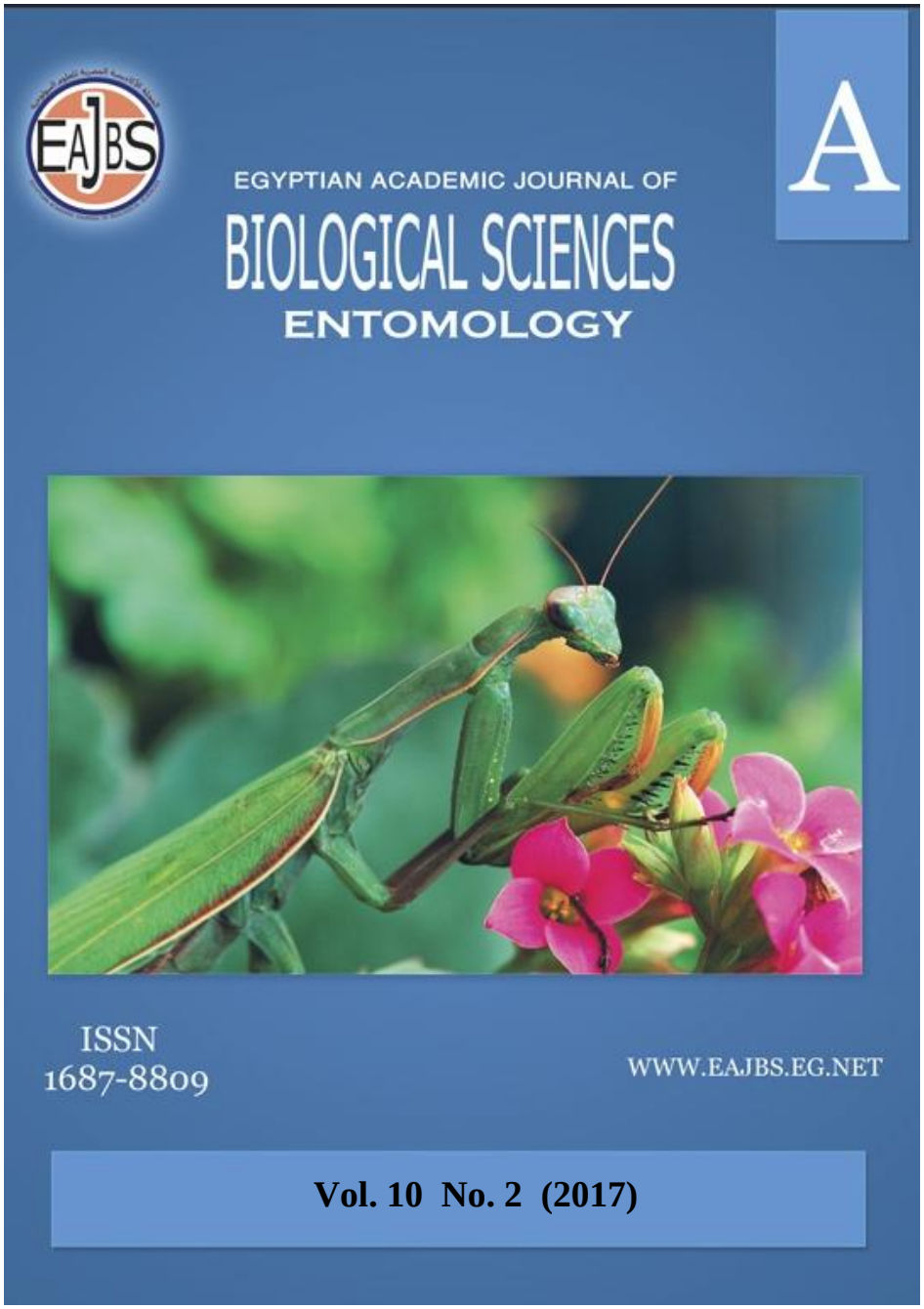

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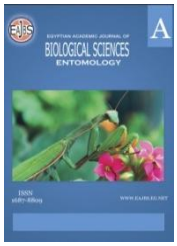

