VE-1
IDENTIFICATION OF ACID HYDROLASES ACTIVITIES DURING EMBRYOGENESIS OF *RHODNIUS PROLIXUS* (HEMIPTERA, REDUVIIDAE)

Fialho E /*, Masuda H, Atella GC**, Silva-Neto MAC*
Deptº de Nutrição Básica e Experimental, Instituto de Nutrição Josué de Castro, UFRJ *Deptº de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro, RJ, Brasil **Deptº de Bioquímica, UERJ, Rio de Janeiro, RJ, Brasil

Phosphorylation/dephosphorylation of proteins is the major post-translational mechanism involved in many cellular processes. In *Rhodnius prolixus* eggs from fertilized females, we detected at least two hydrolytic enzymes, a phosphatase and cathepsin D-like aspartic proteinase, both with an acid pH optimum. Acid phosphatase activity, was enhanced after second day of development and reaches a plateau around day four which is maintained up to the eclosion of the first instar larvae. This enzyme is strongly inhibited "in vitro" by 2 mM of sodium fluoride and 10 mM of tartrate Na/K but not by 1 or 100 nM of okadaic acid. Thus, this enzyme may have lisosomal origin. Aspartic proteinase activity, was measured "in vitro" using hemoglobin or purified vitellin as substrates. The activity of this enzyme also increases with embryo development. Among several proteinase inhibitors tested, pepstatin was able to abolish vitellin (VT) breakdown. When phosphatase activity was assayed in non-fertilized eggs, only a basal level was observed when compared with fertilized eggs, suggesting that this enzyme is activated in a fertilization dependent process. The increase in phosphatase activity coincides with the beginning of VT proteolysis. In addition sodium fluoride, an acid phosphatase inhibitor strongly impaired the action of cathepsin D-like aspartic proteinase suggesting a cooperative action of both enzymes and a possible relationship between protein dephosphorylation and VT degradation. This hypothesis is supported by the fact that non-fertilized eggs, which does not present phosphatase activity, does not present vitellin breakdown.

Supported by CNPq, PADCT, Pronex.

VE-2
HEME ASSOCIATION WITH THE PERIMICROVILLAR MEMBRANES IN THE GUT LUMEN OF *RHODNIUS PROLIXUS* (HEMIPTERA, REDUVIIDAE)

Silva JR, Oliveira MF*, Silva CP, Atella GC*, Oliveira PL* , Masuda H*, Petretski MDA
Lab. de Química e Função de Proteínas e Peptídeos, CBB, UENF, Campos, RJ, Brasil *Departamento de Bioquímica Médica, CCS, UFRJ, Rio de Janeiro, RJ, Brasil

Rhodnius prolixus is a hematophagous insect which ingests large volumes of blood during a single feeding session. High concentrations of heme, iron-protoporphyrin IX, are generated as a result of hemoglobin digestion. Heme can cause damage to various biomolecules and membranes. In the insect midgut, the perimicrovillar membrane (PMVM) is the first structure to come into contact with the gut luminal contents, which contain heme at high concentrations. PMVM, in fact, can protect the microvillar membranes from direct contact with the gut luminal contents. In order to evaluate the association of heme with PMVM we used TEM to detect peroxidase activity in these membranes. We incubated the midgut in 0.1 M Cacodilate buffer pH 6.5 at 37ºC with Diaminobenzidine (DAB). We detected a positive staining, which indicates the presence of heme on these membranes. We suggest that this result is probably due to a heme binding protein or lipid partition in the PMVM. In order to investigate the heme polymerization, we centrifuged the gut luminal content of *R. prolixus*. The heme polymerization activity was associated with the pellet. We found that the heme polymerized successfully. In order to evaluate the participation of the PMVM in the heme polymerization process, we isolated the PMVM of both *R. prolixus* and *Dysdercus peruvianus* (a phytophagous insect) in a discontinuous sucrose density gradient. These membranes were capable successfully polymerizing heme. These results suggest that the PMVM has a role in heme polymerization. The phospholipids present in the PMVM of both *R. prolixus* and *D. peruvianus* were detected. Of the phospholipids detected in *R. prolixus*, 52.5% consisted of Phosphatidylethanolamine (PE), 33.75% of Phosphatidylcholine (PC), and 13.78% of Phosphatidylserine (PS). In *D. peruvianus* the results were 60.03 % PE, 5.83 % PC, 27.29 % PS and 6.85 % a non-identified phospholipid. Ridley (1998) showed the heme polymerase activity can be obtained with PE, PS, PC and PI. Presently we are investigating the role of the PMVM protein moiety.

