Ootaxonomic Investigation of Five *Lutzomyia* Species (Diptera, Psychodidae) from Venezuela

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The eggshell fine structure of five sand fly species from Venezuela belonging to the genus Lutzomyia (L. migonei, L. ovallesi, L. absonodonta, L. gomezi *and* L. panamensis) *was examined by scanning electron microscopy. The chorionic sculpturing of* L. migonei, L. ovallesi, L. absonodonta *and* L. gomezi *was characterized by series of columns arranged in palisade to form sinuous ridges. In inter-ridge areas, the basal layer was covered with fibrous material. The outer chorion of* L. panamensis *had a pattern known as "mountain- or volcano-like". The morphology of the posterior pole and aeropyle had a common structure in the five species, with some species-specific characters. The eggshell features of the five species are compared with those of other phlebotomine sand flies.*

Key words: phlebotomine sand fly - ootaxonomy - eggshell - aeropyle - fine structure - scanning electron microscopy

Sand fly taxonomy was declared to be "an indispensable basis for every work on sand fly biology and on their vector role" by the World Health Organization (WHO 1977). However, the taxonomic status of phlebotomines is much more obscure than that of other insects of medical significance, such as mosquitoes (Lane 1986), although many attempts have been made to find unequivocal characters to distinguish morphologically similar species and to propose stable classifications (Lewis et al. 1977, Artemiev 1991, Ashford 1991).

Scanning electron microscope (SEM) studies of eggshell morphology have shown that differences between related species of insects are reliable taxonomic markers (Hinton 1981, Mazzini 1987, Mazzini et al. 1993a). Ootaxonomy of various families of dipterans is in an advanced stage (for a review, see Margaritis & Mazzini 1998). With regard to sand flies, studies on eggshell morphology have been delayed by the difficulty of finding eggs in nature and of breeding certain species. The eggs of Old World sand flies have only been studied for a limited number of species (Irungo et al. 1985, Lane & El Sawaf 1986, Gebre-Michael & Lane 1991, Fausto et al. 1991, 1992, 1993, Rogo et al. 1992, Ghosh & Mukhopadhway 1996). These studies suggest that differences in egg sculpture of *Sergentomyia* and *Phlebotomus* species are sufficient for separation of the two genera, hitherto based on other taxonomic characters (Perfil'iev 1966). Ootaxonomic investigations have been carried out in many Neotropical species (about 10% of the about 400 known species) demonstrating the usefulness of chorionic ultrastructure in separating closely related species (Ward & Ready 1975, Zimmerman et al. 1977, Endris et al. 1987, Feliciangeli et al. 1993, Sierra et al. 1995, Perez & Ogusuku 1997).

In the present study, the eggshell fine structure of five sand fly species belonging to the genus *Lutzomyia* - *L.* (*Psychodopygus*) *panamensis, L.* (*Lutzomyia*) *gomezi, L.* (Species Group *Verrucarum*) *ovallesi, L.* (Species Group *Migonei*) *migonei,* and *L.* (*Micropygomyia*) *absonodonta* - from Venezuela was examined by SEM. The first four species are known to be anthropophilic and have also been demonstrated or incriminated as vectors of *Leishmania* spp. in different Latin American countries. *L. panamensis* is regarded as a primary vector of cutaneous leishmaniasis (CL) in Venezuela (Rodriguez et al. 1999) and as a secondary vector in Panama, Guatemala and Colombia (Desjeux 1991). *L. gomezi* was reported to be naturally infected with *Le. braziliensis* in Panama (Johnson et al. 1993), Colombia (Young et al. 1987), Ecuador (Gomez & Hashiguchi 1987) and Venezuela (Feliciangeli 1991, Feliciangeli et al. 1994). *L. ovallesi* is recognized as a vector of CL

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in Venezuela (Feliciangeli 1991, Feliciangeli et al. 1994) and Guatemala (Rowton et al. 1992). It was found to be infected with a variant of *Le. panamensis/guyanensis* in Venezuela (Bonfante-Garrido et al. 1991) and with unidentified flagellates in Belize, Panama (Williams 1970) and Colombia (Young et al. 1987). *L. migonei* is a suspected vector of *Le. braziliensis* in the State of Ceará, Brazil (Azevedo et al. 1990). The medical significance of *L. absonodonta* is unknown. Closely allied species in the subgenus *Micropygomyia* are known to feed on lizards.

The chorionic patterns of the five species and other species belonging to different genera of Phlebotominae were compared.

MATERIALS AND METHODS

Newly laid eggs were obtained from naturally blood-engorged sand flies of the following species: *L.* (Species Group *Migonei*) *migonei, L.* (Species Group *Verrucarum*) *ovallesi, L. (Micropygomyia) absonodonta, L.* (*Lutzomyia*) *gomezi, L.* (*Psychodopygus*) *panamensis.*

For scanning electron microscopy, the eggs were fixed for 1 h at 4°C in 4% paraformaldehyde and 5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 (Karnovsky 1965), then rinsed overnight in cacodylate buffer, post-fixed in 1% osmium tetroxide for 1 h and dehydrated in a graded ethanol series. The material was dried by the critical point method using liquid CO_2 in a Balzers CPD 020 apparatus, attached to specimen holders, coated with gold in a Balzers Union MED 010 evaporator and observed with a 5200 Jeol JSM electron microscope.

