

Description of the male genitalia of *Pristomyrmex punctatus* (Smith, 1860) (Hymenoptera, Formicidae, Myrmicinae)

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ABSTRACT. *Pristomyrmex punctatus* (Smith, 1860) is a parthenogenetic ant species. Colonies are usually composed of workers only, or rarely of workers and a few ergatoid queens. Workers and ergatoids reproduce thelytokously, however colonies containing alate males are often found. The habitus of the body of *P. punctatus* has already been observed and described by previous authors. In the present study, a detailed description of the male genitalia is provided.

Keywords: Formicidae, Myrmicinae, *Pristomyrmex punctatus*, male genitalia, external morphology, taxonomy.

INTRODUCTION

The myrmicine genus *Pristomyrmex* is a moderate-sized taxon distributed in the Old World tropics and subtropics, primarily in the Oriental region (Wang 2003). Wang (2003) comprehensively revised the classification of the genus *Pristomyrmex*, and provided descriptions of males for 17 species. Currently, males are described for 19 of the 59 valid, extant species (Bolton *et al.* 2015), or one-third of the total known diversity. *Pristomyrmex punctatus* was described by Smith (1860) based on the material from Batjan Island, Indonesia, and is widely distributed in Southeast and East Asia, including Japan (Wang 2003). *Pristomyrmex punctatus* is a parthenogenetic species, *i.e.*, colonies are usually composed of workers only, or often of workers and a few ergatoid queens (Itow *et al.* 1984). Although workers and ergatoids reproduce via thelytokous parthenogenesis, colonies containing alate males may be found in Japanese populations in early summer, although they are rare and no evidence of sexual reproduction has been found (Itow *et al.* 1984;

Tsuji 1988; Dobata *et al.* 2009). The external morphology of the male of this species was described by Ogata (1991) and Wang (2003). Itow *et al.* (1984) provided an illustration of the dorsal outline of entire male genitalia and mentioned “the copulatory organ is also normal”, but they did not provide a comparative description of the genital capsule, which is necessary for taxonomic purposes. Excluding Itow *et al.* (1984), the male genitalia of the genus *Pristomyrmex*, including *P. punctatus*, have not yet been described. In the present study, a detailed description of the male genitalia of *P. punctatus* is provided.

MATERIALS AND METHODS

Materials

A *Pristomyrmex punctatus* colony containing more than 100 alate males (Fig. 1) was found under a concrete block on the ground in a green tract of the Minami-Osawa Campus of Tokyo Metropolitan University. Twenty males of the



Fig. 1. Male of *P. punctatus* , Scale bar = 1.0 mm.