Morphological Diversity of Buccopharyngeal Armatures in Susceptible and Refractory Sandflies to *Leishmania major*

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ABSTRACT

The morphological diversity of armatures in susceptible and refractory sandflies to *Leishmania* was studied using light microscopy. Large interspecific variations were detected in the size, shape and numbers of the pharyngeal armatures. *Phlebotomus papatasi* females have stout pharynx, narrowing after posterior bulge, armature consisting of numerous small scales with fringe of fine backward-pointing teeth and without cibarial armature or pigment patch. *Phlebotomus sergenti* females have a pharynx with large scales anteriorly, some produced into long broad spines, scales becoming broader and flatter posteriorly with hind margin serrated with fine teeth. Oppositely, *Sergentomyia squamipleuris* females have tapering pharynx abruptly posteriorly, with rows of angular teeth. Cibarium with convex rows of fine, parallel horizontal teeth, undulating row of vertical teeth and pigment patch small, tapering anteriorly, and broad, chitinised, transverse band are present. *Sergentomyia christophersi* females have pharyngeal armature with a few scale-like folds anteriorly and a series of vague ridges posteriorly. Cibarium with four or five long teeth, many small denticles visible, small pigment patch are present.

INTRODUCTION

Leishmania are digenetic protozoa which inhabit two highly specific hosts, the sandfly where they grow as motile, flagellated promastigotes in the gut, and the mammalian macrophage where they grow intracellularly as non-flagellated amastigotes. According to the World Health Organization there are 2 million new cases each year and 1/10 of the world's population is at risk of infection (Gravino, 2004). The vectors of leishmaniasis are phlebotomine sandflies belonging to the genera *Phlebotomus*. In Egypt, *P. papatasi* is proved to be the vector of *L. major* (Wahba *et al.*, 1990 and Hanafi *et al.*, 1998) from rodents to human (El Hossary *et al.*, 2000). Like other blood-sucking nematoceran insects, the cuticle-lined foregut of *P. papatasi* comprises the biting mouthparts, the cibarium and the pharynx. The latter two are modified into pumps flanked by the cibarial and stomodaeal valves, which regulate blood flow into the midgut (Jobling, 1987).

The pharyngeal armature of sandfly consisted of pointed-teeth with various shape, number and arrangement among different species (Guo *et al.*, 2004). The topology of the internal surfaces of the sandfly female's alimentary tract is directly

relevant to the development of *Leishmania* promastigotes within it (Killick-Kendrick, 1999). Accordingly, the present work aims to study the structure diversity of armatures in susceptible and refractory sandflies to *Leishmania* parasite.

MATERIALS AND METHODS

Sandflies: Phlebotomine sandflies were colonized at the Research and Training Center (RTC), Ain Shams University. *Phlebotomus papatasi* and *sergenti* colonies were set up from gravid females caught in CDC light traps from Suez and southern Sinai, governorates, respectively while *Sergentomyia spp.* were collected from North Sinai. Colonies were maintained at 27±2°C and 80% R.H. Adults had permanent access to cotton pad soaked in saturated sucrose solution. Females were allowed to feed on anesthetized (Thiopental) golden hamsters twice per week.

Morphological Diversity of Armatures in Susceptible and Refractory Sandflies to *Leishmania*:

For light microscopy, the adult females were narcotized with carbon dioxide and then the head is removed from the body and mounted ventral side uppermost in a thin film of medium, thus allowing examination at high magnification. Photographs were taken by interference microscopy connected with computer. Variations between the cibarium and the pharynx of *Phlebotomus* as susceptible species and *Sergentomyia* as refractory species were examined under objectives with phase-contrast or interference illumination.

