

Intraspecific variation of the diagnostic characteristics among the Japanese populations in *Nymphonella tapetis* (Pycnogonida, Ascorhynchidae)

Asahi IBE¹⁾ and Katsumi MIYAZAKI²⁾

¹⁾ Graduate School of Science and Technology, Niigata University, 8050 Ikarashi 2-no-cho, Niigata 950–2181, Japan

²⁾ Department of Environmental Science, Faculty of Science, Niigata University, 8050 Ikarashi 2 no-cho, Niigata 950–2181, Japan

E-mail: miyazaki@env.sc.niigata-u.ac.jp (KM)

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ABSTRACT

There is a taxonomic confusion among the three species of the pycnogonid genus *Nymphonella*, especially between the East Asian *N. tapetis* and the Mediterranean *N. lecalvezi*. We examined the five diagnostic characteristics for *N. lecalvezi* using total 65 specimens of *N. tapetis* from six localities in Japan. Wide intraspecific variations were found among the Japanese *N. tapetis* in the four characteristics, and they became inappropriate for diagnosis of *N. lecalvezi*. A distinct difference was confirmed in the remaining one characteristics, which is still a candidate of a diagnostic one.

INTRODUCTION

Nymphonella tapetis Ohshima, 1927 is a curious pycnogonid species showing a larval endoparasitism on some bivalves. Three species have been described in the genus *Nymphonella*: *N. tapetis*, *N. lambertensis* Stock, 1959, and *N. lecalvezi* Guille and Soyer, 1967. These *Nymphonella* species have been discretely recorded from the following areas: East Asia (Japan and South Korea), southern Africa (South Africa and Namibia), and the Mediterranean Sea. The adult morphology is very similar among the species, which causes a taxonomic confusion especially between the East Asian and the Mediterranean forms (*N. tapetis* and *N. lecalvezi*). The Mediterranean *Nymphonella* specimens were identified as *N. tapetis* at the first discovery from the bay of Banyuls-sur-Mer, France (Le Calvez 1950), but later Guille and Soyer (1967) described the specimens from the same locality as a new species, *N. lecalvezi*. They compared the nine Mediterranean specimens with the descriptions and figures in the articles on Japanese *N. tapetis* (Ohshima 1927, 1933, 1935) and found two small morphological differences in appendages. All authors other than Guille and Soyer (1967), however, have identified the Mediterranean

Nymphonella as *N. tapetis* without any discussion. The “Pycnobase”, an online database of pycnogonid taxa follows the major opinion, and *N. lecalvezi* is accepted as a synonym of *N. tapetis* at present (Bamber et al. 2024). Miyazaki (2011) examined the two diagnostic characteristics for *N. lecalvezi* proposed by Guille and Soyer (1967) in 19 specimens of *N. tapetis* from Tokyo Bay, and found an intraspecific variation, which evidently weakened the species validity of *N. lecalvezi*. Subsequently, Munilla (personal communication) insisted three additional diagnostic characteristics which differentiate the Mediterranean *N. lecalvezi* from the Japanese *N. tapetis*. A preliminary examination on these additional diagnostic characteristics has been reported in a conference presentation (Miyazaki et al. 2016), but further study using more specimens from more populations has been required.

In the present study, we examined the two original and the three additional diagnostic characteristics for *N. lecalvezi*. The results of 65 specimens from six localities in Japan were compared with those of the Mediterranean *Nymphonella*.

MATERIALS AND METHODS

A total of 65 adult specimens of *Nymphonella tapetis* were collected from the following six localities (Fig. 1): Hamanaka (Hokkaido, one specimen), Souma (Fukushima Prefecture, seven specimens), Tokyo Bay (Banzu and Futtsu tidal flats, Chiba Prefecture, 23 specimens), Tateyama (Chiba Prefecture, 28 specimens), Misaki (Kanagawa Prefecture, four specimens), and Misumi (Shimane Prefecture, two specimens).

