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Antennal epicuticular structure of camel crickets (Orthoptera: Rhaphidophoridae) for identifying the prey of *Mustella sibrica* Pallas

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ABSTRACT

Camel crickets (Orthoptera: Rhaphidophoridae) are a group of wingless and humpbacked Orthopteran insects. They are closed relatives to katydids and crickets; they were called spider crickets somewhere because of their long legs. Camel cricket plays an important role in the food web in various ecosystems. In the forest ecosystem, they live in the ground level of forestry under litters and fallen leaves, and some of them live beneath the log and hid themselves under loosen tree barks. They feed on decomposing organic substances and serve as decomposers in the food chain. It is a very common insect prey to insectivorous animals in natural and artificial vegetation. Guandaushi (GDS) is the experimental forest station of National Chung Hsing University, as the study site of long term ecological research (LTER) in Taiwan. And the camel cricket became an important subject and a dominant insect group in the LTER studies. They are the major parts of the insect preys of vertebrates, especially to the insectivorous mammals. According to our previous publication, 32.2% of the insect preys of *Mustela sibirica* Pallas are camel crickets in GDS forest ecosystem. There were 5 morpho-species of camel crickets by the characters of antennal epicuticular characters. The camel cricket was collected from various ways according to the LTER study during 1995-1998. The morphology of antennal epicuticular structure from totally 60 individuals was observed by using SEM. The epicuticular sculpture was described and classified into Type I and Type II by the SEM photographs. Type I has 8 patterns and Type II has 5 patterns in this study. The illustration and the pictorial key were provided here as the tool for further application. The morpho-species could not be identified due to the remainder being fragmented while examined. After the study, the SEM antennal epicuticular structures were classified and identified based upon the antennae sample of the morphospecies from the specimens of the 5 camel crickets which were collected from GDS forest ecosystem in this study. This is a part of the fundamental study for the protocol of ecosystem study. The protocol for identifying the insectivorous animal's prey by the insect fragment of food remainders is necessary. One more, there were 7 of the 13 patterns of antennal epicuticular structure applied to analysis the 5 morpho-species provided as the case study for taxonomy.

KEYWORDS

Camel Crickets; Antennal Epicuticle; Ultra-Structure; Insectivorous; Prey

1. INTRODUCTION

The surfaces of insect antennae with various structures, such as sensory organs, hairs setae etc. are important and good characters to taxonomy. We used to describing some special and distinguishable structure applied as good diagnostic characters for the identification. There are many previous studies reported on antennal sensory plaque organs in the taxonomy and phylogeny of fulgoroidea of homoptera [1,2]. And recently, antennal sensory organ classified and evaluated as the taxonomic characters of nocturnal moth by SEM photography is published by our team [3]. Antennal epicuticular structure has been served as insect taxonomic character since long time ago, but not until the practice of the techniques of SEM and TEM, the ultra fine structure becomes possible in many other studies. In general, the number and the position or distribution type of cuticular receptors were used as important characters or land marks structures while studying on antennal morphology [4,5]. The identification using antennal structure is fairly common in identifying some groups of insects such as thrips, spring tails, dipterans etc., but not so common in identifying the fragments which mixed in the inclusion of food remainders pieces by pieces from mammal's digestive ducts or the animal feces. This paper is an application of insect integument structure for investigating the prey of insectivorous animals. Moreover, to establish the character library in such detail, fine scale is special for identifying the fragmented pieces of remainders obtained from the mammal's digestive ducts or their feces.

Camel crickets, Rhaphidophoridae belonging to Orthoptera are a group of middle size insects in Taiwan; the body size in general, is about 30 mm in length. Filiform antennae are about 3 - 5 times longer than body length. Most camel crickets are humpbacked, and both male and female are wingless. The habitats of them are known as living on damp humus, and sometimes in rock caves or tree caves as well. Camel crickets are scavengers or saprophagous [6].

According to Yang *et al*. [7] and Hu [8], the most parts of insect fragments in the feces of *Mustela sibirica* Pallas, 1977 (Carnivora: Mustelidae) and in digestive tracts of *Crocidura tadae kurodai* Jameson & Jones, 1977 (Insectivora: Soricidae) are Rhaphidophoridae in Guandaushi forest ecosystem. Obviously, camel crickets are significant keystone species for insectivorous mammals in GDS forest ecosystem. This study will be very helpful in such food chain or food web investigation to analyze the trophic circulation in the ecosystem.