RESULTS

Common characters of all eggs are elongated, cigar-like form with one side slightly flattened and both poles rounded (Figs 1, 5, 9, 14).

The five species had specific chorionic sculpturing which enabled the species to be distiguished from each other.

L. migonei (Figs 1-4) - The egg had a median width of about 80 µm and a length of about 280 µm (Fig. 1). The eggshell surface was characterised by polygonal design, consisting of longitudinal ridges united by cross-ridges, which defined irregular rectangular areas with a major side parallel to the longitudinal axis of the egg (Fig. 3). Each ridge consisted of a single series of cylindrical columns, about 2 µm high, united at the top (Figs 3-4). In inter-ridge areas, the basal layer was covered in uniformly distributed fibrous material (Figs 3-4).

The posterior pole was delimited by the terminal parts of a few chorionic ridges. Marked protuberances of different size were disposed around the aeropylar openings (Fig. 2).

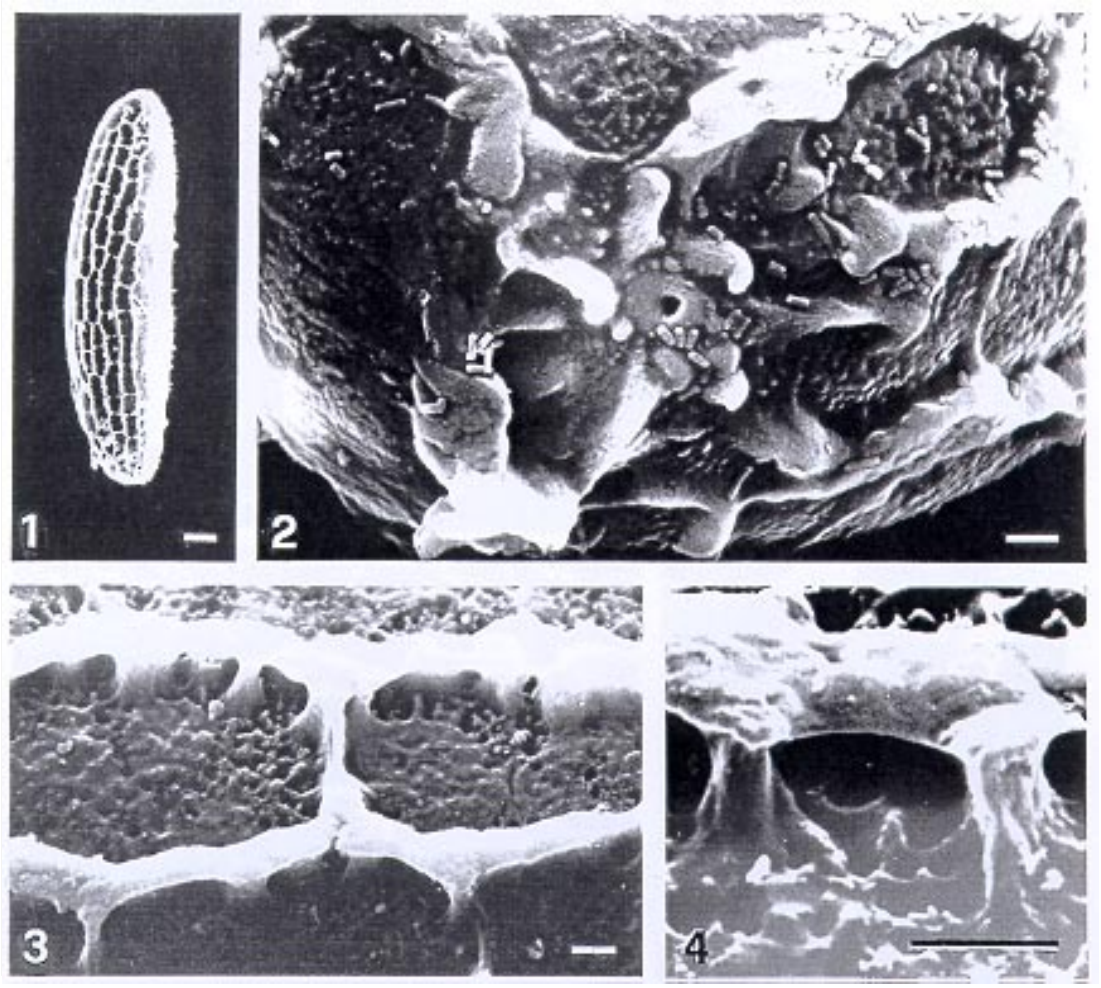
L. ovallesi (Figs 5-8) - The eggs were about 270 µm in length and 80 µm in width (Fig. 5) with a complete basal layer covered with a compact coat of fine fibrous material (Figs 6, 7). The outer chorion had a series of sinuous longitudinal ridges with a few cross-ridges defining rectangular areas of different size (Fig. 6). The ridges consisted of a double series of columns (high about 0.5 µm) linked at the top (Fig. 7). Some of the chorionic ridges extended as far as the polar regions.

