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Biological notes of *Probolomyrmex okinawaensis* Terayama & Ogata collected in Yonagunijima Island, and five species of *Probolomyrmex* collected in Japan and Southeast Asia

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ABSTRACT. A colony of *Probolomyrmex okinawaensis,* composed of one dealate queen and four workers, was collected in Yonagunijima Isl., the westernmost island of Japan, as the first record outside of Okinawajima Isl. The colony was kept in the laboratory for behavioral observations. The ants attacked and fed only on polyxenid millipedes. Mature colony size was small: an alate queen was produced when the number of workers was ca. ten under laboratory conditions. The number of ovarioles was two in both castes. Larvae were found hanging from the glass ceiling of the nest chamber. We also report biological data of five species of this genus collected in Southeast Asia and Japan and discuss our results in relation to the available published information on this genus. The biological characteristics mentioned above are commonly found in these species.

Keywords colony composition, larval hanging, ovary, Polyxenid millipede
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INTRODUCTION

The proceratine ant genus *Probolomyrmex* comprises 30 species (Bolton 2020) and is typically cryptic (Taylor 1965; Hita Garcia & Fisher 2014), making it difficult to collect whole colonies. Even with the recent improvements in collection techniques and intensive research which has provided many specimens for taxonomic study of this genus (Eguchi et al., 2006; Hita Garcia & Fisher 2014; Oliveira & Feitosa 2019), biological information based on colony collection is still scarce, and is available only for *P. bolivensis* Mann, 1923 (Taylor 1965), *P. dammermani* Wheeler, 1928 (Ito 1998), and *P. longinodus* Terayama & Ogata, 1988 (Kikuchi & Tsuji 2005). These species make small colonies, and queens of the latter two species have the smallest ovariole number (1-1) in ant queens. Ito (1998) reported that *P. dammermani* is a specialized predator on polyxenid millipedes, which are soft–bodied and have hooked bristles used for defense against predators including ants (Eisner et al. 1996). Besides *Probolomyrmex*, a few species of the ponerine genus *Thaumatomyrmex* are known to be predators of polyxenid millipedes (Brandão et al. 1991; Rabeling et al. 2012). Except for *P. dammermani,* the feeding habit has not been reported for this genus. Taylor (1965) reported that *P. bolivensis* never accepted any small soil arthropods includ-

ing Collembola, Symphyla, spiders, larval and adult ants, Diptera, Coleoptera, termites, and various arthropod eggs. Hita Garcia and Fisher (2014) mentioned in their revision of Madagascan *Probolomyrmex* the question of whether specialization on Polyxenida is a common characteristic within *Probolomyrmex* or restricted only to *P. dammermani* remains to be investigated.

So far *P. okinawaensis* Terayama & Ogata,1988 has been recorded only from Okinawajima Island (Terayama & Ogata 1988; Terayama et al. 2014). This species is, as in other congeneric species, rarely collected, and no biological information has been reported. We collected a colony of *P. okinawaensis* from Yonagunijima Island (ca. 520 km south from Okinawajima Island), the westernmost of Japan, which represents the first record outside of Okinawajima. Here, we report the collection record and some biological notes of *P. okinawaensis*. This is supplemented by biological information of five species of this genus also collected by us in Indonesia, Japan and Vietnam.

MATERIALS AND METHODS

The colony of *P. okinawaensis* was collected in Mandabaru Forest Park, Yonagunijima on 14 March 2019. In the laboratory, the colony was kept in an artificial nest box measuring 9.5 x 6.2 x 2.8 cm and layered with plaster of Paris in order to keep the humidity sufficiently high. For the nest chamber, two small depressions covered with a slide glass were made in the plaster floor. We kept this colony for more than one year. In the laboratory, we offered the following prey items simultaneously to the captive colony: a small termite nymph (*Reticulitermes speratus* (Kolbe, 1885)), a first instar nymph of the Turkestan cockroach (*Blatta lateralis* Walker, 1868), a springtail (*Sinella* sp.), and a polyxenid millipede (*Eudigraphis kinutensis* (Haga, 1950)). Worker behavior towards these arthropods was then observed. This experiment was replicated five times. One alate queen and one worker, which emerged in the laboratory, were dissected to check ovariole number under a dissecting microscope.

