.330°E, [1100–2400 m], 25.07.1987–25.07.1987, K. Mizuno; 1♀ Sagashio, 35.690°N 138.780°E, [1200 m], 25.07.1957, K. Sato.

**Other records (non type material).** (Togashi 1961, as *P. nigronotata*, specimens not examined) **Ishikawa Pref.:** Mt. Hakusan: 2♀ Naka-hanba-ato, 36.128°N, 136.750°E [1500 m], 07.07.1948; 1♀ 17.07.1954; Taka-hanba-ato, 36.136°N, 136.761°E [1950 m], 1♀ 22.07.1952; Yanagidani 36.128°N, 136.753°E [1500 m], 1♀ 24.07.1955.

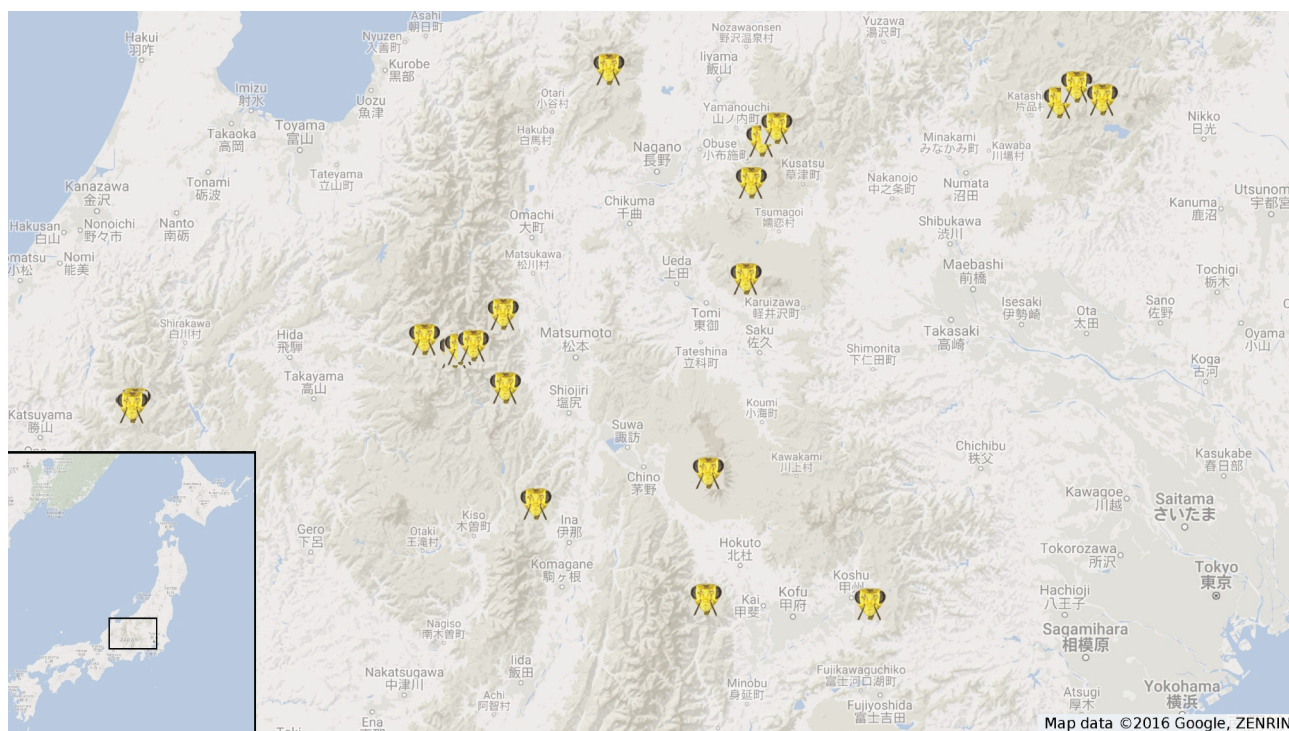
**Distribution.** Japan (Honshu).