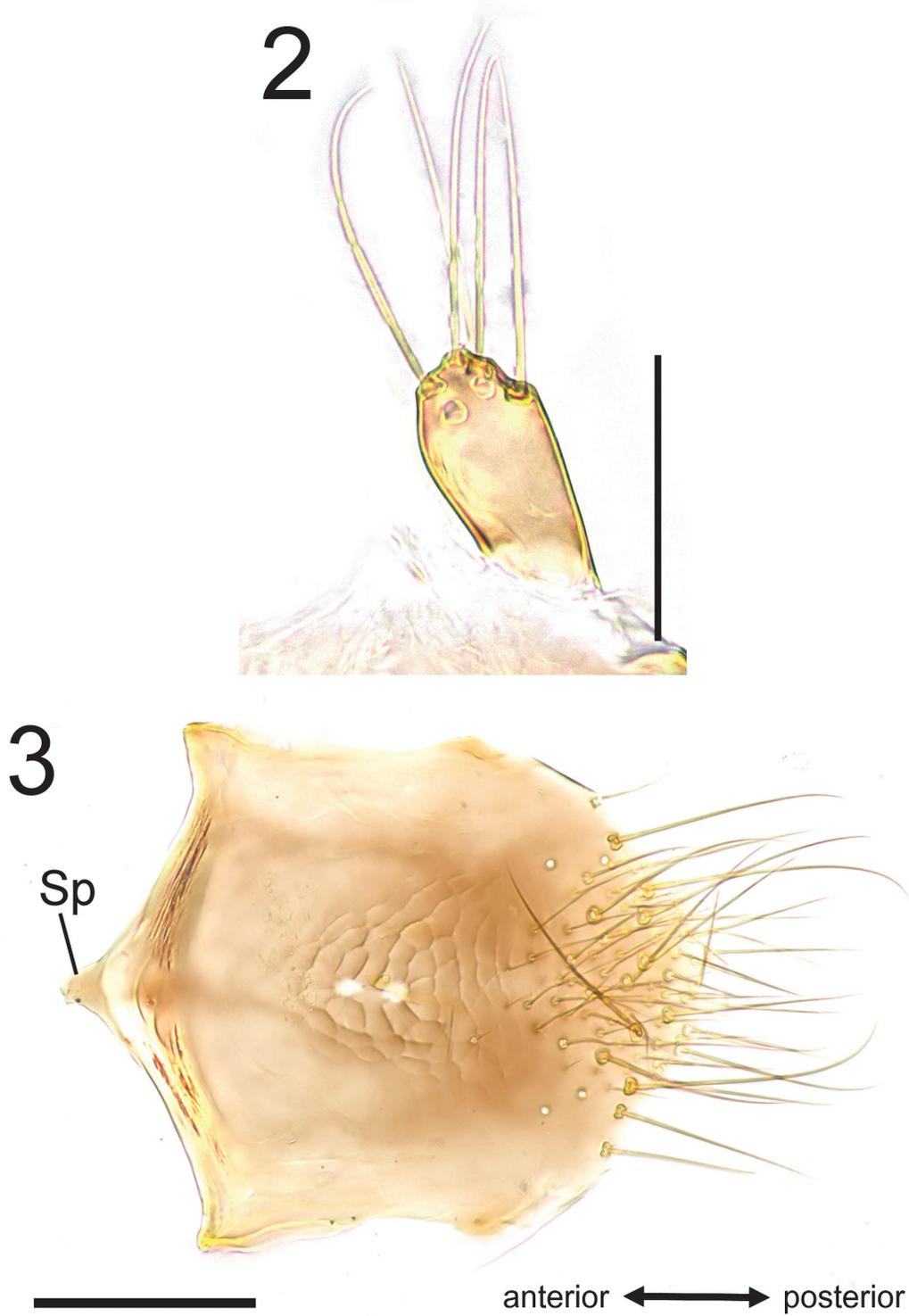
colony were examined in the present study. The detailed collection information of the material is as follows:

Japan: Tokyo: Hachioji-shi: Minami-Osawa: Tokyo Metropolitan University: Minami-Osawa Campus, N35°37'10", E139°22'57", ca. 135 m alt., 1.VIII.2015, K. Eguchi leg., Colony code: Eg01viii15-01.

Methods

Genitalia of 20 males preserved in 80% ethanol were slide-mounted following the steps below. The apical part of the gaster, including the genitalia, was cut off and washed with ca. 500 μ L TE

(pH 8.0) in a sterile disposable dish. The gastral apex was then transferred into 105 μ L of dissolving buffer [100 μ L of 10% Chelex-TE solution and 5 μ L Qiagen Proteinase K (Qiagen, Germany)], and incubated at 56°C for ca. 24 h, then heated at 99°C for 10 minutes to inactivate the Proteinase K. The apical part of the gaster was then picked up using a sterile disposable inoculating loop and transferred into a 1.5 mL microcentrifuge tube filled with in 99% ethanol for dehydration, and then male genitalia was separated from unnecessary parts and cleaned in a small dish filled with 99% ethanol. The genitalia were dissected into several main components using forceps in a small amount of Euparal (WALDECK GmbH & Co. KG) on a slide glass, and covered with a cover-



Figs 2 – 3. Pygostyle and abdominal sternite IX: (2) pygostyle, left side, dorsal view; (3) abdominal sternite IX, ventral view. Scale bars = 0.1 mm. Abbreviations: **Sp** spiculum.