We examined the two original (Guille and Soyer 1967) and the three additional (Munilla, personal communication) diagnostic characteristics for *N. lecalvezi* under a light microscope: original diagnostic characteristic 1: length-relation of the sixth to eighth segments of palp (second head appendage) (Fig. 2); original diagnostic characteristic 2: number of denticulation-pairs of compound spines of oviger (third head appendage) (Fig. 3); additional diagnostic characteristic 1: number of compound spines in the distal four (seventh–10th) segments of oviger (Fig. 4); additional diagnostic characteristic 2: presence/absence of marginal hollow in the lateral process (Fig. 5); additional diagnostic characteristic 3: presence/absence of basal process in the first coxa (first segment of walking leg) (Fig. 6).

RESULTS AND DISCUSSION

No differences between genders seem to be present in the examined characteristics.

Original diagnostic characteristic 1 (Table 1, Fig. 2)

We measured the length of the sixth to the eighth palpal segments in 24 specimens from three localities. In 10 specimens from the two localities, the seventh segment was longest, and the sixth was shortest ($7\text{th} > 8\text{th} > 6\text{th}$). On the other hand the relation of the segment length was " $7\text{th} > 6\text{th} > 8\text{th}$ " in the remaining 14 specimens from the three localities.

Guille and Soyer (1967) measured the palpal segment length of nine Mediterranean *Nymphonella* specimens and showed the length-relation was always " $7\text{th} > 8\text{th} > 6\text{th}$ ". They also showed the relation of Japanese *N. tapetis* was " $7\text{th} > 8\text{th} = 6\text{th}$ " based on the measurement in the figure of Ohshima (1935) and insisted that the difference of the length-relation was one of the diagnostic characteristics for *N. lecalvezi*. Miyazaki (2011) measured 19 *N. tapetis* specimens from Tokyo Bay and reported the relation " $7\text{th} > 8\text{th} > 6\text{th}$ ". In the present study, the seventh segment was always longest, but the relation between the sixth and the eighth was not fixed. The length-relation among the segments is variable, and thus this characteristic is not appropriate for diagnosis of *N. lecalvezi*.



Fig. 1 Sampling localities of *Nymphonella tapetis* examined in the present study.

Table 1 Original diagnostic characteristic 1: length-relation of the sixth to eighth segments of palp.

Locality	Length-relation	
	$7\text{th} > 8\text{th} > 6\text{th}$	$7\text{th} > 6\text{th} > 8\text{th}$
Souma	0	1
Tokyo Bay	3	8
Tateyama	7	5

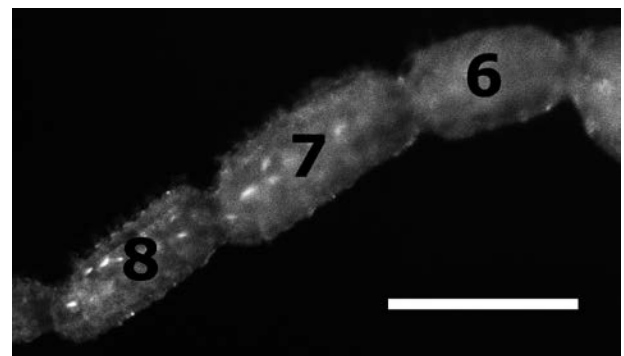


Fig. 2 Middle part of palp of *Nymphonella tapetis*, showing $7\text{th} > 8\text{th} > 6\text{th}$ pattern of segment length. 6–8: sixth to eighth segments of palp. Bar = 250 μm .