**Etymology.** The species is dedicated to Mr. Haruyoshi Kojima (Nagano).



**FIGURE 3.** *Pachyprotasis nigronotata*. A–B (♀, Primorsky Kray) A—dorsally, B—ovipositor: lance and lancet, details of serrulae; C (♂, Estonia)—head and thorax dorsally; D (♂, Korea)—genital capsule ventrally and dorsally; E (♂, Korea)—penis valves. *Pachyprotasis simulans*. F–G (♀, Germany) F—dorsally, G—head and thorax laterally.





**FIGURE 4.** Distribution of *Pachyprotasis kojimai* Taeger & Shinohara **sp. nov.** (Japan, central Honshu).

## Discussion

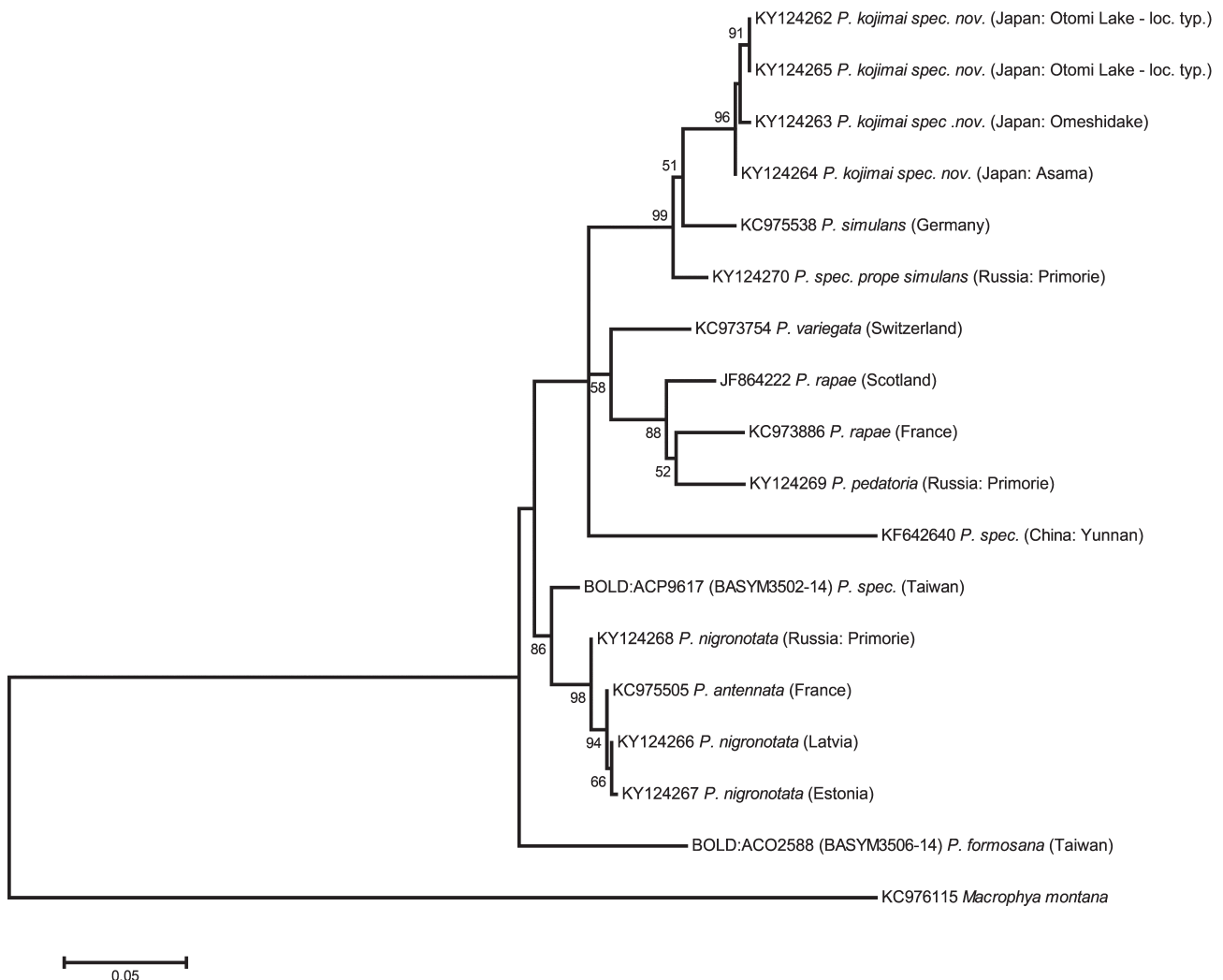
*Pachyprotasis kojimai* seems to be an endemic Japanese species. It is hitherto only known from the mountains of central Honshu (altitude usually between 1000 and 2000 m) (Fig. 4). The flight period of the species apparently is in summer, almost all specimens were collected between late June and the beginning of August.