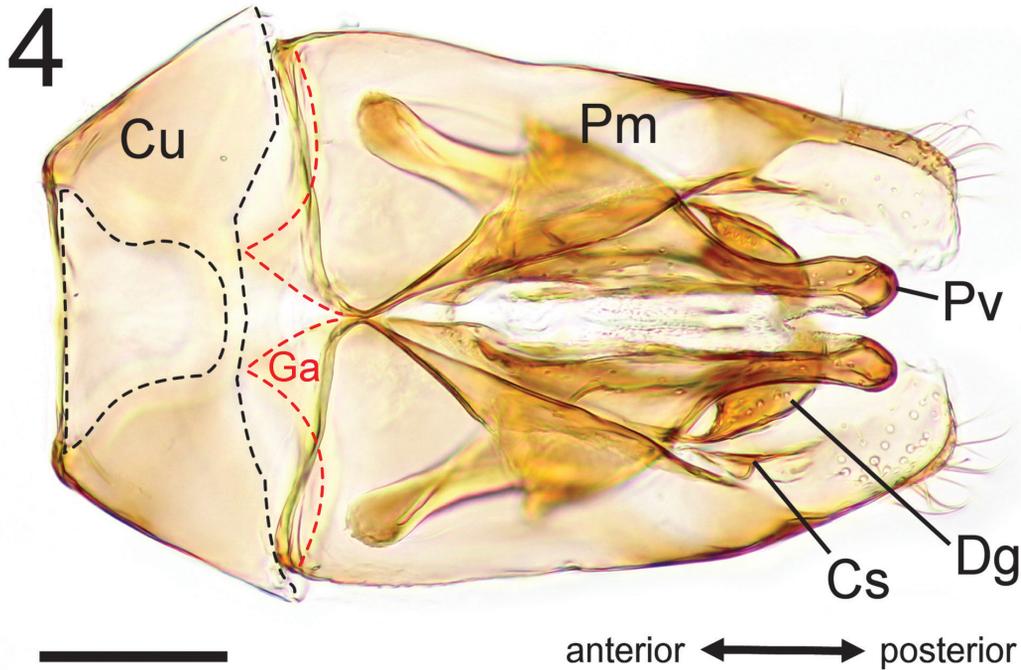


Fig. 4. Entire genital capsule, dorsal view, dashed line indicates outline of ventral surface of cupula (black line) and anteroventral outline of basimere (red line). Scale bars = 0.1 mm. **Cs** cuspis; **Cu** cupula; **Dg** digitus; **Ga** gonostipital arm; **Pm** paramere; **Pv** penisvalva.

slip. These slide-mounted specimens were examined with a Nikon Eclipse E600 microscope.

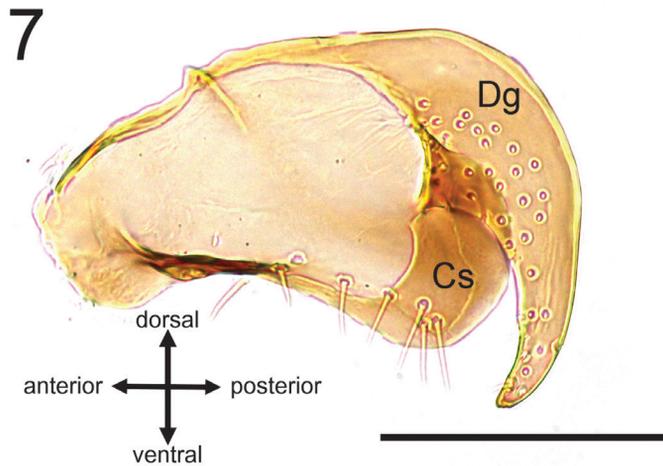
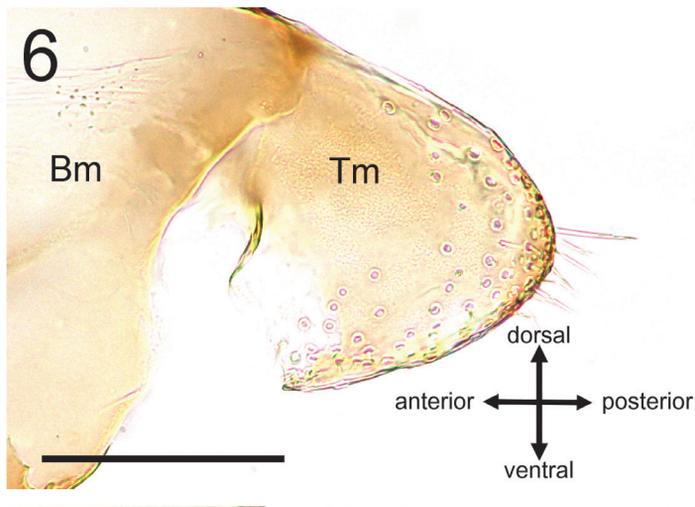
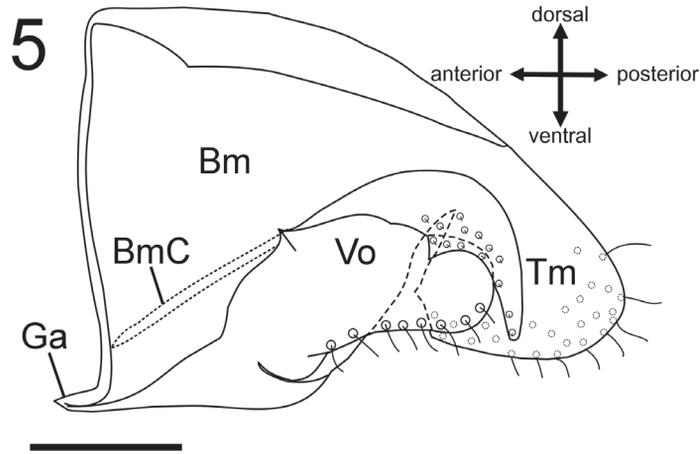
The multi-focused montage of Figure 1 was produced using CombineZM (A. Hadley, <http://hadleyweb.pwp.blueyonder.co.uk/>), and those of Figures 2–4 and 6–9 were produced using Helicon Focus Pro (Helicon Soft Ltd., <http://www.heliconsoft.com/>) from a series of source images taken by a Canon EOS KissX5 digital camera attached to a Nikon ECLIPSE E600 microscope. Artifacts/ghosts and unnecessary objects (dust, fragments of the anterior segments of the gaster, *etc.*) surrounding or covering target objects were erased and cleaned up using the retouching function of Helicon Focus Pro and GIMP2.8 (The GIMP Development Team, <http://www.gimp.org/>). The color balance, contrast and brightness were also adjusted using GIMP2.8.