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VE-3
FINE STRUCTURE OF *TRYPANOSOMA RANGELI* ADHERED TO THE SALIVARY GLANDS OF *RHODNIUS DOMESTICUS* NEIVA & PINTO, 1923 (HEMIPTERA: REDUVIIDADE)

Rodrigues IS, Steindel M*, Soares MJ
Laboratório de Biologia Celular de Microrganismos, Departamento de Ultra-estrutura e Biologia Celular, Instituto

Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Caixa Postal 476, 88040-900 Florianópolis, SC, Brasil

Study of South American triatomine bugs (Hemiptera: Reduviidae) is both of biological and medical interest, since most of them are the invertebrate hosts of *Trypanosoma cruzi*, causative agent of Chagas' disease, and the closely related *Trypanosoma rangeli*. Unlike *T. cruzi*, *T. rangeli* is pathogenic to its insect host, and colonizes and spends part of its life cycle in the salivary glands of the invertebrate vector. Scanty information is available on the histology and ultrastructure of healthy and infected glands, as well as the development of the parasites therein.

Adult *Rhodnius domesticus* (the main invertebrate host of *T. rangeli* in Santa Catarina State, Brazil) of both sexes were infected with *T. rangeli*, strain SC-58. Eight days post-infection the salivary glands were collected and processed for scanning electron microscopy (SEM). The glands were adhered with double face adhesive tape to SEM stubs, coated with a 20 nm thick gold layer in a sputtering device and then observed in a Zeiss DSM-940 scanning electron microscopy.

Observation of the salivary glands by scanning electron microscopy showed that they are usually elongated, unilobular structures, about 1-2 mm long, with an excretion channel arising at the medial/sub-terminal region. Parasites, both epimastigote and trypomastigote forms, were found adhered to the outer surface of the glands in high numbers, usually forming patches. Some glands were split in two pieces, in order to expose their inner surfaces. Epimastigotes and trypomastigotes could be found adhered among the microvilli. Some trypanosomes entering the glands could be also observed. Infected glands, including the microvilli, showed no visible morphological alterations due to the presence of the parasites.

This work has been supported by CNPq and Fiocruz.

VE-4
LIGNANS AND NEOLIGNANS AS INSECT GROWTH REGULATORS OF *RHODNIUS PROLIXUS*: A STUDY OF *TRYPANOSOMA CRUZI* DEVELOPMENT

Maleck M, Schaub GA*, Azambuja P, Gottlieb Otto R**, Garcia ES
Departamento de Bioquímica e Biologia Molecular ** Departamento de Fisiologia e Farmacodinâmica, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil * Department of Special Zoology, Ruhr University, D-44780 Bochum, Germany

Chagas'disease is an endemic protozoan disseminated in Brazil. The natural transmission occurs by excretion by the blood-feeding vector by reduviidae insect whose faeces contained the parasite *Trypanosoma cruzi* in their infective tripomastigote metacyclic forms. In our laboratory using *Rhodnius prolixus* as an insect model, we tested some lignoids (pinoresinol, burchellin, NDGA, licarin A, sesamin, podophyllotoxin) on feeding, moulting, excretion and development of *T. cruzi*. The oral treatment of *R. prolixus* with burchellin and NDGA drastically reduced the number of flagellates excreted (urine and faeces). *In vitro* treatments with these substances did not inhibit the number of parasites. However feeding with higher concentration of pinoresinol and NDGA had antifeedant effect and inhibited the amount blood ingested. The most important results was obtained by burchellin and pinoresinol, both extracted from brazilian plants, *Aniba burchelli* and *Melia azedarach*, respectively, by demonstration of their effect on moulting and parasites growth inhibition. in *Rhodnius* These findings indicate the potential use these compounds growth regulators which could interferes with *Trypanosoma cruzi* development in the digestive tract.