Takeuchi (1952) recorded *P. nigronotata* Kriechbaumer, 1874 for the first time for Japan. No further details are given for this record. Almost certainly, this record and all subsequent records from Japan (Togashi 1961, 1965, 1970; Naito 1982) are based on *P. kojimai*. No specimens of true *P. nigronotata* from Japan are known to us, and therefore *P. nigronotata* is to be removed from the Japanese fauna. The former identifications are very likely only a result of the similarity caused by the very pale coloration of both species. Hitherto, *P. nigronotata* was claimed to be the palest *Pachyprotasis* species (Zhong & Wei 2012). However, the here described *P. kojimai* is even paler: apart from a clearly much more dense punctuation and different genitalia, *P. nigronotata* is distinguished by the upper head and upper thorax with much more black marks (Figs 3A–E). The latter species is widely distributed from Europe to Eastern Siberia and Korea. It is extremely rare in central Europe (the westernmost record is a single female from Wales, UK, see Benson 1947), and it seems to become less rare in an easterly direction.

The phylogenetic relationships within *Pachyprotasis* are considered to be unknown. The groups created by Zhong & Wei (2010a) and Zhong *et al.* (2015) may be used for identification, but very likely these groups do not reflect phylogenetic relationships.

Genetic data (COI) are available only for a few *Pachyprotasis* species. These data were obtained in the course of the project of Schmidt *et al.* (2016) and during the present study. The only additional data available from GenBank concern *P. rapae*, *P. antennata*, *P. simulans* Klug, 1817 and *P. variegata* (Fallén, 1808) (all these taxa are also included in Schmidt *et al.* 2016). Finally, 11 taxa (3 of them unidentified or undescribed) were available for analysis (Fig. 5). In this analysis we found that *P. nigronotata* and *P. kojimai* are obviously not very closely related. Allopatric pairs of closely related species in sawflies from the Asian mainland and Japan are not rare (e.g., Blank *et al.* 2005; Shinohara & Zhou, 2006; Taeger *et al.* 2016), but the situation seems to be different in this case. *Pachyprotasis nigronotata* (from Eastern Europe and Russian Far East) appear in the same cluster as European *P. antennata*, whereas *P. kojimai* forms its own cluster in the group of *P. simulans* (from Europe) and a species similar to *P. simulans* from the Russian Far East. The differences in morphology and color are correlated with the different

barcodes. *P. simulans* (Figs 3F–G) is a dorsally largely black species, with clearly shorter mouthparts in comparison with *P. kojimai*. Nevertheless, differences in the barcode region alone seem to be not sufficient enough to separate different taxa, as demonstrated by Kristiansen (2013) for the different clusters in *P. rapae*. On the other hand, the same barcode is not necessarily an argument for synonymizing taxa. *Pachyprotasis nigronotata* and *antennata* are surely not conspecific, but may not be separated by their barcodes (Fig. 5).



**FIGURE 5.** The maximum likelihood tree (T92+G) showing relationships among 17 samples of 11 species of *Pachyprotasis*. Numbers on branches are bootstrap values (1000 bootstrap replicates), only values > 50% are shown.

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