Observation on Aggregation and Resting on Spider Web Behavior of Crane Fly *Limonia (Euglochina)* sp. from Nanjing Subtropical Rainforest of China

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Abstract: There are several species of crane flies in Nanjing subtropical rainforest, whereas, the *Limonia (Euglochina)* sp. is the only one we observed resting on the spider web (primary thread favor). So, observation on co-occurrence behavior of *Limonia (Euglochina)* sp. with the Araneidae spiders in the same orb-web was conducted. Ultrastructure of the pretarsus of several species of crane flies and one spider species of Araneidae were scanned by SEM technology, in addition, the mechanism of hanging on the orb-web thread by *Limonia (Euglochina)* sp. was discussed in this paper by comparing the tarsal external morphology of *Limonia (Euglochina)* sp. with the others. We also recorded the aggregation regulation of *Limonia (Euglochina)* sp. in the working field in the rainforest.

Key words: crane flies; orb-weaver spiders; co-occurrence; pretarsus; *Limonia (Euglochina)* sp.

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The distribution of crane flies *Limonia (Euglochina)* sp. ranges from low latitude to middle latitude of subtropical rainforest or mountainous area. Nanjing subtropical rainforest is the most northward rainforest in southern China. The location where we discovered *Limonia (Euglochina)* sp. was an orange garden in the rainforest. The same species was also discovered in Taipei Erge mountain[1]. Species of *Limonia (Euglochina)* sp. were observed resting on the orb-web and we failed to find them outside of orb-webs. The host] spiders whoes webs *Limonia (Euglochina)* sp. rest on involved several species of Araneidae. The bodies of the host spiders are relatively small among the orb-weavers and even smaller than that of the *Limonia (Euglochina)* sp.. Special Araneidae species discriminated by *Limonia (Euglochina)* sp. have not been observed yet, so it seems *Limonia (Euglochina)* sp. recognised the orb-webs as a shelter instead of a partner of the "hosts". Few researches have been done on this phenomenon and the relationship between *Limonia (Euglochina)* sp. and the host spiders is still uncertain. The purpose of this study was to further describe this unusual relationship between *Limonia (Euglochina)* sp. and the host spiders.

1 Materials and Methods

The location where we discovered *Limonia (Euglochina)* sp. was in an orange garden in Nanjing subtropical rainforest (26°53′391″ N, 117°05′251″ E, 292 m alt.), Hexi, Nanjing, Fujian Province. A normal dry and wet bulb hygrometer were used to record the dry circumstance temperature (DT) and wet circumstance temperature (WT) in the working field. Based on DT and WT, the relative humidity was approximately computed according to Ling's calculation formula[2]. The specimens, including *Limonia (Euglochina)* sp., one species of host spider, one species of crane flies belong to Tipulida who did not rest on spider web thread, as well as an unidentified species of Dipterous insect who could also hang on the orb-web thread were collected in the rainforest. The specimens dehydrated in 100% alcohol, preserved in 50 mL centrifuge tubes with cotton inside to keep the specimens dry, and all the samples were taken back to the laboratory where the SEM were operated. The apparatus applied for SEM was GSM-6301F.

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2 Results and Discussion

2.1 Relationship between the emergence amount and the relative humidity and temperature

Linonia (Euglochina) sp. species is really abundant in the orange garden in Nanjing subtropical rainforest, we once observed 84 individuals of *Linonia (Euglochina)* sp. on the spider webs while only 3 *L. annulata* were found on the stems of an orange tree. The relationship between the humidity (or temperature) and the emergence amount of *Linonia (Euglochina)* sp. on the orb-webs were presented by two coordinate curves (Fig. 1). The maximal number appeared between 10 a.m. and 11 a.m., meanwhile the relative humidity were 70.8 and 63.2 respectively. This result indicated that *Linonia (Euglochina)* sp. might favor humidity condition. Similarly, the amount of *Linonia (Euglochina)* sp. was highly related with the dry circumstance temperature, and it seem as the temperature around 29°C was in favor of the activity of *Linonia (Euglochina)* sp. (Fig. 2).