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VE-5
HEME TRANSPORT AND DEGRADATION IN *RHODNIUS*: AN ANTIOXIDANT MECHANISM

Paiva-Silva GO, Bouts DMD, Moreira MF, Braz G, Masuda H, Oliveira PL
Departamento de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro, RJ, Brasil

Heme (Fe-protoporphyrin IX) is a well-known generator of reactive oxygen species that cause damage to several classes of biomolecules. Some organisms, as the hematophagous insect *Rhodnius prolixus*, have vertebrate blood as the sole source of food. In this case, the digestion of the meal represents an oxidative challenge due to the large amount of heme release by hemoglobin degradation. One of the antioxidant mechanisms involved in the protections of *Rhodnius* against heme-induced oxidative injuries is a hemolymphatic heme-binding protein (RHBP). It has already been described that deleterious effects of heme are inhibited by its binding to apoRHBP. Here we propose that RHBP participates in the antioxidant defense also by transporting heme to some tissues where it is degradated by heme-oxygenase. The formation of biliverdin, a heme degradation product, in the pericardial and midgut cells is observed when hemolymphatic level of heme is increased. Injection of purified RHBP in the abdominal cavity of this insect leads to the formation of biliverdin in those tissues. This results suggest that RHBP is able

to transport heme and its degradation by heme-oxygenase represents an important intracellular antioxidant mechanisms in this insect.

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VE-6
THE USE OF A RECESSIVE PHENOTYPE FOR EYE COLOUR IN *TRIATOMA INFESTANS* AS A TOOL FOR STUDYING FERTILITY AND FECUNDITY RELATED ASPECTS

Pires H, Abrão DO, Machado EMM, Diotaiuti L
Laboratório de Biologia de Triatomíneos e Epidemiologia a Doença de Chagas, Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto de Lima 1715, 30190002 Belo Horizonte, MG, Brasil

The "wild" eye colour observed in triatomines is black, but some authors have reported the existence of mutants with red or white eyes. The first report about red eyed mutants was that of Noé & Silva (1949), in which they determined that this was a recessive character linked to the X chromosome. The objective of the present work was to study the effect of the number and sequence of matings on the fecundity and fertility of *Triatoma infestans* females, using this recessive character as a marker. It may allow to evaluate what possibility of genetic variability would be expected, for example, in a triatomine colony that has begun from a unique female. Virgin adults were coupled in pairs and separated into five groups:

G1 - Females with red eyes paired with males with the same eye colour. After females began laying eggs, the males were substituted by males with black eyes.
G2 - Females with red eyes paired with males with the same eye colour. After oviposition began, males were removed without substitution.
G3 - Females with red eyes paired with males with the same eye colour during the whole assay.
G4 - Females with red eyes paired with males with the same eye colour. After oviposition began, males were removed, being replaced by males with black eyes only after two months.
G5 - Females with red eyes paired with one male with red eyes and another with black ones during the whole assay.

All couples were kept separated and fed once a week. Eggs were collected once a week. The mean of the eggs laid did not show statistical difference between the groups. In the fifth series, and through the expression of the eye colour genotype, we have observed a frequency of 50% for each character, suggesting that both males contributed in the same proportion to the fertilisation of the eggs. In those series in which one male was replaced by another, we observed a gradual tendency of replacement of red eyed larvae by black ones. We also observed that the dynamics of this process of replacement depends on the moment at which males were substituted. Results suggest that females may mate more than once without having a corresponding rise in their fertility or fecundity.