The posterior pole consisted of a circular area delimited by the end part of chorionic ridges and divided into two semicircular areas by two short transverse ridges (Fig. 8). Each area had an aeropylar opening and several small conical protrusions.

L. absonodonta (Figs 9-11) - The eggs were about 300 µm long and 75 µm wide (Fig. 9). The chorionic sculpture consisted of longitudinal columnar ridges defining elliptical areas of about 35-40 µm in length (Fig. 10). Each area was crossed by transverse fine ridges to form irregular quadrilaterals. As a result the egg surface had a reticular pattern. The basal layer of chorion between the ridges was covered in coarsely arranged fibrous material, that formed minute microvilli (Fig. 10). The borders of the posterior pole region were not well defined. This area was covered by several protuberances randomly distributed around the two aeropylar openings (Fig. 11).

L. gomezi (Figs 12-13) - The surface sculpturing of eggs from Venezuelan females had a pattern of polygons formed by intersecting ridges consisting of columns arranged in palisade completely united at the top (Fig. 12). The areas enclosed by the ridges were four-sided with rounded corners and sides measuring about 15-20 µm (Fig. 12). The basal layer was covered with regularly arranged fibrous material. The posterior pole region was devoid of the structures characterising the rest of the egg surface and was delimited by the ends of the chorionic ridges and irregular circular ridges (Fig. 13). Two non-columnar ridges partially divided the polar region into two semicircular areas in which small protrusions were observed beside each aeropylar opening.

L. panamensis (Figs 14-17) - The egg had a median width of about 100 µm and a length of about 340 µm (Fig. 14). The chorionic pattern was different from that of the other species, consisting of a uniform layer of numerous and tall mountains bearing volcano-like structures regularly disposed (Fig. 15). These structures showed prominent irregular edges, enclosing a central depression in which holes of different sizes were visible (Fig. 16). The posterior pole was surrounded by a circle of short non-columnar ridges. The aeropylar openings were surrounded by small uneven ridges (Fig. 17).



Scanning electron microscopy micrographs of the outer chorionic sculpturing of *Lutzomyia migonei*. Fig. 1: whole egg. The eggshell surface has a polygonal pattern formed by longitudinal ridges and non-columnar cross ridges (Fig. 3). Each ridge consists of a single series of columns united at the top (Fig. 4). The basal layer is covered with uniformly arranged fibrous material (Fig. 4). The posterior pole, delimited by the terminal portion of chorionic ridges, shows protuberances and aeropylar openings (Fig. 2). Bar: Fig.1 = 20 µm; Figs 2-4 = 2 µm