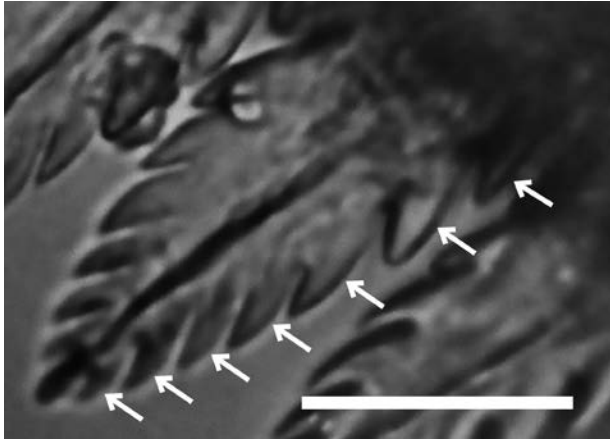
Original diagnostic characteristic 2 (Table 2, Fig. 3)

Nymphonella species have compound spines with denticulation-pairs on the distal four segments of ovigers. We counted the number of the denticulation-pairs in 51 specimens from six localities. The number of the denticulation-pairs was very variable among the Japanese specimens ranging between four and 11.

Guille and Soyer (1967) counted the number of denticulation-pairs between four and seven in the Mediterranean specimens, whereas Ohshima (1935) wrote that the number of Japanese *N. tapetis* was about

Table 2 Original diagnostic character 2: number of denticulation-pairs of compound spines of oviger.

Locality	No. of denticulation-pairs
Hamanaka	6–8
Souma	4–7
Tokyo Bay	6–10
Tateyama	6–10
Misaki	6–11
Misumi	7–9

Fig. 3 Compound spine of oviger of *Nymphonella tapetis*, with seven denticulation-pairs (arrows). Bar = 25 μ m.

eight. This difference of the denticulation-pair number was regarded as a base of establishment of *N. lecalvezi* as a new species (Guille and Soyer 1967). Miyazaki (2011) reported, however, the number was seven or eight in *N. tapetis* from Tokyo Bay, which showed an overlap of the range. The present study extends the range in the Japanese *N. tapetis*, and it becomes completely overlapped between the Japanese and the Mediterranean specimens. Thus, this characteristic is also not appropriate for diagnosis.

Additional diagnostic characteristic 1 (Table 3, Fig. 4)

We counted the number of compound spines in the distal four (seventh–10th) segments of oviger in 65 specimens from six localities. The number of spines is variable among the specimens and widely ranges from four to 16.

According to Munilla (personal communication), the number of ovigerous compound spines in seventh segment is 11, in eighth is nine, in ninth is nine, and in 10th is 11 (11 : 9 : 9 : 11) in the Japanese *N. tapetis*, whereas 10 : 8 : 7 : 8 in the Mediterranean *Nymphonella*. The present study, however, shows a wide intraspecific variation of the compound spine number, and this characteristic cannot be used for diagnosis.

Additional diagnostic characteristic 2 (Table 4, Fig. 5)

Munilla (personal communication) insists that the Mediterranean *Nymphonella* has a marginal hollow in

Table 3 Additional diagnostic characteristic 1: number of compound spines in seventh to 10th segments of oviger.

Locality	No. of compound spines			
	7th	8th	9th	10th
Hamanaka	11	9	9	10
Souma	5–10	4–9	5–8	5–12
Tokyo Bay	9–15	7–14	7–12	8–16
Tateyama	7–13	7–10	8–10	9–13
Misaki	10–13	8–10	8–10	11–12
Misumi	13	9–10	11–12	12