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VE-7
THE VISUAL SYSTEM OF *TRIATOMA INFESTANS*: THE COMPOUND EYE

Insausti TC, Lazzari CR
Departamento de Ciencias Biológicas, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina. E-mail: lazzari@bg.fcen.uba.ar

As those of other Hemiptera, triatomine compound eyes are focal apposition eyes with an open rhabdom. The photoreceptors (Ph) of each ommatidium are arranged in a ring of rhabdom, formed by six retinula cells, which surrounds the central double rhabdomere of the seventh cell. An eighth thinner cell does not possess a rhabdomere and is located in the peripheral ring. Densely packed pigment granules are located into the retinula, inside two primary pigment cells (PPC), which surround the soft crystalline cone formed by four cells, and inside 24 secondary pigment cells that surround the whole ommatidium. Pigments located in the distal end of the Phs and in the PPCs form an "iris" in the focal plane of the corneal lens in front of the rhabdom distal tip. These eyes would have two types of image channel, one with high sensitivity and poor resolution (the peripheral rhabdomeres) and other with low sensitivity and high resolution (the two central rhabdomeres). In contrast to neural superposition eyes, the gain in sensitivity, conveyed by the peripheral rhabdomeres, must occur at the expense of resolution. This proposed mechanism, with one photopic and one scotopic system, is supported experimentally and by the way these eyes adapt to changes in light intensity. In the dark-adapted state, the pigment cells and retinular pigments that form the "iris", withdraw to a peripheral position and the dilated cone leaves all the rhabdomeres exposed. During light adaptation, the pigment cells contract, the rhabdom is pressed down and the cone cells form a narrow thread transmitting light to the central rhabdomere. This mechanism allows a very direct control of the acceptance angle and hence, the rhabdoms light flux.

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VE-8
ADIPOKINETIC HORMONE (AKH) AND LIPID MOBILIZATION IN *PANSTRONGYLUS MEGISTUS* (HEMIPTERA:REDUVIIDAE)

Canavoso LE* , Rubiolo ER
Departamento de Bioquimica Clinica, Fac. Ciencias Quimicas, Universidad Nac. Cordoba, Argentina, *Department of Biochemistry, University of Arizona, Biological Sciences West Building, Tucson, Arizona, USA

The aim of this study was the investigation of the effects of AKH and flight in the levels of lipid, lipoproteins and carbohydrate in the hemolymph of reduviid bugs. In resting and untreated insects the hemolymph of *P. megistus* presents an average lipid content of 3.27 mg/ml. Diacylglycerols (DG) represent the main lipid class (65%). Carbohydrate are present at 0.53 mg/ml. Lipids are transported by high density lipophorin (HDLp, 1.130 g/cm^3), which is present at 4.0-4.9 mg of protein/ml. HDLp is an homogeneous population of particles, ranging in size from 8-14 nm. Adult insects were injected with a maximal dose of AKH-I from *Schistocerca gregaria*, and the hemolymph levels of total lipids, DG, carbohydrates, and lipophorin determined at different times. Lipid increases were detected 1 h after AKH-I injection and maximum levels were obtained at 3.5 h (7.15 mg/ml), affecting principally DG. The total content of lipophorin did not change during the time course, but a partial interconversion of HDLp into low density lipophorin (LDLp) was observed. The LDLp is an heterogeneous lipoprotein fraction containing particles larger than 25nm and an average density of 1.07 g/cm^3. No changes in carbohydrate levels were detected after the AKH injection. As in AKH injected insects, the lipid and LDLp contents also increased in insects subjected to flight. However, contrarily to the results obtained with AKH injected insect, the levels of carbohydrates were dramatically reduced after 5 min of flight. Also, the hyperlipemic response induced by AKH-I was slower than that observed during flight.

VE-9
SALIVARY HEME PROTEINS ELECTROPHORESIS AS A TOLL FOR DISTINGUISHING *RHODNIUS* SPECIES

Soares RPP, Sant'ana MRV, Gontijo NF, Romanha AJ*, Diotaiuti L*, Pereira MH
Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil, * Centro de Pesquisas René Rachou,Fiocruz, Belo Horizonte, MG, Brasil