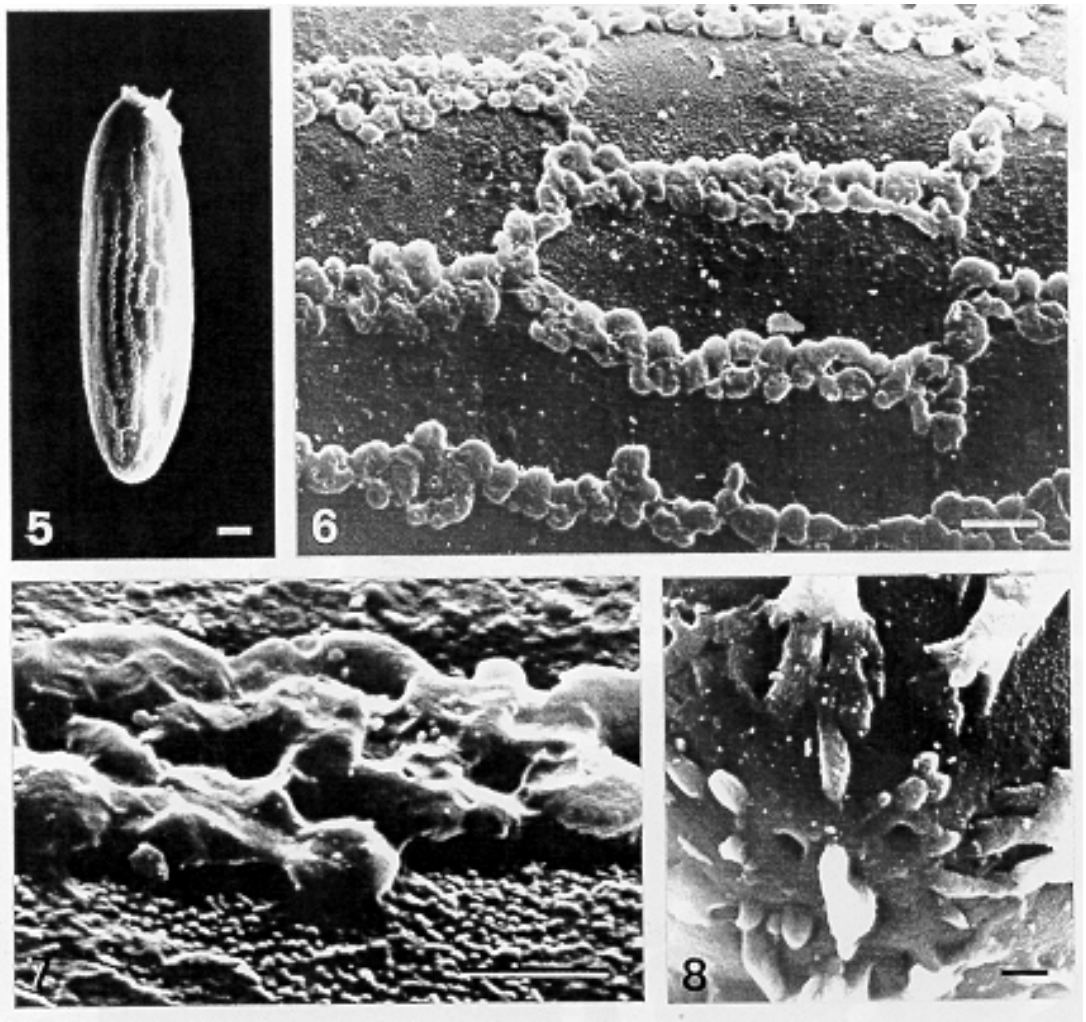
DISCUSSION

The eggshell sculptures of the five species showed species-specific characters, useful for species identification. However, they had some features in common with eggs of other New and Old World sand fly species. Morphological categories, based on the chorionic patterns of *Lutzomyia* eggs, have been proposed by different authors. Ward and Ready (1975) proposed three categories: "volcano-like or mountain-like", "polygonal", and "parallel ridging". The latter was divided by Endris et al. (1987) into "connected ridges" and "unconnected ridges". With the recent discovery of other chorionic patterns, three new categories were added: "elliptical" (Feliciangeli et al. 1993), "verrucose" and "disperse" (Pérez & Ogusuku 1997). With regard to chorionic patterns described in the Old World species, Gebre-Michael and Lane (1991) divided the category "unconnected ridges" into the groups: "fragmented chained" and "complete chained". The latter was defined as "reticular" by Fausto et al. (1992).

The five species described in this paper can be grouped in the following categories: *L. migonei* "connected parallel ridges"; *L. ovallesi* "connected parallel ridges" (with few connections); *L. absonodonta* "reticular"; *L. gomezi* "polygonal"; *L. panamensis* "volcano-like or mountain-like".

Most of patterns (*L. migonei, L. ovallesi, L. absonodonta,* and *L. gomezi)* have prominent ridges consisting of one or more series of columns. These patterns, common in New World species, are the only ones present in Old World species belonging to genera *Phlebotomus* and *Sergentomyia* (Fausto et al. 1992, 1993). However, *Phlebotomus* eggs show great variability in the arrangement of the columns, and the eggs of *Sergentomyia* species are morphologically uniform, with only a polygonal pattern and none of the fibrous material usually covers the basal layer between the ridges. These results are in line with comparative spermatology data, suggesting that the genus *Sergentomyia* followed a different evolutionary path to *Phlebotomus* and *Lutzomyia* (Dallai et al. 1984, Mazzini et al. 1993b, Fausto et al. 1995).

