SCANNING ELECTRON MICROSCOPY OF THE CIBARIAL ARMATURE OF SPECIES IN THE *ANOPHELES DIRUS* COMPLEX (DIPTERA: CULICIDAE)

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Abstract. The structure of the cibarial armature of mosquitoes has been found to be useful for taxonomic identification. We used a scanning electron micrograph to examine the cibarial armature of 4 of 5 species in the *Anopheles dirus* complex existing in Thailand: *Anopheles dirus* Peyton & Harrison, and *An. cracens* Sallum & Peyton, *An. scanloni* Sallum & Peyton, and *An. baimaii* Sallum & Peyton. In all species examined, there was only 1 row of large teeth or cones (modes = 12) characteristic of the Neomyzomyia series. The cones usually have anterior spines and a fimbriated or deeply cleft tip. No significant differences were observed among the 4 species examined, thus the cibarial armature has little value for taxonomic differentiation among these species. However, they appear different from closely related species in the Leucosphyrus complex reported previously.

INTRODUCTION

The foregut of many hematophagous insects is characterized by the presence of sclerotized teeth and spines which are organized into rows or groups protruding from the gut wall into the lumen called "cibarial armature". This structure has multiple functions, including defense against filarial infections and a role in blood-meal hemolysis (McGreevy *et al*, 1978; Coluzzi *et al*, 1982; Clements, 1992). In Phlebotomine sandflies (Diptera: Psychodidae), the cibarial armature is one of the major characters that has been used for taxonomic identification (Lewis, 1978). The structure of the cibarial armature in female mosquitoes under light microcopy has been found to be useful for the recognition of taxonomic series within the subgenus *Cellia* of *Anopheles* (Reid, 1968) and also useful for the subgeneric or specific identification of *Culex* species (Sirivanakarn, 1978). In the subgenus *Anopheles* the armature is missing. In the Myzomyia series of the subgenus *Cellia* the armature is differentiated into 2 rows, the so-called rods and cones; in the Neocellia series the crest of the cone has a double row of spines, whereas in the Pyretophorus, Paramyzomyia and Cellia series the cones have well developed roots. In contrast, in the Neomyzomyia series only 1 row of teeth or cones is present. However, it is difficult to describe fine structures using light microscopy (Christophers, 1933; Reid, 1968). Examination with a scanning electron microscope (SEM) revealed several novel characteristics that are useful for reconstructing the phylogeny of *Anopheles*

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(Anthony *et al*, 1999). We found a SEM useful for identifying members of the Minimus subgroup of the Myzomyia series (Somboon *et al*, 2000, 2001).

The Leucosphyrus group in the Neomyzomyia series is divided into 3 subgroups (Sallum *et al*, 2005), one of which, the Leucosphyrus subgroup, includes the Dirus complex and the Leucosphyrus complex; the latter of which includes species such as *An. leucosphyrus* Dönitz and *An. balabacensis* Baisas. Information regarding the fine structure of the cibarial armature of this series and others is very limited.

The Dirus complex consists of 7 members, some of which are important malaria vectors in southern and Southeast Asia (Sallum *et al*, 2005). Morphological identification in this complex is difficult due to overlapping characteristics. In the present study, we used a SEM to examine the cibarial armature of 4 of the 5 species of the Dirus complex found in Thailand: *Anopheles dirus* Peyton & Harrison, *An. cracens* Sallum & Peyton, *An. scanloni* Sallum & Peyton and *An. baimaii* Sallum & Peyton (formerly *An. dirus* species A, B, C and D, respectively) to determine if this structure is useful for identifying members of the complex. Unfortunately, specimens of the 5th species, *An. nemophilous* Peyton & Ramalingam, were not available during the study.

MATERIALS AND METHODS

The *Anopheles dirus* females used originated from western Thailand and were identified using the molecular method of Walton *et al* (1999). They were preserved as dry specimens in silica gel. *Anopheles cracens* has been maintained in our laboratory for a number of years (Prapanthadara *et al*, 2000) and a number of these individuals were taken from the colony. *Anopheles scanloni* and *An. baimaii* were collected from forested areas in Kanchanaburi and Mae Hong Son, respectively. Adult female mosquitos were collected at night by human-baited landing catches and from cow sheds. *Anopheles dirus s.l.* females were sorted from other anopheline mosquitos following Rattanarithikul *et al* (2006). They were kept alive and returned to the insectary in Chiang Mai. Those that had not blood fed were allowed to feed on a guinea pig. The gravid females were placed individually in small cups and allowed to lay eggs. After oviposition, the females were killed and preserved individually by desiccation with silica gel in small plastic tubes. They were then identified to the species level with a method that utilizes allele-specific amplification of the ribosomal DNA internal transcribed spacer 2 (ITS2) region as described by Walton *et al* (1999). Their progeny were reared to the adult stage and a number of 4-5 day-old females from each brood were sampled for dissection of the cibarial armature.