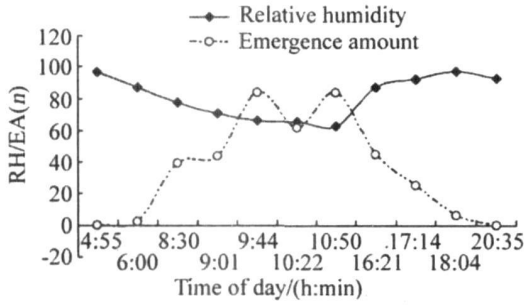

Fig. 1 Relationship between the relative humidity (RH) and emergence amount (EA) on the orb-webs in the orange woods in Nanjing subtropical rainforest

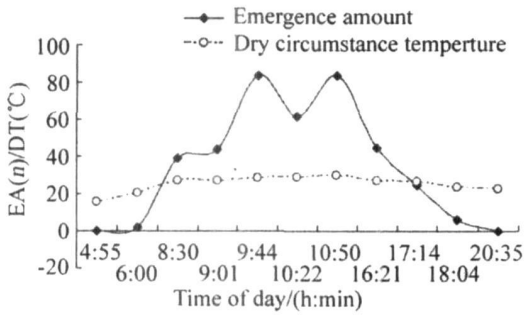

Fig. 2 Relationship between the dry circumstance temperature (DT) and emergence amount (EA) on the orb-webs in the orange woods in Nanjing subtropical rainforest

2.2 Evolutionary pressure, aggregation and mating behavior of *Linonia (Euglochina)* sp.

Comparing with *Linonia (Euglochina)* sp. and *Helius* sp., both of them camouflaged with mimic color similar to the brown trunk where they usually rested, the former had relative colorful body and looked conspicuous on the orb-webs. None of predatory behaviors upon *Linonia (Euglochina)* sp. was observed during the investigation. *Linonia (Euglochina)* sp. probably benefited from the spider web against the predators, although the spiders were considered as the main natural enemies of crane flies[3]. Generally, *Linonia (Euglochina)* sp. prefered the primary thread of the orb-web, which was a non-sticky thread serves as scaffold during the orb-web constructing. *Linonia (Euglochina)* sp. do can rest on the inner place of the web but this seldom occur, apparently the crane flies tried to keep away from their danger hosts as possible as they could. In addition, since *Linonia (Euglochina)* sp. could "walk" freely on the web and we have never seen one of them got trapped by the sticky capture thread. So, the host spider who had provided shelters for *Linonia (Euglochina)* sp. was probably impalpable for the crane flies. Thereby, lacking of enemies and selective pressure (or evolutionary pressure), *Linonia (Euglochina)* sp. preserved the vivid body color and looked conspicuous in their living circumstance.

Aggregation on the same thread or the same web was the typical behavior in *Linonia (Euglochina)* sp. species[1], and both sexes were involved. The adjacent two males often fight each other with their hind legs (Fig. 3 i) which always raise behind but do not touch the thread. Observation on relationship between the temperature and the aggregation manners showed that the higher the temperature, the more aggregation manners the *Linonia (Euglochina)* sp. practised (Tab. 1).

Linonia (Euglochina) sp. is polyandrous insect, males compete with each other for mating with females in their society. All the courtship and copulation behaviors occurred on the orb-web (mostly on the primary thread). The female, relative smaller in body size, hung on the web thread while several stronger males fought for mating over her. Once succeed in standing atride the female, the male curled its abdomen under female's; this process lasted for only two or three seconds. Generally,

Tab. 1　Number of aggregation manners under different conditions

time of day / (hr: min)	t /℃	RH	Number of aggregation manner							
			1[a]	2	3	4	5	6	7	8
5: 00	15. 9	97. 00	0[b]	0	0	0	0	0	0	0
6: 00	20. 4	87. 54	2	0	0	0	0	0	0	0
8: 30	27. 0	77. 50	34	1	1	0	0	0	0	0
9: 00	27. 0	77. 50	27	7	1	0	0	0	0	0
10: 00	28. 5	70. 75	49	5	7	1	0	0	0	0
10: 30	29. 1	66. 20	33	7	2	1	1	0	0	0
11: 00	30. 0	63. 15	34	2	5	2	2	1	1	0
16: 30	27. 1	87. 42	26	6	1	1	0	0	0	0
17: 00	26. 9	92. 60	14	2	1	1	0	0	0	0
18: 00	23. 8	97. 20	6	0	0	0	0	0	0	0
20: 30	22. 8	93. 00	0	0	0	0	0	0	0	0

[a] The number of *Limonia (Euglochina)* sp. resting on a thread

[b] Number of manners observed in aggregation of *Limonia (Euglochina)* sp. under different condition.

all males have chances to mate with the female but only the last male has the greatest likelihood of fertilizing the ova[4]. The post-copulatory guarding behavior[5] of *Limonia (Euglochina)* sp. have not been observed in the present study because of failing to find their oviposition habitats in the rainforest.