Morphological terminology follows Boudinot (2013).

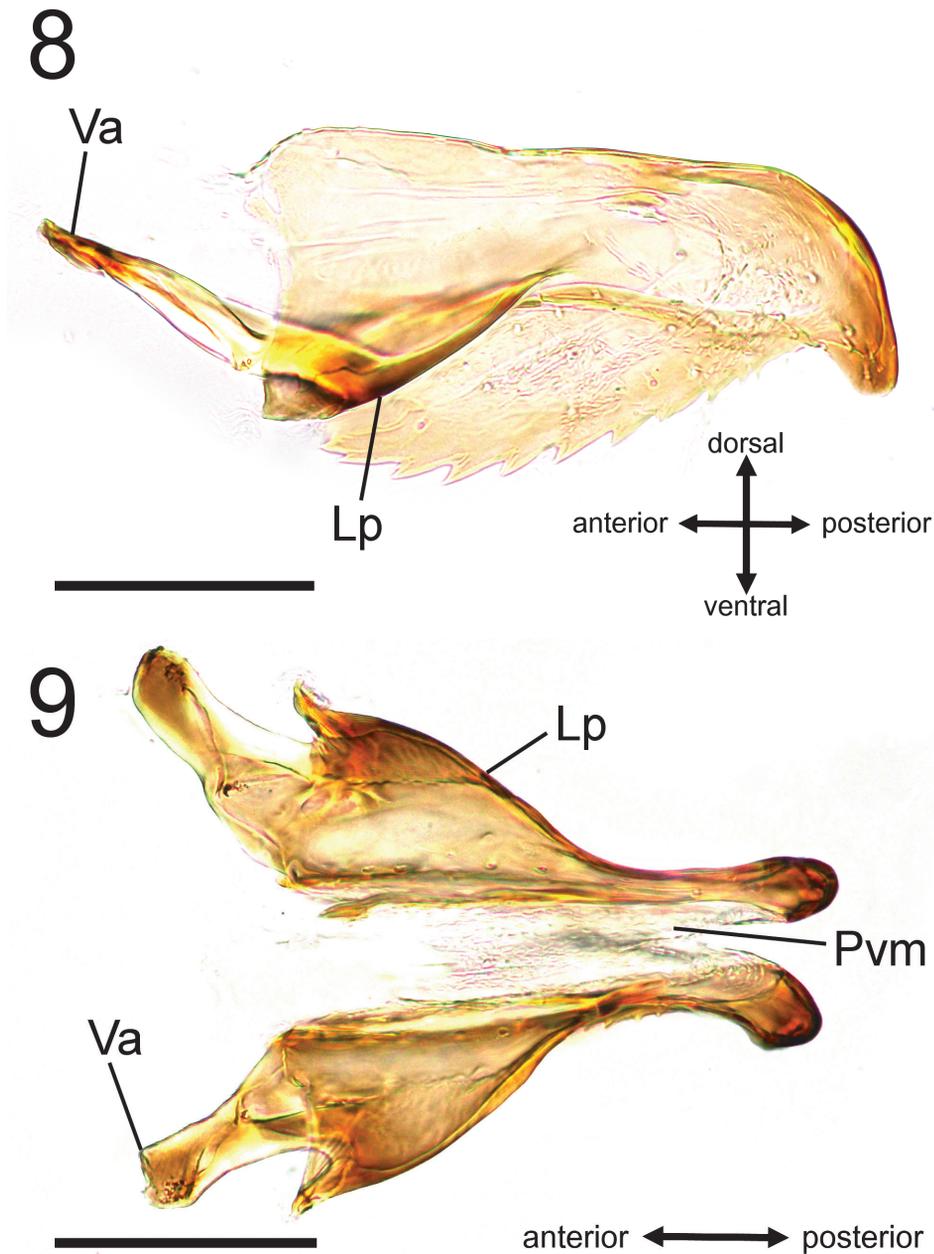
MALE GENITALIA OF *PRISTOMYRMEX PUNCTATUS*

Description

Pygostyle digitiform with 3–8 long setae in its apical third (Fig. 2). Abdominal sternite IX sub-hexagonal in outline (Fig. 3); spiculum (anterior apophysis of the sternite IX, Sp in Fig. 3) short; anterior margins meeting in an obtuse angle basal to the spiculum; anterolateral sternal corners weakly produced anterolaterally; lateral margins slightly concave; posterolateral sternal corners somewhat produced anterolaterally; posteromedian part ventrally with long setae. Genital capsule longer than wide. Cupula wider than long, weakly constricted anteriorly (Fig. 4), with its mesoventral part shortened anteroposteriorly as a transverse bridge (the anterior and posterior margins of cupula indicated by black dashed line in Fig. 4). Telomere distinctly differentiated from basimere by ventral notch (the notch indicated by dashed line in Fig. 5); oblique carina present on



Figs 5 – 7. Paramere and volsella. (5) paramere and volsella, right side, inner view; (6) anterior part of paramere, right side, inner view; (7) volsella separated from paramere, right side, inner view. Scale bars = 0.1 mm. **Bm** basimere; **BmC** carina of lower basimere; **Cs** cusps; **Dg** digitus; **Ga** gonostipital arm; **Tm** telomere; **Vo** volsella.