For fresh specimens, the cibarial armature was dissected from heads in a drop of distilled water, and dehydrated through a graded ethanol series and then mounted on stubs. For dry specimens, the head was placed in 1 ml distilled water with a drop of glass washing solution (Lipon-F, Lion, Thailand) and kept in a refrigerator overnight (this is useful to reduce debris covering the armature). The heads were then washed in distilled water and dissected as above. After being sputter-coated with gold, the specimens were examined and photographed in a JEOL scanning electron microscope (JSM-840AN; JEOL, Akishima, Japan).

RESULTS

Fig 1 shows the SEM of the cibarial armature of *An. dirus* (a, b), *An. cracens* (c, d), *An. scanloni* (e, f), and *An. baimaii* (g, h). In all species, there was only one row of large teeth characteristic of the Neomyzomyia

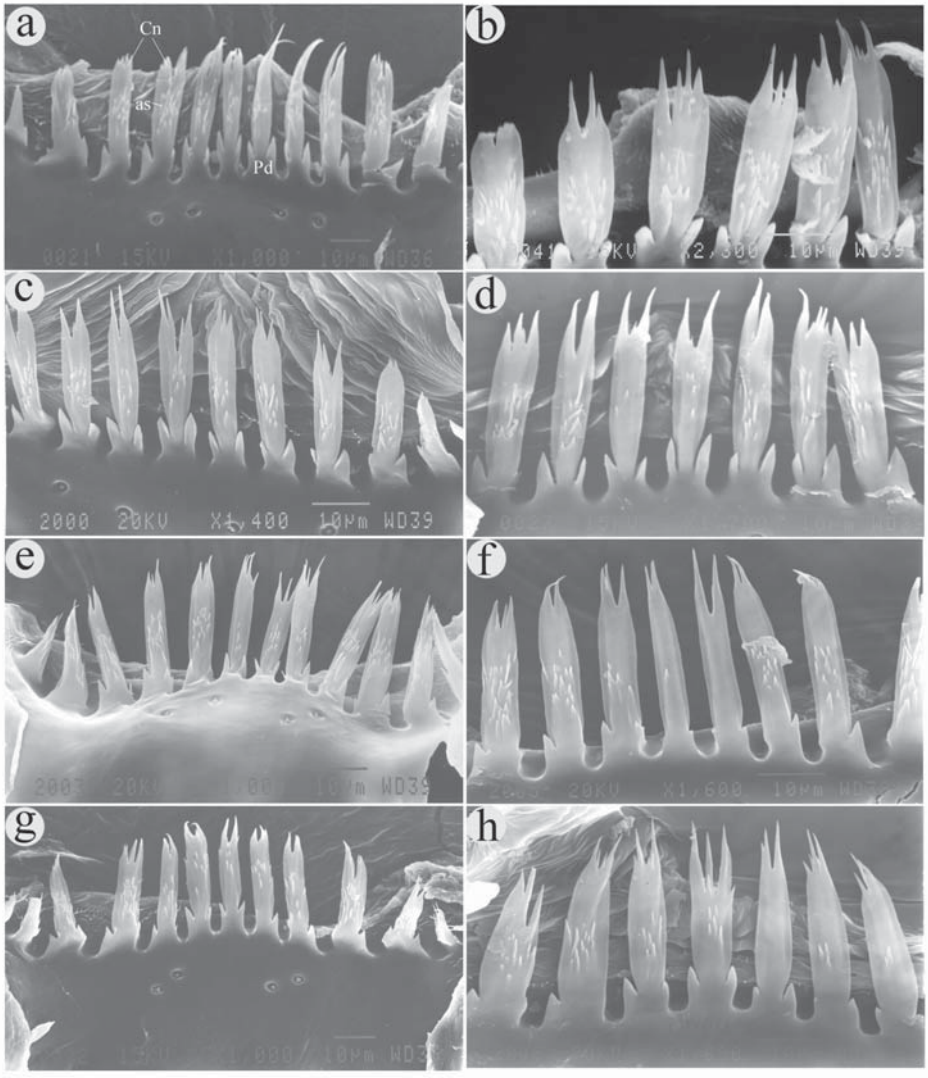

Fig 1–Scanning electron micrographs illustrating anterior aspects of the cibarial armature of members of the Dirus complex. a and b, *An. dirus*; c and d, *An. cracens*; e and f, *An. scanloni*; g and h, *An. baimaii*. as, anterior spines of cone filament; Cn, cone; Pd, pediment.