2. MATERIALS AND METHODS

The study was conducted at the Guandaushi forest (24˚04'N, 121˚01'E), Huisun Experimental Forest Station of National Chung Hsing University, Nantou. The camel crickets specimens were collected by pitfall traps (24 × 15 × 11 cm) and fragments were collected from the feces of *Mustela sibirica* Pallas, 1977 (Carnivora: Mustelidae) while the Long Term Ecological Research investigated the insectivores animal's prey insects (**Table 1**). All the possible specimens became as available in this study. We tend to provide the taxonomic identification tools for the applied entomological study in the future.

Preparation: The procedures for preparing to collect the antennal of camel cricket are described as following.

1) Antennal Sample: Cutting off 5 - 10 segment of antenna from the individual specimen which was collected from field by pitfall trap, and put into 70% alcohol solution for temporality preservation.

2) Cleaning: Ultrasonic (43 KHz) for 15 minutes to clean up the specimens to get rid of the contamination on the surface.

3) Dehydration: A gradient series of acetone and alcohol (70%, 85%, and 100%) at room temperature for dehydration.

4) Coating: Using gold in each specimen.

5) Observation: SEM (TOPCON ABT-150s) applied for this study.

Measurement: The measuring parts and the landmarks of the process or producing on the antennal surface are shown in **Figures 1** and **2**. The surface and the face of electronic gun of SEM should be parallel to each other to prevent from the projection of the SEM photographs. The method followed previous study of Yang and Yen (2001) for sure the metric character is technically useful and reasonable.

3. RESULTS AND DISCUSSION

Five morpho-species of camel crickets have been found in this study (**Table 1**). However, it does not mean that there are 5 species of camel cricket in the LTER site, GDS subtropical forest ecosystem, Taiwan. The morphology of antennal epicuticular structure was observed by using SEM.

Totally 60 individuals were examined and described as detail as possible for the potential usage of the insectivores animal's prey study in the future. There were 13 patterns of antennal epicuticular structure discriminated from the various surface structures. They were classified as two main groups, Type I the lobe-like and Type II the setae-like. The lobe-like group, Type I including 8 types were subdivided into 2 subgroups: complete lobe (Type I a-1) and separate lobes (Type I b-1). The setae-like group, Type II including 5 patterns was divided into 3 subgroups, *i.e.*, short setae-like, long ridged setae-like and needle-like surface sculptures. The arrays of the surface sculpture were distinguished and described as linear, reticular and sinuate types. This result will make the application of insect fragments identification for investigating the insectivore animal's prey became as possible and easier.

The patterns and array types of epicuticular structure could be the significant character sets for identifying camel crickets in GDS forest ecosystem. The key to types of antennal epicuticular structure of camel crickets in this study is provided in the following section (key to types and patterns of antennal epicuticular structure of camel

Table 1. Camel cricket samples collected from pitfall traps in GDS forest ecosystem, 60 specimens.