Figs 8 – 9. Penisvalva. (8) penisvalva, left side, outer view. (9) pair of penisvalvae, dorsal view. Scale bars = 0.1 mm. **Lp** lateral apodeme of penisvalva; **Pvm** penisvalva membrane; **Va** valvura.

lower of basimere (BmC in Fig. 5); gonostipital arm (median anteroventral extension of the basimere indicated by red dashed line in Fig. 4 and Ga in Fig. 4 and 5) in ventral view almost as long as basal width, with its lateral and mesal margins

converging and forming an acute apex; telomere in lateral view almost as long as high, with setae on the outer surface of its posterior part (Fig. 6); ventral ridge of volsella with setae (Fig. 5); cuspis in lateral view roundly lobate and short,

not reaching posterior margin of digitus (Fig. 7); digitus in lateral view claw-shaped, entirely hooked ventrad (Fig. 7); very short setae with distinct sockets scattered ventrally on digitus. Valviceps in lateral view with posterior apex hooked ventrad, anteroventral corner not produced; ventral margin with 9–13 denticles (Fig. 8); foveae (recognized as tunnels running inside valviceps) sparsely present on apical and ventral area of valviceps (some of apical foveae probably with short setae but obscure in optical microscope observation); valvura directed dorsoanterolaterally (Va in Fig. 9); lateral apodeme produced ventrolaterally (Lp in Fig. 9), forming wing-like structure which hold basal part of volsella in genital complex; penisvalva membrane densely spinate (Pvm in Fig. 9).