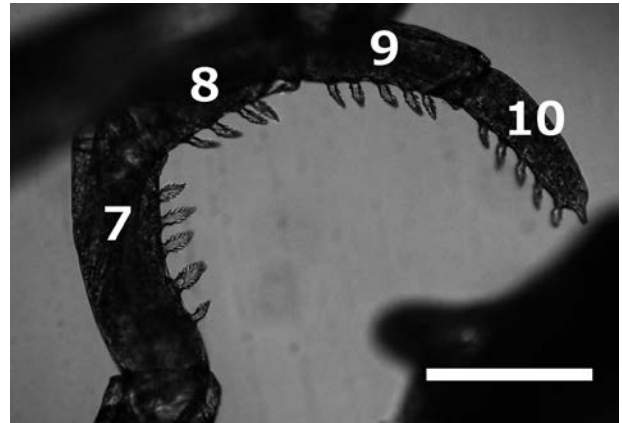
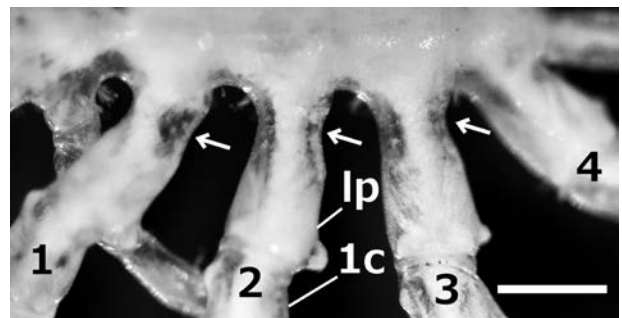
Fig. 4 Distal four segments of oviger of *Nymphonella tapetis*, showing 5 : 4 : 5 : 5 pattern of compound spine-number. 7–10: seventh to 10th segments. Bar = 200 μ m.

Table 4 Additional diagnostic characteristic 2: presence/absence of marginal hollow in lateral process.

Locality	Present	Absent
Hamanaka	1	0
Souma	3	4
Tokyo Bay	21	2
Tateyama	25	2
Misaki	4	0
Misumi	2	0

Fig. 5 Lateral processes of trunk of *Nymphonella tapetis*, showing marginal hollows (arrows). 1–4: first to fourth walking legs, 1c: first coxa, lp: lateral process. Bar = 500 μ m.

each lateral process, but the hollow is absent in Japanese *N. tapetis*. We recognized the hollows in 56 specimens out of 64 observed ones, and this insistence cannot be valid for diagnosis.

Additional diagnostic characteristic 3 (Table 5, Fig. 6)

Munilla (personal communication) also insists that the basal process in each first coxa is present in the Japanese *N. tapetis*, whereas it is absent in the Mediterranean *Nymphonella*. We confirmed the presence of the process in all 65 specimens examined. Thus, this characteristic is still a candidate of a diagnostic one, but further examination on enough number of the Mediterranean specimens is necessary to confirm it.

In the present study, we examined many specimens from six localities in Japan on the five characteristics, which were said to be distinguishable ones between the Japanese and the Mediterranean *Nymphonella*. We found wide intraspecific variations among the Japanese *N. tapetis* in the four characteristics out of the five, and they became inappropriate for diagnosis

Table 5 Additional diagnostic characteristic 3: presence/absence of basal process in first coxa.

Locality	Present	Absent
Hamanaka	1	0
Souma	7	0
Tokyo Bay	23	0
Tateyama	28	0
Misaki	4	0
Misumi	2	0

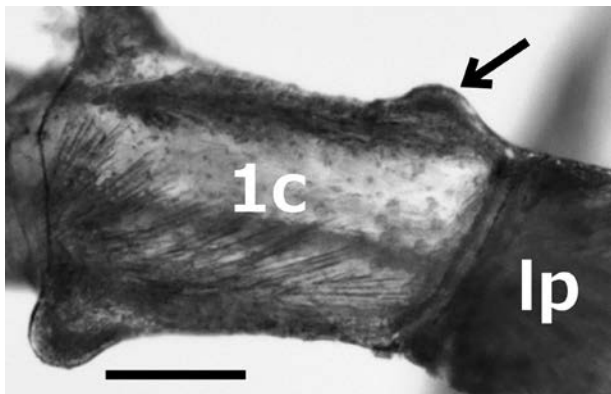


Fig. 6 First coxa of *Nymphonella tapetis*, showing basal process (arrow). 1c: first coxa, lp: lateral process. Bar = 250 μ m.

of the Mediterranean *N. lecalvezi*. One distinct difference (presence/absence of the basal process in first coxa) still remains, and further morphological and molecular phylogenetic study is required for the taxonomic conclusion between the Japanese and the Mediterranean *Nymphonella*.

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