RESULTS AND DISCUSSION

Leishmania parasites develop as extracellular promastigotes that are attached to the alimentary canal wall of the sand fly vector (Killick- Kendrick, 1979). The cibarium and the pharynx, which function as pumping organs, are lined with cuticle (Warburg, 2008). The author found also that the cibarium and the pharynx bear diverse types of cuticular spines and appendages. He added that sand fly pharyngeal armatures consist of pointed-teeth with a variety of shape, number and arrangement among different species. To evaluate the relationship between *Leishmania* development and the presence of sandfly foregut armatures, the foregut of females of *P. papatasi*, *P. sergenti*, *S. squamipleuris* and *S. christophersi* were examined using light microscope for the structure of pharyngeal pump and cibarium in each of them.

In the present study, light microscope photos showed the features of pharyngeal pump in the four selected sandfly species. Large variations were detected in the size, shape and numbers of the pharyngeal armatures in the various tested species. *Phlebotomus papatasi* females have fleshy pharynx, tapering after posterior bulge, armature consisting of many small scales with fringe of fine backward-pointing teeth (Fig. 1A) and without cibarial armature or pigment patch (Fig. 1B). *Phlebotomus sergenti* females have a pharynx with large scales anteriorly, some produced into long broad spines, scales becoming broader and flatter posteriorly with hind margin serrated with fine teeth (Fig. 2A). Oppositely, *S. squamipleuris* females have tapering pharynx abruptly posteriorly, with rows of angular teeth (Fig. 3A). The size and shape of the cibarial pump varied from one species to another being markedly larger and more pointed in *S. squamipleuris* (Fig. 3B) and *S. christophersi* (Fig. 4B) than the two *Phlebotomus* tested species (Fig. 1B, 2B). Cibarial armatures are arranged in two rows of tiny cones and rods and sharp long pointed spines (Fig 3B,

4B). Structure of the pharyngeal armatures in these *Leismania* refractory species are characterized by developing three to four rows of small weak warts with flattened edges (Fig 3A). In *S. squamipleuris*, cibarium with convex rows of fine, parallel horizontal teeth, undulating row of vertical teeth and pigment patch small, tapering anteriorly, broad, chitinised, transverse band are present (Fig. 3B).

Pharyngeal armatures in *S. christophersi* reflect its refractoriness to *Leishmania* by having minute scattered spines (Fig. 4A), few scale-like folds anteriorly and a series of vague ridges posteriorly. Moreover, cibarium with well developed large sharp strong blades with pointed spines arranged in two rows of eight blades in the first row and five to six small spiny blades are present (Fig. 4B). Such differences may provide the morphological proof for identification of species (Randrianambinintsoa *et al.*, 2013 and Méndez-de Daboín *et al.*, 2015). Léger *et al*. (2005) described *S. goodmani*, the pharynx being narrow, and the cibarial armature comb-like. The presence of anterolateral teeth of the pharyngeal armature in *P. chabaudi*, never observed in *P. riouxi* helped to differentiate between the two closely related species (Bounamous *et al*., 2008). The inclusion of *P. barguesae n. sp.* in the subgenus *Euphlebotomus* is acceptable on the basis of characters of the male genitalia and of female pharyngeal armature (two kinds of teeth) (Depaquit *et al*., 2009).

In the context of large size parasite transmission, it has been suggested that the shape and density of the cuticular spines can influence the capacity to kill microfilaria. Thus, *Culex pipiens* that lack developed pharyngeal or cibarial pumps is more competent vector of *Wuchereria bancrofti* than *Anopheles multicolor* and *Aedes caspius* having respectively well developed cibarial and pharyngeal armatures that kill microfilariae (Shoukry and Soliman, 1995). Cuticular pharyngeal armature in mosquitoes has also been shown to provoke haemolysis (Chadee *et al.*, 1996). However, in the case of *Leishmania* and other protozoan parasites, it is doubtful that mechanical disruption plays a role in preventing infection. *Leishmania* promastigotes frequently colonize the pharynx, cibarium and mouthparts of infected sand fly females by attaching to the cuticular surfaces (Killick-Kendrick *et al.*, 1988 and Walters *et al.*, 1989).