coll. Date (yyyy-mm-dd)	Sampling plot	coll. method	Phenon no.	morpho-species	Antennal Type/pattern	Illust. Index (Figure)	Elevation (m)	Notes (collector)
19980424	4-2	pitfall	1	sp1	Ib-4	F	1100	SH Wu
19980409	4-2	pitfall	2	sp1	Ib-4	F	1100	SH Wu
19980409	3-2	pitfall	3	sp1	Ib-4	F	1200	SH Wu
19980409	4-1	pitfall	4	sp1	Ib-4	F	1100	SH Wu
19980409	1-1	pitfall	5	sp1	Ib-3	E	1150	SH Wu
19980409	3-2	pitfall	6	sp2	Ib-5	G	1200	SH Wu
19980409	3-2	pitfall	7	sp2	Ib-5	G	1200	SH Wu
19980730	4-3	pitfall	8	sp2	Ib-5	G	1100	SH Wu
19980730	4-3	pitfall	9	sp2	Ib-5	G	1100	SH Wu
19980730	1-3	pitfall	10	sp5	Ib-2	D	1150	SH Wu
19980730	4-2	pitfall	11	sp2	Ib-5	G	1100	SH Wu
19980409	1-1	pitfall	12	sp3	Ib-5	F	1150	SH Wu
19980730	2-3	pitfall	13	sp3	Ia-2	B	1250	SH Wu
19990425	8-2	pitfall	14	sp3	Ib-4	F	1550	SH Wu
19990425	8-2	pitfall	15	sp3	Ib-4	F	1550	SH Wu
19990425	9-5	pitfall	16	sp3	Ib-4	F	1610	SH Wu
19980428	1-1	pitfall	17	sp3	Ib-4	F	1150	SH Wu
19990425	I-2	pitfall	18	sp4	Ib-4	F	ND	SH Wu
19990131	9-4	pitfall	19	sp4	Ib-4	F	1610	SH Wu
19990425	9-5	pitfall	20	sp4	Ib-4	F	1610	SH Wu
19990131	1-3	pitfall	21	sp5	Ib-2	D	1150	SH Wu
19990425	3-2	pitfall	22	sp1	Ib-4	F	1200	SH Wu
19990131	8-3	pitfall	23	sp4	Ib-4	F	1550	SH Wu
199808-	H30-3B	pitfall	5-001	sp5	Ia-1	A	1500	HY Wu
199808-	H30-3B	pitfall	6-002	sp5	Ia-2-1	B-1	1500	HY Wu
199710-	W40-1B	pitfall	9-001	sp1*	Ib-4	F	1350	HY Wu
199710-	W40-3B	pitfall	15-001	sp5	Ia-1	A	1350	HY Wu
199808-	H37-2B	pitfall	26-002	sp5	Ia-1	A	1550	HY Wu
199804-	H48-2B	pitfall	52-005	sp1*	Ib-4	F	1600	HY Wu
199802-	L79-2B	pitfall	133-001	sp2	Ib-4-1	F-1	1550	HY Wu
199712-	H30-2B	pitfall	159-001	sp5	Ia-1	A	1500	HY Wu
199712-	H37-1B	pitfall	161-001	Sp2	Ib-4-1	F-1	1550	HY Wu
199712-	H48-1B	pitfall	163-001	sp5	Ia-1	A	1600	HY Wu
199712-	L72-1B	pitfall	172-001	sp5	Ib-2	D	1650	HY Wu
199710-	H30-2B	pitfall	177-001	sp5	Ia-1-1	A-1	1500	HY Wu
199810-	L97-1B	pitfall	205-001	sp5	Ib-2	D	1680	HY Wu
199810-	L97-1B	pitfall	206-002	sp5	Ib-2	D	1680	HY Wu
199810-	L97-1B	pitfall	207-003	sp1*	Ib-4	F	1680	HY Wu
199810-	L97-2B	pitfall	208-001	sp5	Ia-2-	B-2	1680	HY Wu
199810-	L79-1B	pitfall	209-001	sp5	Ia-2-2	B-2	1550	HY Wu
199810-	L26-3B	pitfall	211-001	sp5	Ib-2	D	1610	HY Wu
19971024	H40	remainder	221-001	(sp1)**	Ib-3	E	1350	JT Yang
19971217	H09	remainder	237-001	(sp5)	Ib-2	D	1400	JT Yang
19960614	H63-1	remainder	110-001	(sp 5)	Ia-1	A	1600	JT Yang
19960728	H63-1	remainder	122-001	(Sp 5)	Ia-2-2	B-2	1600	JT Yang
19960730	H60	remainder	126-002	(Sp 5)	Ia-2-2	B-2	1600	JT Yang
19961015	H56	remainder	151-001	***	Ib-1	C	1600	JT Yang
19970813	H58	remainder	206-001	(sp5)	Ia-1	A	1600	JT Yang
19971021	LTER40	remainder	215-002	(sp 5)	Ia-2-2	B-2	1600	JT Yang
19971214	H46	remainder	227-000	(sp 1)	Ib-3	E	1600	JT Yang
19961017	LTER82	remainder	153-002	(sp 5)	Ia-1	A	1620	JT Yang
19960813	LTER49	remainder	204-002	(sp 5)	Ia-1	A	1630	JT Yang
19971217	LTER49	remainder	239-000	(sp 1)	Ib-3-1	E-1	1630	JT Yang
19960723	LTER67	remainder	120-003	(sp 5)	Ia-1	A	1640	JT Yang
19960912	LTER82	remainder	127-002	(sp 5)	Ia-2-1	B-1	1650	JT Yang
19971024	LTER90	remainder	222-001	(sp 1?)	Ib-3-2	E-2	1670	JT Yang
19971216	LTER90	remainder	236-002	(sp 1)	Ib-3	E	1670	JT Yang
19980212	LTER90	remainder	249-002	(sp 1?)	Ib-3-2	E-2	1670	JT Yang
19970319	LTER105	remainder	185-001	-	Ib-6	H	1700	JT Yang
19971214	LTER99	remainder	232-002	(Sp1)	Ib-3	E	1690	JT Yang