series. The number of cone filaments in the 4 species varied from 9-15 (all modes = 12) (Table 1) of which 2 or 3 lateral ones were small and not fully developed. The pediment of the cones had 2 distinct, lateral teeth. The tips of the cones were mostly fimbriated or had a deep cleft. The middle part of the anterior aspect of the cones usually had spines (up to 20), but some cones lacked spines. Lateral spines were rarely. The average proportions of the cones with anterior spines in the 4 species analyzed by the chi-square test were not significantly different ($\chi^2 = 4.636$, df = 3, $p > 0.10$). The average numbers of the total anterior spines of the four species analyzed by an analysis of variance were also not significantly different ($F = 2.146$; df = 3, 53; $p > 0.10$) (Table 1).

Table 1
Average numbers of cones and anterior spines in the Dirus complex.

Species	No. examined	Mode of cones (range)	Average % cones with anterior spines (range)[a]	Average no. of total anterior spines (range)[b]
An. dirus	5	12 (11-14)	81.9 (66.7-100)	59.0 (45-72)
An. cracens	16	12 (9-15)	69.8 (53.3-90.9)	54.2 (22-82)
An. scanloni	16	12 (10-14)	76.9 (53.8-100)	49.8 (25-77)
An. baimaii	20	12 (10-14)	74.5 (53.8-100)	44.1 (16-65)

[a]$\chi^2 = 4.636$, df = 3, $p > 0.10$; [b]$F = 2.146$, d.f. = 56, $p > 0.10$

DISCUSSION

Using light microscopy, Reid (1968) described the general characteristics of the cibarial armature of members of the Neomyzomyia series as having a single row of rather large cibarial teeth with fimbriated or tips with deep clefts, as was observed in this study. For most studies few details of the cibarial armature are described and the taxonomic significance of this structure is overlooked. For example, Reid (1968) briefly described the cibarial armature of *An. leucosphyrus* as having 13-17 rather long teeth and that of *An. balabacensis* as have 12-20 cibarial teeth, variable in shape but often long and strap-like with small spicules on the stem, the tips were fimbriated or had a deep cleft. However, these earlier descriptions of the cibarial armature in these two species cannot be used for comparison to those in this study because they were of mixed origin and the species identifications cannot be confirmed. What Reid (1968) considered to be *An. leucosphyrus* and *An. balabacensis* are now known to be species complexes. Early records of *An. balabacensis* in Southeast Asia are now known to correspond to several species of Dirus complex (Peyton and Harrison, 1979; Peyton, 1990; Sallum *et al,* 2005).

The cibarial armature in the Dirus complex under SEM appears different from that of *An. leucosphyrus* reported by Anthony *et al,* (1999); the latter has numerous anterior spines except near the tip of cone, whereas those in the Dirus complex are fewer in number and mostly in the middle part of the cones, rarely extending to the pediment (Fig 1). In the Leucosphyrus Group, Reid (1968) reported anterior spines on the cones of only *An. balabacensis* but not the others. This may indicate that descriptions of the cibarial armature based on light microscopy in previous reports are incomplete and should be re-investigated.

Identification of the adult and immature stages of species of the Dirus complex by external morphology is difficult due to overlapping characteristics (Sallum *et al,* 2005). Our study demonstrates the fine structure of the cibarial armature is indistinguishable morphologically among the four species examined and is therefore not useful for separating the species from each other. The cibarial armature of the other species in the Dirus complex, *An. nemophilous*, *An. elegans* (James), and *An. takasagoensis* Morishita, is not known. Morphologically, the Dirus complex is distinguishable from the Leucosphyrus complex (Peyton, 1990). Our study also shows that the cibarial armature of the Dirus complex is distinguishable from at least one member of the Leucosphyrus complex, *An. leucosphyrus*, as reported by Anthony *et al* (1999). Although more information is

needed, our results indicate that the structures of the cibarial armature carry a phylogenetic signal as suggested by Anthony *et al* (1999). Our results also support the taxonomic classification of the Leucosphyrus subgroup by Harbach (2004) and Sallum *et al* (2005).

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