The genus *Rhodnius* Stal (Hemiptera, Reduviidae) currently includes 13 species widely distributed in South and Central America and recognized on morphological features. These species exhibit different degrees to adaptation to the human environments becoming potential vectors of *Trypanosoma cruzi*. Distinction between these species of *Rhodnius* is of considerable importance because of their different epidemiological significance as vectors of *T. cruzi*. It was studied the interspecific variability in the genus *Rhodnius* based on salivary heme proteins (nitrophorins) electrophoresis in starch gel. We compared salivary proteins profiles of 8 *Rhodnius* species: *R. prolixus, R. robustus, R. neglectus, R. nasutus, R. ecuadoriensis, R. pallescens, R. pictipes* and *R. domesticus*. Salivary glands from each insect were dissected out, washed in 0,9% NaCl and transferred to 5ml of running buffer on a microscope slide in a humid chamber where they were maintained until all insects had been prepared. The prepared glands from each insect were then disrupted with dissection needles, and the salivary contents applied to a starch gel electrophoresis plate. Electrophoresis was carried out at 300V for 150min. The running buffer was 0.15M glycine/NaOH, pH 9.5; the gel buffer was the same but diluted 1:10. After electrophoresis, the gels were immersed for 10 minutes in a staining solution of 0.3 mg/ml tetramethylbenzidine in a 1:1:1 mixture of ethanol, acetic acid and water. They were then transferred to 2% hydrogen peroxide until the bands began to develop. Because band development is transient, the gels were viewed by videocamera to record the bands and the subsequent tapes transferred to a computer file.It was possible to distinguish all species studied with this technique. This approach also distinguished *R. prolixus* from *R. robustus* and *R. neglectus* from *R. nasutus*, species with extreme phenotypical similarity. The technique has demonstrated to be sensitive in differentiating *Rhodnius* species, and it also could be useful in interpopulation studies in this genus

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VE-10
AGGREGATION IN *TRIATOMA INFESTANS*: CHEMICAL AND SPECTRAL INTERACTIONS

Lorenzo Figueiras NA, Reisenman CE, Giurfa M*, Lazzari CR
Departamento de Ciencias Biológicas, Universidad de Buenos Aires, Argentina *Institut für Neurobiologie, Freie Universität Berlin, Germany, (E-mail: aliciaf@bg.fcen.uba.ar.)

The aggregation behaviour of triatomines is mediated by several factors, i.e., thigmotaxis, chemical attraction, arresting chemotaxis and negative phototaxis. The present work analyses how aggregation is modulated by the convergence of chemical factors and spectral qualities of the light associated with potential refuges. Second-instar larvae of *Triatoma infestans* were presented with three identical refuges of filter paper disposed on an experimental, circular arena. The distribution of bugs on the three refuges was measured under two basic treatments: 1) different spectral lights (human blue, green and red), each associated with one refuge and a clean filter paper; 2) the same as 1) with the addition of faeces to a filter paper. Since faeces provide the source of aggregation chemicals in triatomines, we tested whether the presence of such chemical modifies the pattern of spectral preferences in these insects. The choice of spectral qualities was the same under both treatments: bugs significantly avoided green light, independently of the presence of the chemical attractant and did not exhibit any preference for blue or red lights. Thus, the green photoreceptor seems to have an enhanced participation in this behavioural context. The spectral properties of an object (e.g., artificial refuge, baited trap) affect, therefore, its probability to be approached. This result is important for the elaboration of targets or baited traps aimed to capture or detect Chagas disease vectors.

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VE-11
CHARACTERIZATION OF THE LIPOPHORIN RECEPTOR FROM THE FAT BODY OF *RHODNIUS PROLIXUS*

Pontes EG, Grillo LAM, Entringer PF, Gondim KC
Departamento de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro, RJ, Brasil

In insects lipids are transported in the hemolymph by lipophorin, a major lipoprotein. It was shown in different insect species that lipophorin acts as a reusable shuttle for lipids, that is, it transfers lipids to the sites of storage or utilization, but the apoproteins of the particle are not accumulated in these tissues, and the lipoprotein can be reloaded with more lipids and reutilized. For delivering or taking up lipids, lipophorin interacts with the tissues by means of specific binding sites at cell surface. In *Rhodnius prolixus* females, lipophorin transfers phospholipids to the developing oocytes and, at the different days after blood meal it is preferentially reloaded at the midgut or fat body, and we are studying lipophorin receptors from the fat body.