**The morpho-species could not identify due to the remainder is fragmented while examined. After the study, compared to the SEM antennal epicuticular structures classification and identify to the morpho-species shown in parenthesis ( ). ***The SEM observation only by fragment and not able to identify to morpho-species known in this study.

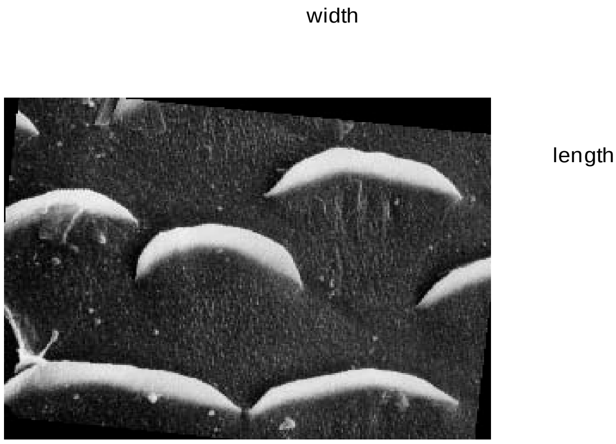


Figure 1. The land marks for measuring the antennal epicuticular structure of complete lobe-like patterns (Type I).

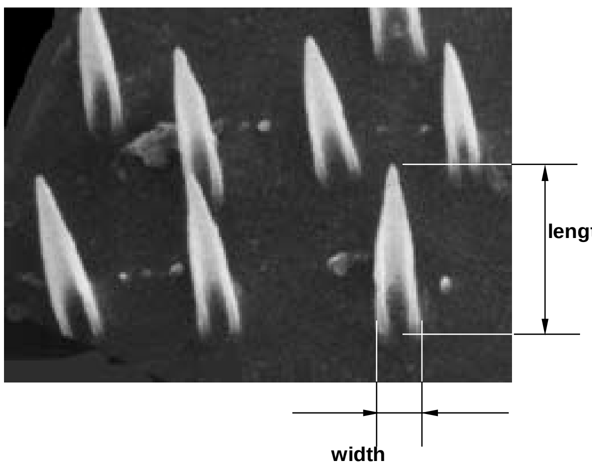


Figure 2. The land marks for measuring the antennal epicuticular structure of setae-like patterns (Type II).

crickets in GDS forest ecosystem). It is useful to other relevant studies, such as food web of terrestrial predator project study. There have been many projects under Long Term Ecological Research (LTER) in GDS forest ecosystem focused on insectivorous animal's feeding behavior like as Kam *et al*. [9], Yang *et al.* [7] and Hu *et al*. [10]. In general, animal prey investigation is based on the community inventory, but the accuracy prey which is intaken by predators must be evident by remainder in feces or inclusions in digestive ducts. This study used both the material collected directly from the remainder and from the pitfall trapped camel crickets for comparison and it makes the study possible from both remainder of feces and inclusions of intestine and fauna inventory. The camel cricket is common and dominant ground living insects in different ecosystem. The antenna fragments remanded in both feces and digestive ducts as the dominant parts of insect fragments. The materials are easy to collect for identification. The antennal surface is easier to observe by SEM. The characters of the epicuticular structure were defined in this paper and classified as 13 patterns. The morpho-species could not be identified due to the remainder is fragmented while examined. After the study (**Table 1**), compared to the SEM antennal Epicuticular structures classification and identify to the morpho-species are shown in the **Table 1**. However, some SEM observation is lacking the comparison from the pitfall trapped specimen and still not able to identify to morpho-species in this study. Significantly, the community inventory is some very supported background information in this case. As a biological taxonomist, we would like to suggest the fauna inventory and taxonomic revision work is very important for such applied ecological studies.