In related studies, *P. fengi*, a suspected visceral leishmaniasis vector in China, had distinct pharyngeal armature (Leng and Zhang, 1994). Within the *fallax*-group, *S. fallax* was distinguished readily by its heart-shaped pharynx and the variation in armature between the dorsal and lateral plates (Benabdennbi *et al*., 1996). For comparison, females of *P. sergenti* and *P. similes* differ by the armature morphology of the pharynx and by the rings number of the spermatheca (Depaquit *et al*., 1998). Females of *P. fertei* and *P. huberti* have a characteristic pharyngeal armature bearing about ten cibarial teeth (Depaquit *et al*., 2002). The female of *P. fertei* was described from Madagascar (Depaquit *et al*., 2004), having pharyngeal armature with small teeth and cibarium with 15 to 30 denticles.

CONCLUSION

The present study supports the hypothesis that armature structures of sand flies are directly related to their vectorial capacity to *Leihmania* parasite.

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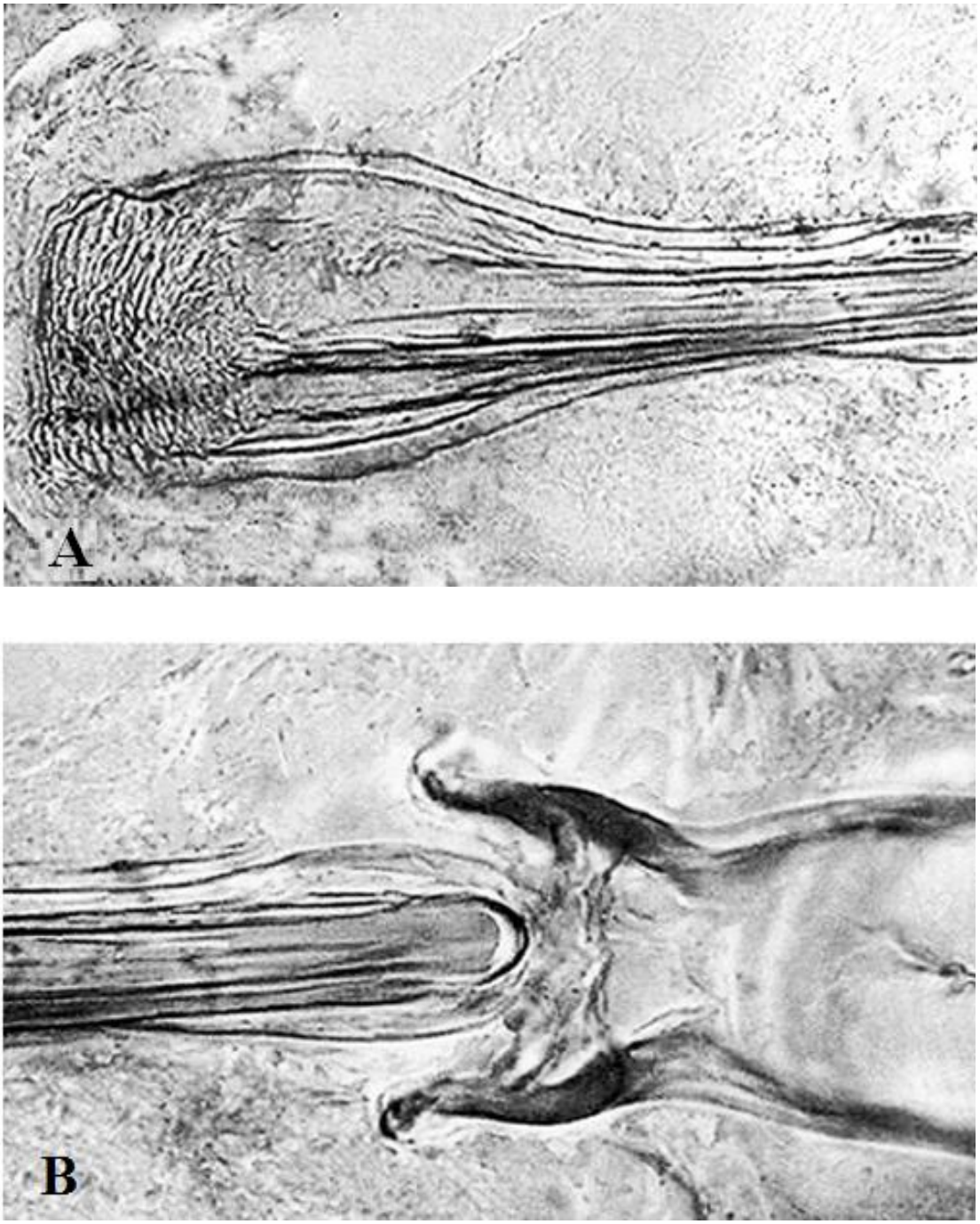

