Additional distribution record of *Sergentomyia* (*Neophlebotomus*) *squamirostris* (Newstead) (Diptera: Psychodidae) from Tokyo, Japan

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**Abstract:** This is the first record of *Sergentomyia squamirostris* in Tokyo, Japan. Sand flies were collected from the western part of Tokyo, in August and September 2016, one male and seven females from Nishitama District and two females from Hachioji city. Morphological examination of the cibarium, pharynx, and spermathecae in female, and the genitalia, coxite and style in male revealed that all these specimens belonged to *S. squamirostris*.

Key words: sand fly, *Sergentomyia squamirostris*, Tokyo

**INTRODUCTION**

Phlebotomine sand flies are primarily present in the warm zones of Asia, Africa, Australia, southern Europe, and the Americas (Killick-Kendrick, 1999). Their distribution extends northwards, to latitude just above 50°N, in southwest Canada (Young and Perkins, 1984), northern France, and Mongolia (Lewis, 1982). Their southern distribution ends at latitude 40°S; however, they are absent from New Zealand and the Pacific islands (Lane, 1993). Although the classification of phlebotomine sand flies in the Old World remains controversial, the three genera, *Phlebotomus*, *Sergentomyia*, and *Chinius* are widely accepted by modern taxonomists (Maroli et al., 2013). *Sergentomyia* (*Neophlebotomus*) *squamirostris* Newstead, 1923, is found on Honshu, Shikoku and Kyushu of Japan, and China (Nakamura, 2014). In Japan, the distribution of *S. squamirostris* has been reported in Aomori, Akita, Gunma, Saitama, Gifu, Kyoto, Tottori, Yamaguchi, Ehime, Tokushima and Fukuoka Prefectures (Newstead, 1923; Sinoda, 1950; Esaki, 1953; Ogata, 1958; Sanjoba et al., 2011; Sanjoba, 2012; Sumino, 2014). However, further entomological survey, to elucidate the detailed geographical distribution and ecology of phlebotomine sand flies in Japan, is needed. Moreover, the distribution of phlebotomine sand flies in Europe is widely reported to be changing. This change can be attributed to either discovery of sand flies, previously overlooked, in some areas, or a true expansion of their range, as a result of climate or environmental changes (Özbel, 2010; Alten et al., 2015). Phlebotomine sand flies are of considerable medical importance as vectors of human pathogens, such as zoonotic *Leishmania* spp. and several arboviruses, mainly the *Phlebovirus*, *Vesiculovirus*, and *Orbivirus* spp. (Depaquit et al., 2010). Therefore, routine surveillance for phlebotomine sand flies is also necessary in Japan.

**MATERIALS AND METHODS**

Field surveys to collect sand flies were conducted at six sites, in Nishitama District in August and in Hachioji city in September of 2016 (Fig. 1) using sticky paper traps made by coating A4 size paper with castor oil (Alten et al., 2015). The sticky paper traps (10–40 per surveyed locality), were placed in roadside drainage pipes (Fig. 2), between 3 and 5 p.m. and collected after 17–20 h. Collected sand fly specimens were immediately transferred to 75% ethanol, in the field, and labeled accordingly. The preserved flies were first immersed in 10% potassium hydroxide for 2 h, and then washed with water four times, and subsequently immersed in 70%, 90%, 95%, and 99% ethanol, successively for 10 min each. The specimens were then mounted in Euparal solution (Waldeck GmbH & Co. KG., Germany), for species identification. In addition, a few specimens were cleared and mounted in Swan’s solution. Morphological identification was carried out using published keys and description (Newstead, 1923; Raynal, 1937; Sinoda, 1950; Tokunaga and Komyo, 1954; Leng, 1963), based on the features of cibarium, pharynx, and spermathecae in females and the genitalia, coxite and style in the male. Morphological characteristics of the wings and antennal segments, both in the females and the male, were also examined. All specimens were observed using BX51 and SZ61 microscopes (Olympus Co., Japan), recorded using a DP73 microscope camera (Olympus Co., Japan), and measured using cellSens standard 1.7 (Olympus Co., Japan). Specimens examined (voucher specimens) in this study are deposited in Laboratory of Molecular Immunology, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.
Ten sand flies, one male and nine females (no blood fed female) were collected from three of the six sites surveyed in August and September of 2016 (Fig. 3). Detailed information on sampling sites, altitudes, and number of collected sand flies is given in Table 1.