2. 3　Morphological mechanism of hanging on spider web silk thread

In 1970, Foelix's experiment verified Nielsen's hypothesis[6] that the spiders help the silk thread by the accessory claw against the notches between the serrated bristles (Fig. 3 h)[7]. While comparing ultrastructure of the pretarsus between *Limonia (Euglochina)* sp. and their host spider, we believed *Limonia (Euglochina)* sp. grasping the silk thread by their serrated claws and the medial bristles (Fig. 3 b, e). The serrations of claw guaranteed the *Limonia (Euglochina)* sp. holding the thread fast and prevented the forelegs from sliding out of silk thread. Moreover, they served as notches of serrated bristles on the tip of spider's legs, providing the positive force as the accessory claws in spider's legs (Fig. 3 a). The medial bristles (Fig. 3 b, e) in pretarsus of *Limonia (Euglochina)* sp., associated with the serrated claws, supported the silk thread while SC grasping, might also serve as a mechanoreceptor. The sketch drawings (Fig. 3 i, j) tried to further indicate how did the *Limonia (Euglochina)* sp. grasp the orb-web silk thread. *Limonia (Euglochina)* sp. could walk on the silk thread by exchanging its two forelegs with the association of midlegs (Fig. 3 c). The hindlegs (Fig. 3 f) did not touch the thread but function basically as weapon in combat with another crane fly. The unguitractor plate structure is still unidentified and the actual function is unknown, we speculated that it might be a particular form of unguitractor plate (Fig. 3 b, e, f).

As compared the foreleg or midleg pretarsus of *Tipula yamata* (Fig. 3 g), a wide distributed crane fly in China who can not rest on the spider web, with that of the *Limonia (Euglochina)* sp., it was obvious that the *Tipula yamata*'s pretarsus lacked of necessary morphological structure to help them grasping the spider orb-web thread but could easily grasp the rough surface such as trunk, wall and so on. However, another morphological mechanism by which some species could hold the silk thread might be present. As it were showed in Fig. 3 d and the sketch drawing (Fig. 3 k) that the hairy rotiform empodium on tip of the pretrasus of an unidentified Dipterous insect might explain why they could also hang on silk thread of the spider web.