Fig. 1: Pharyngeal armature in *Phlebotomus papatasi* a susceptible species for *Leishmania* as shown by light microscope (A, Pharynx, B, Cibarium).

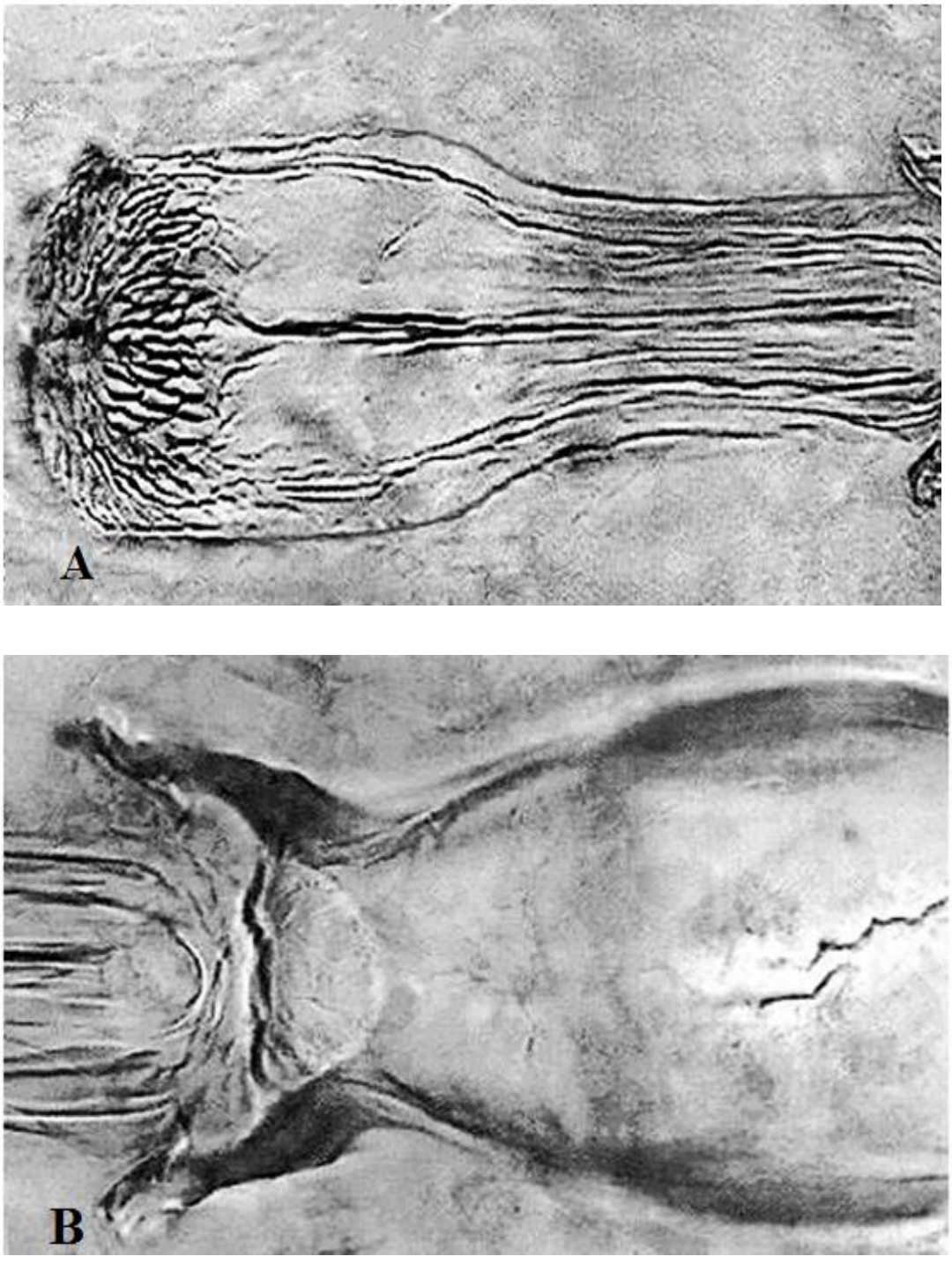

Fig. 2: Pharyngeal armature in *Phlebotomus sergenti* a susceptible species for *Leishmania* as shown by light microscope (A, Pharynx, B, Cibarium)