**Male.**

**Genitalia:** Coxite with tuft of internal setae. Style with stout spatulate ended four spines, two spines placed on terminal, and two spines placed on well-marked tubercle slightly superior to the middle of segment. One accessory spine on inner proximal surface. Aedeagus slender, long, strongly pigmented, and with rounded tip. Bilaterally symmetric sharp processes on basal part of aedeagus. Paramere is single without accessory processes, covered with numerous setae. Surstyle as long as or slightly shorter than coxite.

**Wings:** Length = 2,059 µm, width = 443 µm, alpha = 369 µm, beta = 355 µm, delta = 215 µm, gamma = 614 µm, pi = 95 µm (n = 1).

**Female.**

**Head:** Armature, length approximately one-fourth of pharyngeal length, consists of lance-like scales. Thin spicules from side wall of pharyngeal cavity may be found. Cibarium with 32–42 teeth (average 36.4, n = 9). Pigment patch large and oval. Ascoid bilateral, with geniculated shape on III to XIV or XV. Ascoid on III located near distal segment, does not reach next articulation. Ascoid on other segments located proximally, little before articulation.

**Wings:** Length = 2,237–2,990 µm (average 2,438.4 µm), width = 571–688 µm (average 632.5 µm). Wing index details shown in Table 2.

**Genitalia:** Spermathecae subcylindrical. Faint, sometimes imperceptible, internal wrinkles on the tip of spermathecae. Knob short and cylindrical. Ducts faintly striated and join a common duct.
This study reports the presence of the phlebotomine sand fly *S. squamirostris* in Tokyo Prefecture, Japan, for the first time. In this study, sand flies were collected using sticky paper traps. In principle, sticky paper trap is an interception trap to catch sand flies in their habitat while light trap is an attraction trap with a light source. Animal burrows and rock crevices are used as diurnal resting or breeding sites by many sand fly species and sticky paper traps, hung across the entrances, mounted sail-like on small sticks, or rolled into tubes, can be used to intercept such sites (Alexander, 2000). In Europe, female resting sites, such as roadside drainage holes, are often shared by males (Ready, 2013). In the present study, female sand flies were collected at a high rate from roadside drainage pipes, therefore, they can be presumed to be the resting sites of *S. squamirostris*, in the localities surveyed in the western part of Tokyo. While light traps have a limited use in ecological studies of sand flies (Alexander, 2000), regular resting site collections by sticky paper traps can be used to measure population change (Chaniotis et al., 1971). The surveyed areas in this study were located in close proximity to human habitat, therefore, future studies need to identify the blood source of female sand flies.

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**Table 1.** Information on surveyed sites in Tokyo, Japan, number of sticky paper traps used, and number of sand flies collected.

| Locality information | Number of traps | Number of sand flies collected |
|----------------------|-----------------|-------------------------------|
| Altitude (m)         | Total | Male | Female |
| Nishi-tama 3          | 730   | 0    | 0      |
| 2                    | 40    | 0    | 0      |
| 3                    | 20    | 1    | 0      |
| Hachioji 4            | 35    | 0    | 0      |
| 5                    | 15    | 2    | 0      |
| 6                    | 10    | 0    | 0      |
| Total                | 140   | 10   | 9      |

*The decimal system.

**Table 2.** Wing measurements for *Sergentomyia squamirostris* females collected from Tokyo (n=9) (in µm).

| Measure      | Average | Maximum | Minimum | Standard error |
|--------------|---------|---------|---------|----------------|
| Length       | 2,438.4 | 2,990   | 2,237   | 74.9           |
| Width        | 632.5   | 688     | 571     | 12.6           |
| Alpha        | 531.5   | 631     | 479     | 16.3           |
| Beta         | 410.5   | 456     | 339     | 11.9           |
| Delta        | 323.3   | 435     | 260     | 18             |
| Gamma        | 671.7   | 730     | 599     | 11.8           |
| PI           | 94.7    | 125     | 81      | 5.2            |

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