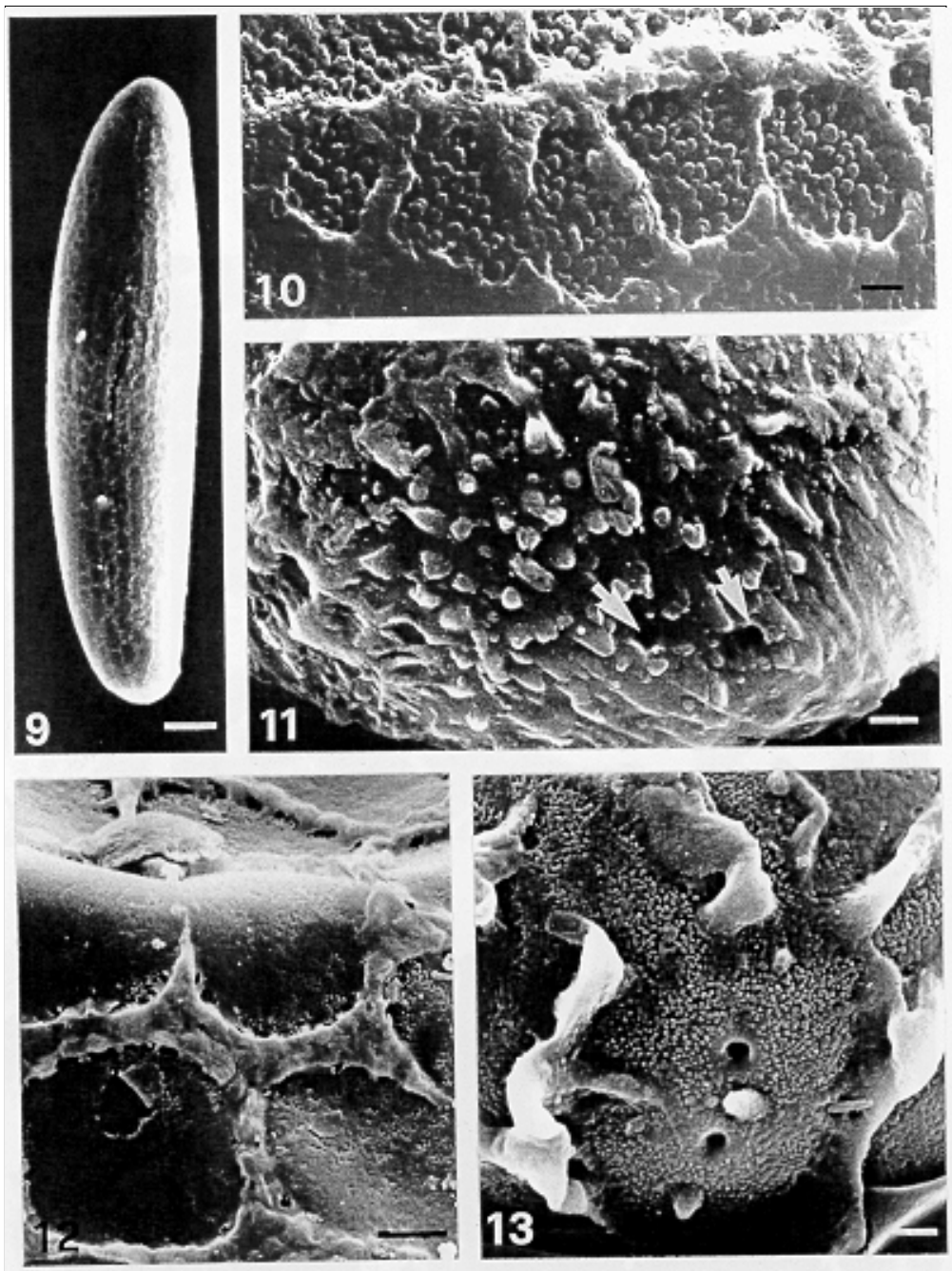
The "volcano or mountain-like" pattern described in *L. panamensis* eggs has only been observed in species of the *Lutzomyia* genus. This particular pattern may have evolved in response to a damp microhabitat, i.e. swamp forest, in which these species are abundant (Ward & Ready 1975). As shown in Fig. 16, the prominent "volcano-like" structures protecting the aeropylar openings, are probably the basis for a well developed plastron and reflect its habitat preference. The chorionic sculpturing of *L. panamensis* from Panama (Zimmerman et al. 1977) is very similar to that of *L. panamensis* from Venezuela, whereas two different chorionic patterns have been described for *L. gomezi* from Panama (Zimmerman et al. 1977) and Colombia (Sierra et al. 1995) (elongated hexagonal polygons) and Brazil (Ward & Ready 1975)



Scanning electron microscopy micrographs of egg of *Lutzomyia ovallesi*. Fig.5: whole egg. The chorion shows sinuous longitudinal ridges with few cross-ridges forming rectangular areas (Fig. 6). The ridges consist of a double series of columns linked at the top (Fig. 7). Some of chorionic ridges reach the polar regions. The posterior pole is divided into two semicircular areas, having aeropylar openings and small conical protrusions, by two short transverse ridges (Fig. 8). Bar: Fig. 5 = 20 µm; Fig. 6 = 5 µm; Figs 7, 8 = 2 µm

(regular pentagonal polygons). We found that eggshell sculpture of the Venezuelan specimens was of the Brazilian type.