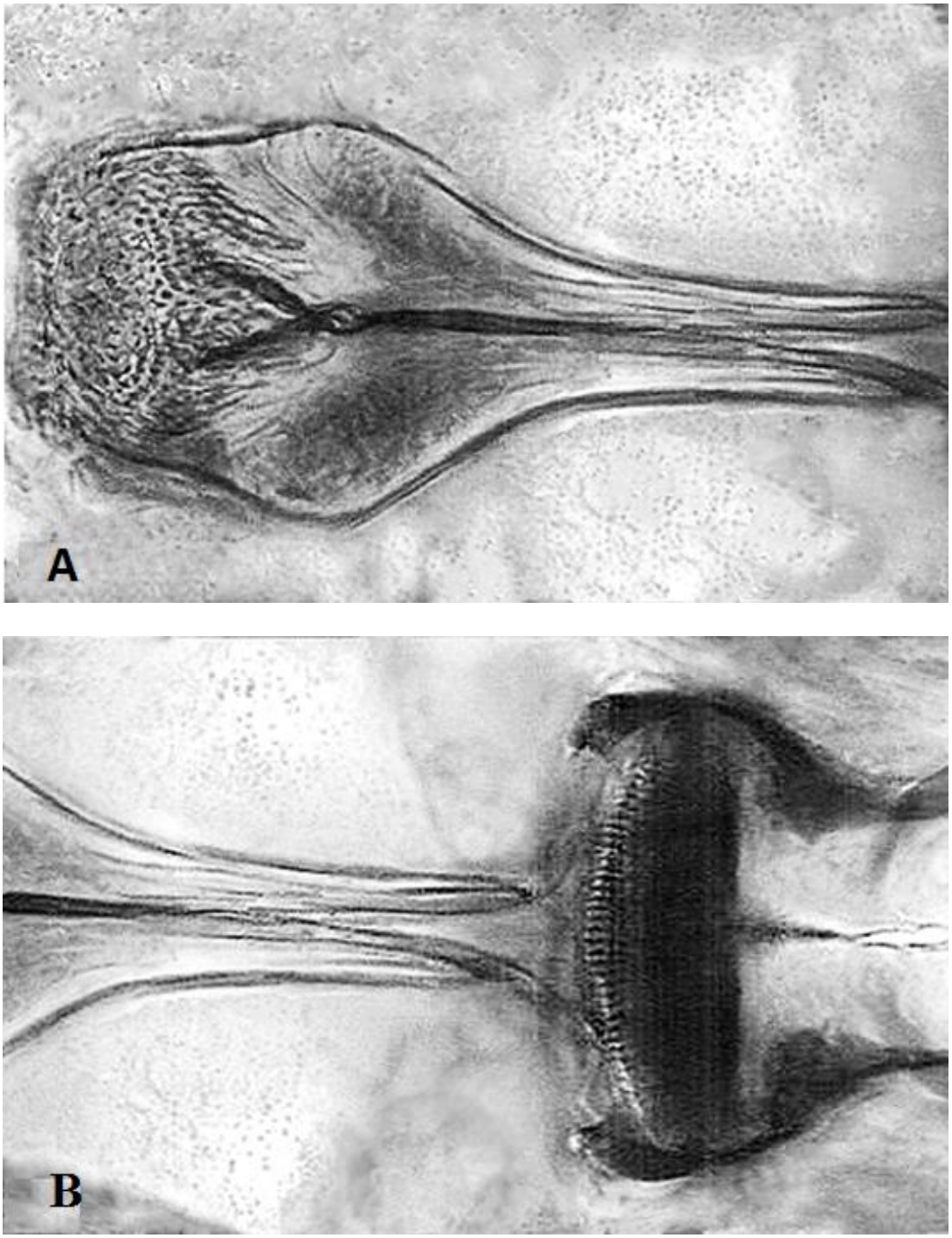

Fig. 3: Pharyngeal armature in *Sergentomyia squamipleuris* a refractory species for *Leishmania* as shown by light microscope (A, Pharynx, B, Cibarium)