Remarks

General constitution of formicid male genitalia as shown by previous authors (Krafchick 1959; Ogata 1987; Ogata 1991) was preserved in irregularly produced males of *Pristomyrmex punctatus*, and conspicuous deletion, deformation, or ornamentation of the main components was not recognized. Gonostipital arm which was claimed as a synapomorphy of the Myrmicinae by Ogata (1991) is present in the male genitalia of *Pristomyrmex punctatus*. But this structure is not a synapomorphy of the Myrmicinae because of its presence in other ant subfamilies and in other hymenopteran lineages (Schulmeister 2003; Boudinot 2013). Based on a comparison of the male genitalia of *P. punctatus* with those of other myrmicine genera described and illustrated by Ogata (1991), the general features of male genitalia of Myrmicinae can be summarized as follows: (1) cupula wider than long; (2) gonostipital arm present, but variable in shape; (3) digitus strongly curved ventrad (except for *Solenopsis japonica*: both of digitus and cuspis reduced); (4) cuspis simple and reduced, shorter than digitus and never protruding dorsad above digitus in lateral view, or absent. In order to understand morphological synapomorphies of Myrmicinae in male genitalia, more detailed and comprehensive comparison across taxa is needed.

In agreement with previous observations by Itow *et al.* (1984), the male genitalia of *P. punctatus* seem to be functional. Further comparison of the male genitalia between *P. punctatus* and amphigonic sister species, *e.g.*, members of *P. punctatus* group (*sensu* Wang 2003), might provide valuable information for our better understanding of the origin of parthenogenesis in the genus.

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REFERENCES

- Bolton B. 2015. AntCat.org: An online catalog of the ants of the world. Available from: <http://antcat.org> (accessed 8 December 2015).
- Boudinot B. 2013. The male genitalia of ants: musculature, homology, and functional morphology (Hymenoptera, Aculeata, Formicidae). *Journal of Hymenoptera Research*. 30: 29–49.
- Dobata S, Sasaki T, Mori H, Hasegawa E, Shimada M and Tsuji K. 2009. Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. *Proceedings of the Royal Society of London B* 276(1656): 567–574.
- Itow T, Kobayashi K, Kubota M, Ogata K, Imai HT and Crozier RH. 1984. The reproductive cycle of the queenless ant *Pristomyrmex pungens*. *Insectes Sociaux* 31(1):87–102.
- Krafchick B. 1959. A comparative study of the male genitalia of North American ants (Formicidae) with emphasis on generic differences. Thesis submitted to the Faculty of the Graduate School of the University of Maryland. 79 pp.
- Ogata K. 1987. A Generic Synopsis of the Poneroid Complex of the Family Formicidae in Japan (Hymenoptera). Part 1. Subfamilies Ponerinae and Cerapachyinae. *Esakia* : occasional papers of the Hikosan Biological Laboratory in *Entomology* 25: 97–132.

- Ogata K. 1991. A Generic Synopsis of the Poneroid Complex of the Family Formicidae in Japan (Hymenoptera). Part 2. Subfamily Myrmicinae. Bulletin of the Institute of Tropical Agriculture Kyushu University. 14: 61–149.
- Schulmeister S. 2003. Genitalia and terminal abdominal segments of male basal Hymenoptera (Insecta): morphology and evolution. *Organisms Diversity and Evolution* 3: 253–279.
- Smith F. 1860. Catalogue of hymenopterous insects collected by Mr. A. R. Wallace in the islands of Bachian, Kaisaa, Amboyna, Gilolo, and at Dory in New Guinea. *Journal of the Proceedings of the Linnean Society of London. Zoology*. 5(s1): 93-143.
- Tsuji K. 1988. Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens*: comparison of intranidal and extranidal workers. *Behavioral Ecology and Sociobiology*. 23(4): 247–255.
- Wang M, 2003. A monographic revision of the ant genus *Pristomyrmex* (Hymenoptera: Formicidae). *Bulletin of the Museum of Comparative Zoology* 157(6): 383–542.

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