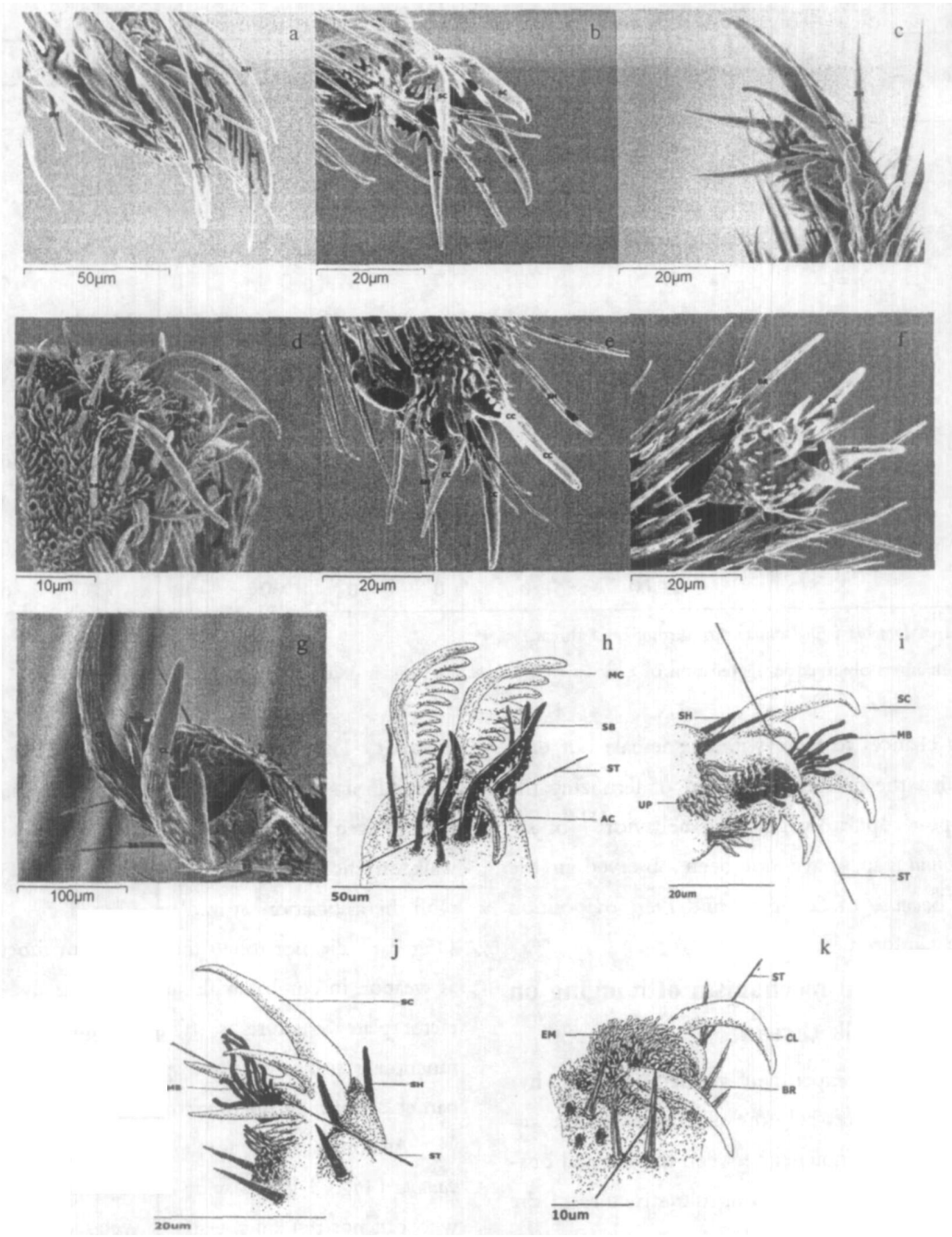

Fig 3 Ultrastructure of the pretrasus of different species and the sketch drawings to present how *Limonia (Euglochina)* sp. grasped the silk thread on the orb-web

a. Foreleg pretrasus of an Araneidae spider; b, c. Lateral view of the foreleg and midleg pretrusus of *Limonia (Euglochina)* sp. separately; d. Foreleg pretrasus of an unidentified Dipterous insect; e. Ventral view of the foreleg pretrusus of *Limonia (Euglochina)* sp.; f. The hindleg pretarus of *Limonia (Euglochina)* sp.; g. Foreleg pretarsus of *Tipula yamata*; h. Sketch drawing imitate Foelix; i~k. Sketch drawings of foreleg in *Limonia (Euglochina)* sp. and the way how *Limonia (Euglochina)* sp. grasped the silk thread on the orb-web with its midleg respectively.

MC = Main Claw, AC = Accessory Claw, SB = Serrated Bristle, SH = Straight Hair, SC = Serrated Claw, MB = Media Bristle, UP = Unguitractor Plate, CL = Claw, BR = Bristle, EM = Empodium, ST = Silk thread of the spider web

When several *Limonia (Euglochina)* sp. assembled on the same silk thread of orb-web, one *Limonia (Euglochina)* sp. often began to "vibrate" in a specific frequence, then the others on the same thread followed the "initiator" to perform the vibration behavior. Based on this observation we considered they communicated with

each other by the thread they were hanging and the medial bristles on pretarsus (Fig. 3 b, c) served as the mechanoreceptor. Since the spiders have never attacked the crane flies who rested actively on their webs, the *Limonia (Euglochina)* sp. seem did nothing harmful to the hosts. However, we trended to explicate this phenomenon with inquilinism rather than symbiosis because the crane flies seemed get protection from the spider web but contributed nothing to the host spiders.

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南靖亚热带雨林美刺亮大蚊栖息蜘蛛网的行为学观察

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摘要: 在南靖和溪亚热带雨林中分布有数种大蚊, 美刺亮大蚊 (*Limonia (Euglochina)* sp.) 是雨林中唯一观察到在圆蛛科蜘蛛网上栖息的大蚊. 因此我们对美刺亮大蚊和圆蛛科蜘蛛在蜘蛛网上的同现行为进行了观察研究. 借助扫描电镜技术, 在比较了其他大蚊、蜘蛛等节肢动物前跗节超微结构的基础上, 对美刺亮大蚊栖息于蜘蛛网行为学的形态学机制进行了初步解析. 文中也对美刺亮大蚊的聚集行为与雨林温湿度的关系进行了记录.

关键词: 大蚊; 圆蛛; 同现行为; 前跗节; 美刺亮大蚊