We collected additional colonies of *Probolomyrmex* during 1992 to 2019. Colony composition and ovariole numbers of *P. itoi* Eguchi et al., 2006 (Colony code, locality and date: FI92-153, Sitiung, 7 Jan. 1992; FI97-510, Ulu Gadut, 26 March 1997, both in West Sumatra, Indonesia), *P. vieti* Eguchi et al., 2006 (FI92-305, Kebun Raya Bogor, West Java, Indonesia, in Jan. 1992; FI93-60, the same place, 19 Jan 1993), *P. watanabei* Tanaka, 1974 (FI97-414, Ulu Gadut, West Sumatra, Indonesia, 26 March 1997) and *P. longiscapus* Xu & Zeng, 2000 (FI15-60, Ban Chu, Na Hang, Tuyan Quang, northern Vietnam, 13 March 2015) were examined just after collection. One colony of *P. itoi* collected in Ulu Gadut, and five colonies of *P. longinodus* collected in Yonagunijima in March 2019, were kept in the laboratory to check prey preferences.

RESUTLS

***P. okinawaensis*:** The colony (colony code: FI19-67) nested in an empty shell of unidentified land snail fallen on the forest floor and contained one dealated queen, four workers, 13 larvae and three eggs. Workers of *P. okinawaensis* attacked and fed only on the polyxenid millipedes in all experiments. Attacking and the subsequent behavior for prey feeding were very similar to *P. dammermani* reported by Ito (1998): foraging workers approached a polyxenid slowly from its side, grasped the dorsal side of the anterior segments with their mandibles, and stung the prey ventrally. The worker then retrieved the paralyzed prey by holding the front part of the ventral appendages of the polyxenid and carrying the prey underneath its body. When the prey size was large, the ant worker pulled an antenna or leg of the millipede and drag it to the nest chamber. There, a few workers immediately stripped off the setae. After complete stripping, workers fed on the polyxenid. Then, the worker passed it on to the larvae which were hanging from the glass ceiling of nest chamber by the tubercle on the abdominal tip. A few larvae fed on a fragment of polyxenid together.

Table 1. Colony composition and some biological characteristics of *Probolomyrmex*. We summarise here the data from colonies collected in this study in comparison to previous published information on this genus. DQ: dealate queen, W: worker, AQ: Alate queen, M: Male. Y: occurrence of each behavior. ne: not examined. Queens and workers had the same number of ovarioles/individual in all species. Number of colonies examined in each case for species without colony code shown in parentheses alongside number of workers.

Species	Colony code	Locality	No. individuals per colony				No. ovarioles	Polyxenid predation	Larval hanging from ceiling
			DQ	W	AQ	M			
P. itoi [1]	FI92-153	Sumatra	2	6			2	Y	Y
	FI97-510	Sumatra	1	5					
P. longiscapus [1]	FI15-60	Vietnam	1	12			2	ne	ne
P. okinawaensis [1]	FI19-67	Japan	1	4			2	Y	Y
P. vieti [1]	FI92-305	Java	1	1	1		2	ne	ne
	FI93-60	Java	1	5					
P. watanabei [1]	FI97-414	Sumatra	1	12		1	2	ne	ne
P. bolivensis [2]		Panama	3	22 (1)	4		ne	ne	Y
P. dammermani [3]		Java	1	13.8 ± 5.9 (5)			2	Y	Y
P. kelleri [4]		Venezuela	2	16 (1)			ne	ne	ne
P. longinodus [1, 5]		Japan	5~13 [5]	22 ± 14 (5) [5]			2 [5]	Y [1]	Y [1]

[1] The present study, [2] Taylor (1965) , [3]Ito (1998), [4] Oliveira & Feitosa (2019), [5] Kikuchi & Tsuji (2005)

Under laboratory conditions, the number of workers increased to 30 workers during one year. When the number of workers was ca 10, a new alate queen was produced. Both castes had 2 ovarioles (1-1). Unfortunately, the colony was suddenly decimated, therefore reproductive condition of the dealate queen collected in the field could not be determined.