It makes identification become as much easier while study on the feeding behavior of insectivore animals. In addition, this study could provide some information and inspiration for the taxonomic study of Rhaphidophoridae to find and evaluate the better and good characters for diagnosis in between species and developing stages by the intra-specific variations.

Key to types and patterns of antennal epicuticular structure of camel crickets (Orthoptera: Rhaphidophoridae) in GDS forest ecosystem:

1a Antennal epicuticular surface sculpture in lobe like - 2
1b Unlike above --- 9
2a Each surface sculpture with single complete lobe --- 3
2b Each surface sculpture comprising of several separate lobes -- 4
3a Lobe surface sculpture wider than middle long about 5.1: 1.0 (n = 3) (**Figure 3(a)**) ------------------- **Type I a-1**
3b Lobe surface sculpture wider than middle long about 9.4: 1.0 (n = 1) (**Figure 3(b)**) ------------------- **Type I a-2**
4a Margin of lobe surface sculpture round --------------- 5
4b Margin of lobe surface sculpture angulate or irregular -- 6
5a Surface sculpture comprises of 3 - 5 separate lobes, some lobes with ridge indistinct (**Figure 3(c)**) ----------- -- **Type I b-1**
5b Surface sculpture comprise of 5 - 10 separate lobes, without ridge (**Figure 3(d)**) --------------------- **Type I b-2**
6a Setae-like surface sculpture in between lobes -------- 7
6b No-setae-like surface sculpture in between lobes ---- 8
7a Lobe surface sculpture without lanceolate surface sculpture (**Figure 3(h)**) ------------------------- **Type I b-6**
7b Lobe surface sculpture mixed with lanceolate surface sculpture (**Figure 3(g)**) ------------------------- **Type I b-5**
8a Indistinct margin of lobe surface sculpture free from antennal surface (**Figure3(f)**) ------------------- **Type I b-4**
8b Distinct margin of lobe surface sculpture free from antennal surface (**Figure 3(e)**) ---------------- **Type I b-3**
9a Needle-like or lanceolate surface sculpture arranged sinuately -- 10
9b Needle-like or lanceolate surface sculpture not arranged sinuately -- 12