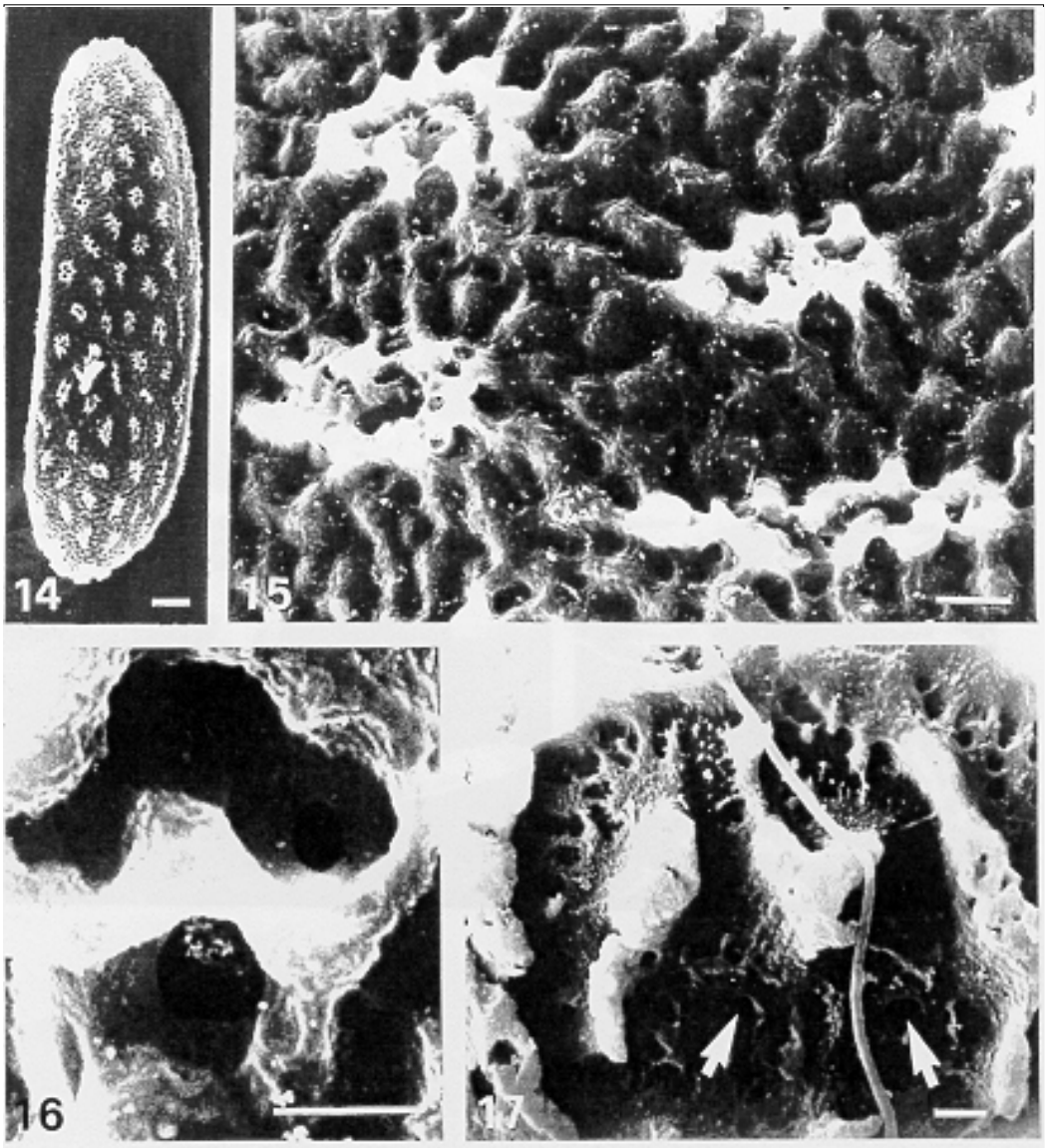
Almost nothing is known about the breeding sites of the present and previous species described, and there is not yet enough data to attempt a taxo-



Scanning electron microscopy micrographs of eggs of *Lutzomyia absonodonta* (Figs 9-11) and *L. gomezi* (Figs 12,13). Fig.9: whole egg. The chorionic sculpture has short longitudinal united by cross-ridges to form irregular quadrilaterals; the basal layer is covered with arranged coarsely fibrous material, forming minute microvilli (Fig. 10). The posterior pole is characterised by protuberances randomly distributed around the two aeropylar openings (arrows) (Fig. 11). Fig. 12: polygonal pattern due to intersecting ridges, in which most of polygons are quadrilaterals with rounded corners. An irregular circular ridge delimits the polar region where small protrusions can be observed beside two aeropylar openings (Fig.13). Bar: Fig. 9 = 20 µm; Figs 10, 11, 13 = 2 µm; Fig. 12 = 5 µm

nomic and evolutionary interpretation. However, the similar exochorion pattern in *L. gomezi* from Panama and Colombia and the other pattern shared by *L. gomezi* from Venezuela and Brazil is of interest and may be related to the Andean barrier between them. *L. gomezi* may of course form a species complex (Feliciangeli 1997) and this should be clarified by isoenzymes and DNA fingerprinting of different populations.

Comparison of the chorionic patterns of the present species and other *Lutzomyia* species (Ward & Ready 1975, Zimmerman et al. 1977, Endris et al. 1987, Feliciangeli et al. 1993) shows characters in most cases compatible with systematic position. Species of subgenus *Psychodopygus* have "volcano-like or mountain-like" patterns, like *L. panamensis. L. ovallesi* has similar chorionic sculpturing ("connected parallel ridges") to *L. evansi* ("polygonal") (Feliciangeli et al. 1993) and to other species belonging to the *Verrucarum* group. However, chorion sculpturing pattern of *L. verrucarum* from a Peruvian Andes valley combines "con-