Other Asian species: Colonies of *P. itoi, P. longiscapus* and *P. watanabei* nested in dead woods fallen on the ground of forest floor. Two colonies of *P. vieti* were found under stones inside artificial small forests in the Bogor Botanical Gardens. As in *P. okinawaensis*, *P. longinodus* colonies were collected from empty shells of land snails fallen on the forest floor. Data on the biology of the five species of *Probolomyrmex* collected in Southeast Asia and Japan are summarised in Table 1 alongside published information of species reported on so far. Colony size of the four southeast Asian species was small, with fewer than 10 workers being common. One (colony code FI92-153) of the two colonies of *P. itoi* had two mated queens, but only one queen had active ovaries with developing oocytes and yellow bodies, indicating that this colony was functionally monogynous. The remaining three species studied by us were monogynous, with one mated dealate queen. Ovariole numbers of both queens

and workers were two (1-1) in all four species. Specialization on polyxenids was confirmed for *P. itoi* and *P. longinodus.*

DISCUSSION

All species of *Probolomyrmex* so far reported make small colonies, often less than 10 workers (Table 1). The maximum number of workers observed was 47 in *P. longinodus* (Kikuchi & Tsuji 2005). The field colony of *P. okinawaensis* had only four workers. Under laboratory conditions, they produced an alate queen when the number of workers was ca. ten, indicating that even mature colony size is small in this species. The number of dealate queens per colony reported in two Neotropical species was two or three (Taylor 1965; Oliveira & Feitosa 2019), though these queens were not dissected. Colonies of *P. longinodus* contained multiple dealate queens, though only one queen/colony was mated (Kikuchi & Tsuji 2005). *Probolomyrmex itoi* exhibits monogyny or functional monogyny. The remaining five species including *P. okinawaensis* had only one dealate queen per colony. Therefore, at least in the Oriental regions, monogyny is a common social structure in *Probolomyrmex.*

The ovariole numbers of both queens and workers was found to be two (1-1) in all seven species of *Probolomyrmex* for which data are currently available. The majority of poneroid ants including Proceratiinae have six or eight ovarioles (Peeters & Ito 2015), which is the basic number for Aculeata (Iwata 1955). Besides *Probolomyrmex*, the reduction to two ovarioles in ant queens has been reported for only two species, *Gnamptogenys cribrata* (Emery, 1900) and *Acanthomyrmex minus* Terayama et al., 1998 (Terayama et. al. 1998; Ito & Gobin 2008). Other species of these two genera have four to ten ovarioles (Ito & Gobin 2008; Yamada et al. 2018). *Probolomyrmex* is the only genus in which queens of all species within the genus appear to have been reduced to two ovarioles. This may relate to their small colony size, but some other unknown reasons may also exist.

As in *P. bolivensis* and *P. dammermani* (Taylor 1965; Ito 1998), larvae of *P. okinawaensis* were always attached to the ceiling by the tubercle on the abdominal tip. This behavior was also observed in *P. longinodus*. Larvae attached to the ceiling in artificial nests under laboratory conditions are often observed in some ant species, e.g. *Odontomachus simillimus* Smith, 1985, and *Hypoponera* spp., however, these are typically attached by the tubercles of the dorsal side (Peeters & Hölldobler 1992). Attaching by the tubercle on the abdominal tip seems to be a unique feature in *Probolomyrmex*.

Probolomyrex okinawaensis is a specialized predator on Polyxenida. The behavioral characteristics of attacking and feeding are almost identical to *P. dammermani* reported by Ito (1998). We also observed that *P. itoi* and *P. longinodus* fed on polyxenid millipedes only. The Oriental *Probolomyrmex* contains two species groups, the *longinodus* group and the *greavesi* group (Eguchi et al. 2006). *Probolomyrmex dammermani*, *P. longinodus,* and *P. itoi* belong to the *longinodus* group while *P. okinawaensis* belongs to the *greavesi* group. Prey specialization on polyxenid millipedes is thus found in both species groups, and this may indicate the widespread occurrence of the prey specialization in this genus. Polyxenida contains only ca 160 species in the world (Nguyen Duy-Jacquemin & Geoffroy 2003). The density of these millipedes seems to vary from place to place: they often make an aggregation under bark or stones (Karasawa et al. 2020), resulting in very high abundance locally, whereas their density is very low in some areas (Nguyen et al. 2016; Santos-Silva et al. 2018). Such very narrow preference to prey showing heterogeneous distribution may partly account for the rareness and uniform biological characteristics in this genus. *Probolomyrmex* is the only genus of the Proceratiinae tribe Probolomyrmecini (Bolton 2020) because of their unique morphological characteristics. The present paper shows that their biological characteristics are also unique among ants.

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