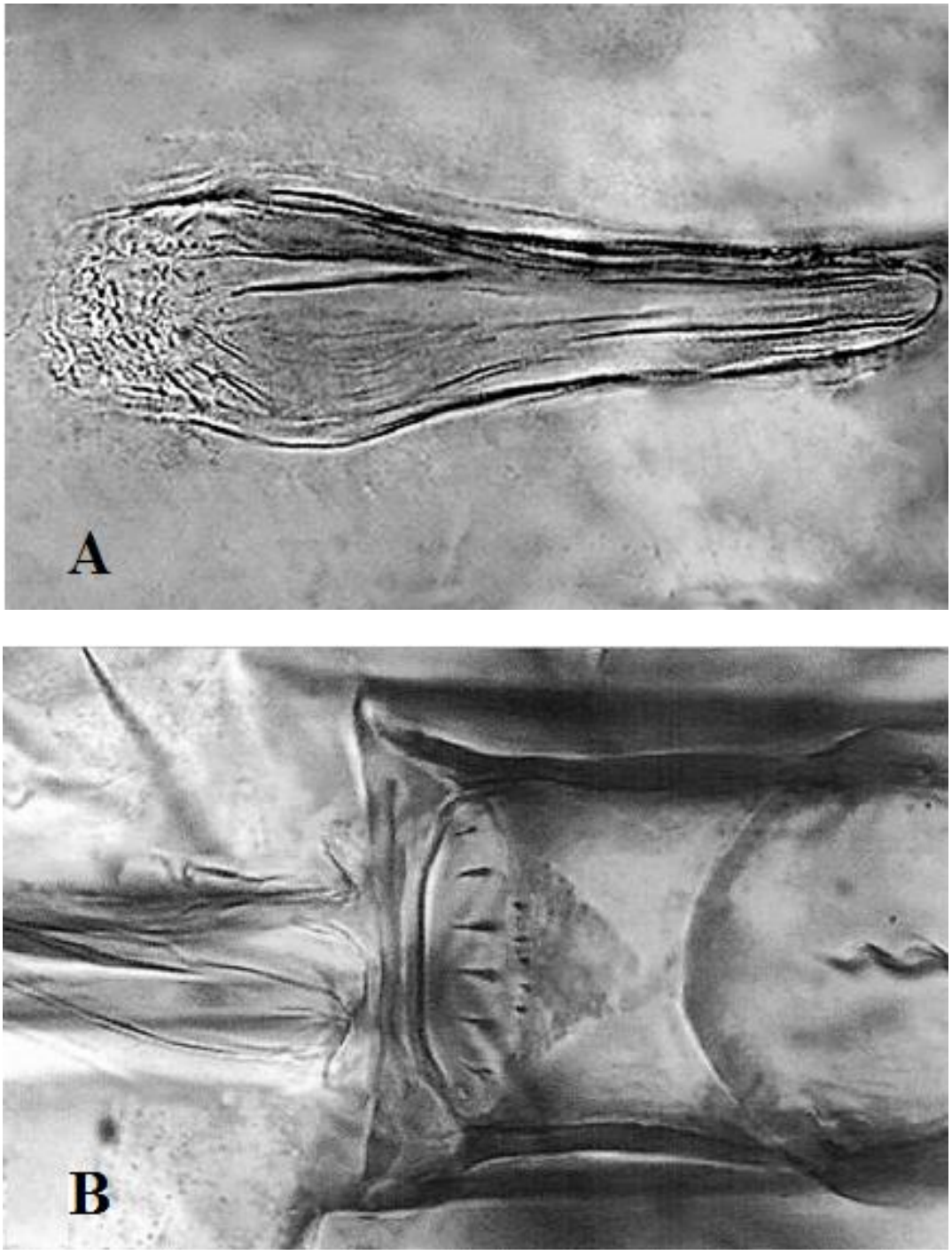

Fig. 4: Pharyngeal armature in *Sergentomyia christophersi* a refractory species for *Leishmania* as shown by light microscope (A, Pharynx, B, Cibarium)

ARABIC SUMMERY

الإختلافات الظاهرية للنتوءات البلعومية في ذباب الرمل الناقل وغير الناقل لطفيلي الليشمانيا

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تمت دراسة الاختلافات الشكلية للنتوءات البلعومية في ذباب الرمل الناقل و غير الناقل لطفيلي الليشمانيا. حيث ظهرت اختلافات كبيرة في حجم و شكل و أعداد النتوءات البلعومية . تميزت إناث فليبوتوماس باباتاسي ببلعوم غليظ وضيق بعد الإنتفاخ الخلفي وتتكون النتوءات البلعومية من حراشيف صغيرة متعددة مع حافة من أسنان رقيقة متجهه للخلف وبدون نتوءات أوبقع صبغية في المنطقة بين البلعوم والخرطوم, كما كان لاناث فليبوتوماس سيرجنتي بلعوم بحراشيف عريضة أمامية يمتد البعض منها على شكل شويكات طويلة و عريضة و تزيد الحراشيف عرضا في الناحية الظهرية بحافة خلفية مسننة باسنان دقيقة. وبالعكس فإن إناث سرجنتومايا اسكوامبليورس تتميز ببلعوم شريطي شديد الإنحدار للخلف مع صفوف من أسنان ناتئة , كما تواجدت صفوف محدبة من أسنان رقيقة متوازية أفقيا وصف متموج من أسنان عمودية وبقعه صبغية صغيرة تبرز للأمام كشريط كيتيني علي شكل عصبة مستعرضة في المنطقة بين البلعوم والخرطوم. كما تميزت اناث سرجنتومايا كريستوفرسي بنتوءات بلعومية بها عدد قليل من الحراشيف الأمامية و مجموعة من التموجات الخلفية. بالاضافة لوجود أربع أو خمس أسنان طويلة بنتوءات دقيقة و بقعة صبغية صغيرة في منطقة السيبيريوم.