Scanning electron microscopy micrographs of an egg of *Lutzomyia panamensis.* Fig.14: whole egg. The chorionic pattern consists of a uniform layer of mountains with regular "volcano-like" structures (Fig. 15). Prominent irregular edges delimit a central depression in which holes of different sizes are visible (Fig. 16). The posterior pole, surrounded by a series of short non-columnar ridges, shows aeropylar openings (arrows) and small uneven ridges (Fig. 17). Bar: Fig.14 = 20 µm; Fig. 15 = 5 µm; Figs 16, 17 = 2 µm

nected ridges" pattern with "reticular" pattern (Pérez & Ogusuku 1997). Slight contrasts of eggs morphology described in *L. verrucarum* from different localities could be associated with geographical isolation of sand fly populations in different Andean valleys (Pérez & Ogusuku 1997). The eggs of *L. venezuelensis*, the only species of *Micropygomyia* subgenus previously studied (Feliciangeli et al. 1993), are in the "elliptical" category, and those of *L. absonodonta*, belonging to the same subgenus, in the "reticular" category. These two categories are very similar by virtue of a common basal structure. Connected parallel ridges give *L. migonei* eggs a polygonal aspect similar to those described, using light microscopy, for the eggs of *L. lenti* and *L. bahiensis* (Species Group *Migonei*) (Feliciangeli et al. 1993).

The morphology of the posterior pole, described for the first time in *Phlebotomus* eggs (Fausto et al. 1992), has not yet been used much as an ootaxonomic character of sand fly eggs: most morphological descriptions of *Lutzomyia* eggs lack this structural detail. However, as reported for other species (Fausto et al. 1992, 1993, Pérez & Ogusuku 1997), the posterior poles of the present five species show species-specific morphology, which could be important for sand fly ootaxonomy.

However, more information, especially regarding the egg morphology of other genera, is needed to indicate phylogenetic relationships among the sand fly taxa.

REFERENCES

Artemiev MM 1991. A classification of the subfamily Phlebotominae. *Parassitologia 33*: 69-78.

Ashford RW 1991. A new morphological character to distinguish *Sergentomyia* and *Phlebotomus*. *Parassitologia 33*: 79-83.

Azevedo CR, Rangel EF, Queiroz RG 1990. *Lutzomyia migonei* (França, 1920) naturally infected with perypilarian flagellates in Baturité, a focus of cutaneous leishmaniasis in Ceará State, Brazil. *Mem Inst Oswaldo Cruz Rio de Janeiro 85*: 479.

Bonfante-Garrido R, Spinetti H, Cupillo E, Momen H, Grimaldi G 1991. *Lutzomyia ovallesi* (Diptera: Psychodidae) as a vector of cutaneous leishmaniasis in Venezuela. *Parassitologia 33*: 99-104.

Dallai R, Baccetti B, Mazzini M, Sabatinelli G 1984. The spermatozoon of three species of *Phlebotomus* (Phlebotominae) and the acrosomal evolution in Nematoceran Dipterans. *Int J Insect Morph Embryol 13*: 1-10.

Desjeux P 1991. Information on the epidemiology and control of the leishmaniases by country and territory. WHO/LEISH/91.30.

Endris RG, Young DG, Perkins PV 1987. Ultrastructural comparison of egg surface morphology of five *Lutzomyia* species (Diptera: Psychodidae). *J Med Entomol 24*: 412-415.

Fausto AM, Maroli M, Mazzini M 1991. Ootaxonomy investigation of three sandfly species (Diptera, Psychodidae). *Parassitologia 33*: 225-228.

Fausto AM, Maroli M, Mazzini M 1992. Ootaxonomy and eggshell structure of *Phlebotomus* sand flies. *Med Vet Entomol 6*: 201-208.

Fausto AM, Maroli M, Mazzini M 1993. Scanning electron microscopical study of the eggshell of three species of *Sergentomyia* (Diptera, Psychodidae). *Insect Sci Appl 14*: 483-488.

Fausto AM, Mazzini M, Maroli M, Feliciangeli D 1995. Spermatozoon of the sandfly *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera, Psychodidae). *Boll Zool 62*: 339-343.

Feliciangeli MD 1991. Vectors of leishmaniasis in Venezuela. *Parassitologia 33*: 229-236.

Feliciangeli MD 1997. Hourly activity of *Lutzomyia ovallesi* and *L. gomezi* (Diptera: Psychodidae), vectors of cutaneous leishmaniasis in northcentral Venezuela. *J Med Entomol 34*: 110-115.

Feliciangeli MD, Castejon OC, Limongi J 1993. Egg surface ultrastructure of eight New World phlebotomine sandfly species (Diptera: Psychodidae). *J Med Entomol 30*: 651-656.

Feliciangeli MD, Rodriguez N, Bravo A, Arias F, Guzman B 1994. Vectors of cutaneous leishmaniasis in north-central Venezuela. *Med Vet Entomol 8*: 317-324.

Gebre-Michael T, Lane RP 1991. Scanning electron microscopy of eggs of *Phlebotomus (Synphlebotomus) martini* and *P. (Syn.) celiae* (Diptera: Phlebotominae). *Parassitologia 33*: 261-266.