10a Only short needle surface sculpture (Figure 3(i)) --- **Type II a-1**

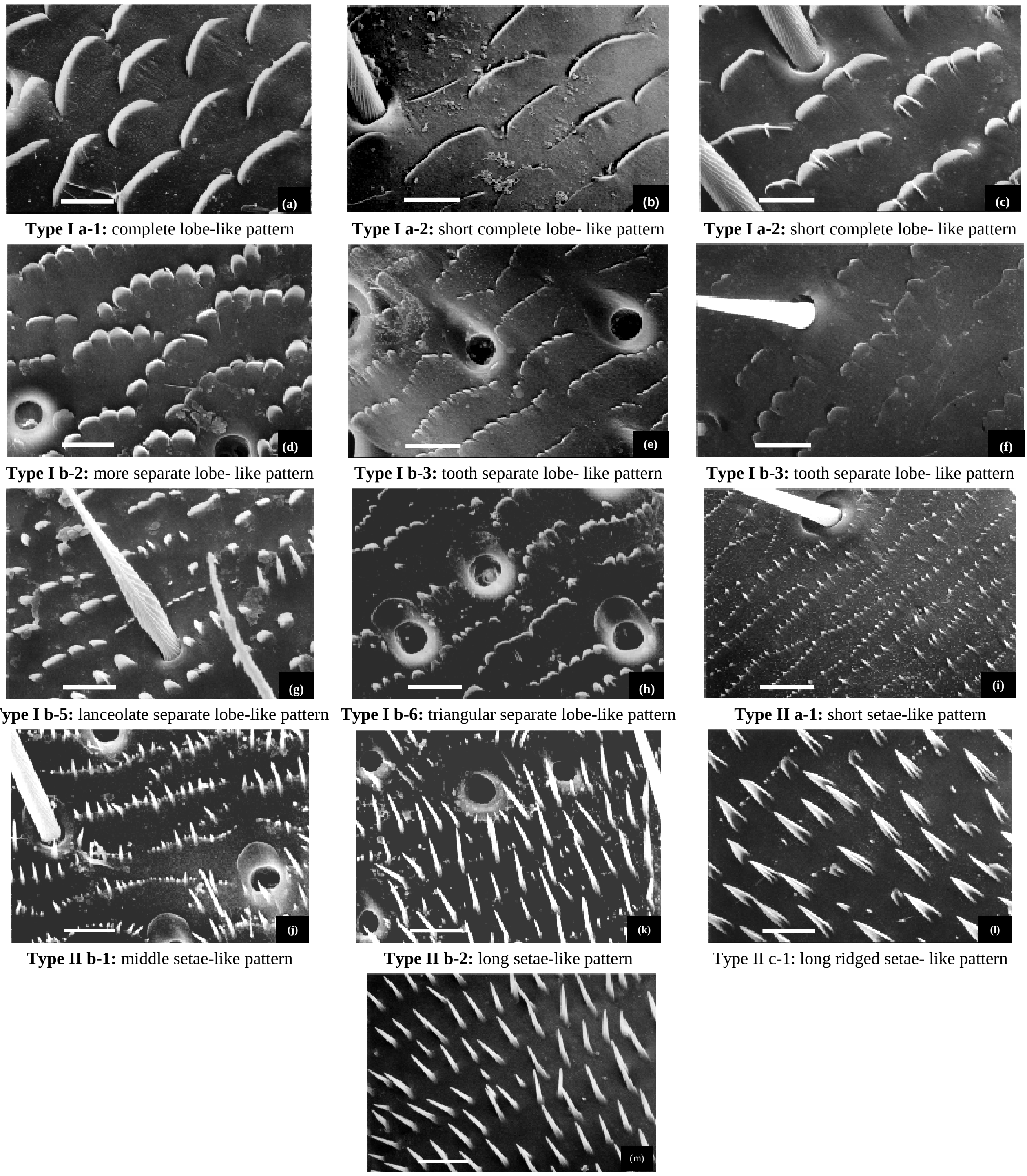
10b Both short and long needle surface sculpture --- 11

11a Long needle about twice as long as short ones (Figure 3(j)) -- **Type II b-1**

11b Long needle much longer than short ones (Figure 3(k)) -- **Type II b-2**

12a Lanceolate surface sculpture with ridge (Figure 3(l)) --- **Type II c-1**

12b Lanceolate surface sculpture without ridge (Figure 3(m)) --- **Type II c-2**



Type I a-1: complete lobe-like pattern
Type I a-2: short complete lobe- like pattern
Type I a-2: short complete lobe- like pattern
Type I b-2: more separate lobe- like pattern
Type I b-3: tooth separate lobe- like pattern
Type I b-3: tooth separate lobe- like pattern
Type I b-5: lanceolate separate lobe-like pattern
Type I b-6: triangular separate lobe-like pattern
Type II a-1: short setae-like pattern
Type II b-1: middle setae-like pattern
Type II b-2: long setae-like pattern
Type II c-1: long ridged setae- like pattern
Type II c-2: needle-like pattern

Figure 3. Various types of antennal epicuticular structure of camel crickets (scale bar = 10 μ).