Ghosh KN, Mukhopadhway JA 1996. A comparison of chorionic sculpturing of four Indian phlebotomine sand flies (Diptera: Psychodidae) by scanning electron microscopy. *Parasitology 3*: 61-68.

Gomez E, Hashiguchi Y 1987. Natural infection of sand flies with *Leishmania* promastigotes. In Y Hashiguchi, *Studies on New World Leishmaniasis and its Transmission, with Particular Reference to Ecuador*, Kyowa Printing & Co, Kochi, Japan, p. 70-78.

Hinton HE 1981. *Biology of Insect Eggs*, Vols I-III, Pergamon Press, Oxford, 1125 pp.

Irungu LW, Mutinga MJ, Kokward ED 1985. Chorionic sculpturing of eggs of some Kenyan phlebotomine sand flies. *Insect Sci Appl 7*: 45-48.

Johnson PT, McConnell E, Hertig M 1993. Natural infection of leptomonad flagellates in Panamanian *Phlebotomus* sand flies. *Exp Parasitol 14*: 107-122.

Karnovsky MJ 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol 27*: 137A-138A.

Lane RP 1986. Recent advances in the systematics of phlebotomine sand flies. *Insect Sci Appl 7*: 225-230.

Lane RP, El Sawaf B 1986. The immature stages of *Phlebotomus langeroni* (Diptera: Psychodidae). *J Med Entomol 23*: 263-268.

Lewis DJ, Young DG, Fairchild GB, Minter DM 1977. Proposal for a stable classification of the phlebotomine sand flies (Diptera: Psychodidae). *Syst Entomol 2*: 319-332.

Margaritis LH, Mazzini M 1998. Structure of the egg. In *Microscopic Anatomy of Invertebrates*, Vol. 11C: *Insecta*, Wiley-Liss, New York, p. 995-1037.

Mazzini M 1987. An overview of the egg structure in Orthopteroid insects. In B Baccetti, *Evolutionary Biology of Orthopteroid Insects*, Vol. II, Ellis Horwood Ltd., Chichester, England, p. 358-372.

Mazzini M, Carcupino M, Fausto AM 1993a. Egg chorion architecture in stick insects. *Int J Insect Morph Embryol 22*: 391-415.

Mazzini M, Fausto AM, Maroli M 1993b. Fine structure of spermatozoon of the sandfly *Sergentomyia minuta* (Diptera, Psychodidae). *Boll Zool 59*: 343-347.

Perez EJ, Ogusuku E 1997. Chorion patterns on eggs of *Lutzomyia sand flies* from the Peruvian Andes. *Med Vet Entomol 11*: 127-133.

Perfil'ev PP 1966. Phlebotomidae (sandflies). In *Fauna of the U.S.S.R.*, 382 pp. (English translation, Jerusalem, 362 pp., 1968).

Rodriguez N, Aguilar CM, Barrios MA, Barker DC 1999. Detection of *Leishmania braziliensis* in naturally infected individual sand flies by the polymerase chain reaction. *Trans R Soc Trop Med Hyg 93*: 47-49.

Rogo LM, Kokwaro ED, Mutinga MJ, Khamala CP 1992. Differentiation of vector species of phlebotominae (Diptera: Psychodidae) in Kenya by chorionic sculpturing of their eggs. *J Med Entomol 29*: 1042-1044.

Rowton ED, de Mata M, Rizzo N, Porter CH, Navin TR 1992. Isolation of *Leishmania braziliensis* from *Lutzomyia ovallesi* (Diptera: Psychodidae) in Guatemala. *Am J Tropl Med Hyg 46*: 465-468.

Sierra D, Uribe S, Velez ID 1995. Egg surface ultrastructure of New World phlebotomine sandfly *Lutzomyia gomezi* (Diptera: Psychodidae), Second International Symposium on Phebotomine Sand Flies, Mérida, Venezuela, p. 96.

Ward RD, Ready PA 1975. Chorionic sculpturing in some sandfly eggs (Diptera, Psychodidae). *J Entomol 50*: 127-134.

WHO-World Health Organization 1977. Scientific working group on leishmaniasis. Report of first meeting, TDR/ LEISH-SWG(1)/77.3, Geneve.

Williams P 1970. Phlebotomine sand flies and leishmaniasis in British Honduras (Belize). *Trans R Soc Trop Med Hyg 64*: 317-364.

Young DG, Morales A, Kreutzer D, Alexander B, Corredor A, Tesh R, Ferro de Carrasquilla C, De Rodriguez C 1987. Isolation of *Leishmania braziliensis* (Kinetoplastida: Trypanosomatidae) from cryopreserved Columbian sand flies (Diptera: Psychodidae). *J Med Entomol 24*: 588-589.

Zimmerman JH, Newson HD, Hooper GR, Christensen HA 1977. A comparison of egg surface structure of six anthropophilic phlebotomine sand flies (*Lutzomyia*) with the scanning electron microscope (Diptera: Psychodidae). *J Med Entomol 13:* 574-579.