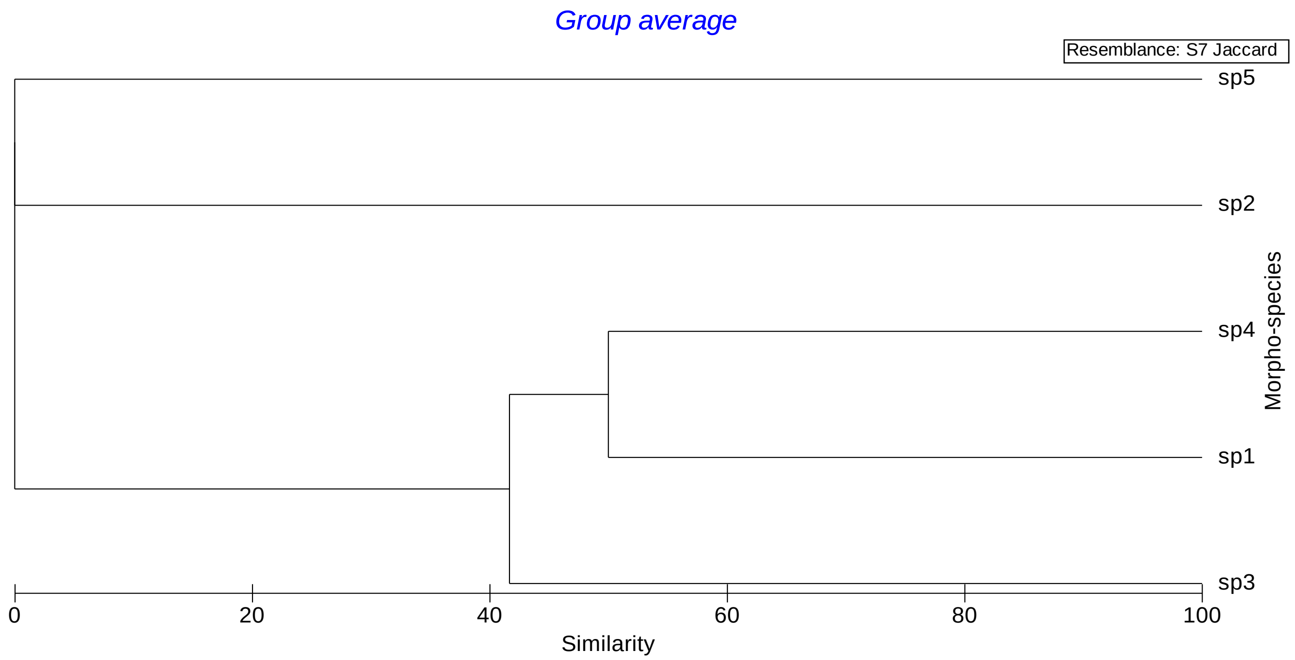


Figure 4. Apply the clustering analysis with Jaccard coeficience to analysis the 5 morpho-species by the 7 patterns of antennal epicuticular structure for example to show the usage of the potential taxonomic character which was provided in this paper. It is the case of application which based on the antennal epicuticular structure of camel crickets.

4. CONCLUSIONS

The camel cricket is sorted as 5 morpho-species by antennal epicuticular structure based on this study. However, the camel cricket fauna of GDS forest ecosystem is still unknown. It is necessary to do much effort to do the taxonomic revision to verify the fact of the camel cricket's biodiversity in the forest ecosystem. The variation of the SEM morphology is sorted as two types and each is with 5 and 8 patterns respectively.

It is just the basic taxonomic character description and definition for the insect diagnosis for both intra- and interspecies in morphology. Taxonomists have to evaluate the good character for good taxonomy based on this study. After the study, the SEM antennal Epicuticular structures classification made the identification of the materials from the remanded fragments to the morpho-species become possible which is shown in the **Table 1**. The exception antennal pattern actually makes us aware that the community inventory to ecosystem study is supported background information in this protocol while the diet of insectivore animal's feeding behavior is studied. As a biological taxonomist, we would like to suggest and emphasize the fauna inventory and taxonomic revision work should be very important for such applied scientific studies.

So, the result of the SEM morphology in this paper is just served as basic and important data and information to the protocol for the taxonomist while applying the taxonomic methodology to do identification.

The clustering analysis for example is showing the further application's possibility to the antennal epicuticular structure (**Figure 4**). Although, the evaluation to such taxonomic characters is necessary while studying the revision work to camel crickets. This is the possible way for taxonomist to do co-laboratory study and publish paper with ecologist and others by sharing the credits.

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