Four or five days after meal, fat bodies were dissected from adult females, homogenized and a membrane preparation was obtained. Purified lipophorin was radiolabelled in the protein moiety (^{125}I-lipophorin), and the conditions for a filtration binding assay were established. When ^{125}I-lipophorin was incubated with the membrane preparation in the presence of bovine serum albumin, lipophorin binding was observed. ^{125}I-lipophorin was bound to specific sites at the membranes as the interaction was dramatically inhibited in the presence of an excess of non radioactive lipophorin. The binding assay was performed for 90 min to allow the system to achieve equilibrium, at 28^{o}C. The obtained nonspecific binding was 10-15 % of the specific one. ^{125}I-lipophorin binding to the membrane preparation was very sensitive to pH, and it was maximal between pH 6.0 and 7.0. Other biochemical properties of this receptor are under investigation, what is important for the understanding of lipid metabolism in *Rhodnius prolixus.*

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VE-12
SUSCEPTIBILITY OF DIFFERENT SPECIES OF TRIATOMINES TO A WILD STRAIN OF *TRYPANOSOMA CRUZI*

Dost CK, Santello FH, Toldo MPA, Levy AMA, Albuquerque S, Prado Jr JC
Faculdade de Ciências Farmacêuticas, USP, Av. do Café s/n, 14049-903 Ribeirão Preto, SP, Brasil

Trypanosoma cruzi, the etiologic agent of Chagas' disease is transmitted to humans by reduviid bugs through contamination of the skin with feces containing metacyclic trypomastigotes. The ability of the vector to spread the pathogen depends not only on its capacity of domiciliation, but also the possibility to offer an adequate environment for the development of the parasite. It is well known that *T. cruzi* undergoes changes in the distinct parts of the intestinal tract, and this can be influenced by many factors as ingestion of blood and starvation. In this work we study the susceptibility of different species of triatomines to a wild strain of *T.cruzi* (CG) that was isolated from *Panstrongylus megistus* captured in Ribeirão Preto, near the urban area SP, Brazil. Considering a possible potential epidemiological role to be played by this strain, groups of 10 third instar nymphs, fed on chickens, and maintained at 28^{o} C and 70% RH were used to perform xenodiagnosis. Parasistemia of *C.callosus* used for xenodiagnosis was similar for all groups, as evaluated by Brener's method on blood collected from these animals. After 45 days of starvation, the intestinal tract of the triatomines were dissected and diluted in 100ml of physiological saline. The number of flagellates were evaluated in Neubauer's hemocytometer. Results are shown in the following table:

	stomach	midgut	hindgut	rectum
R. prolixus	10000	0	845000	260000
T. infestans	180000	415000	250000	710000
T. matogrosensis	215000	395000	590000	150000
T. lectularia	30000	240000	360000	260000

The results show that CG strain of *T.cruzi* adapted and developed perfectly in the species tested. Different forms were predominant at different anatomic sites of the vector. The presence of trypomastigotes in the rectum can be of extreme importance in the dissemination of the parasite. It seems that *T.infestans* is the group most sensitive to CG strain, showing a higher ability to generate more metacyclic forms, followed by *R.prolixus* e *T.lectularia*. Although *T.matogrosensis* displays a high number of adherent and free epimastigotes in gut and midgut, it seems to be not as effective as the other triatomines, since the parasite did not show a capacity to undergo changes to produce forms in the rectum. Other species are being tested to clarify the role of this strain in the epidemiology of the disease.

VE-13
MITOCHONDRIAL DNA SEQUENCES OF CHAGAS' DISEASE VECTORS: PHYLOGENETIC INFERENCES

García Beatriz A
Cátedra de Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, C.C. 35, Suc. 16, 5016 Córdoba, Argentina

DNA sequence comparisons of 12S, 16S, and cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) genes were used to infer phylogenetic relationships among thirteen species of *Triatoma*, eleven belonging to the *infestans* complex (*T. infestans*, *T. guasayana*, *T. sordida*, *T. platensis*, *T. brasiliensis*, *T. rubrovaria*, *T. vitticeps*, *T. delpontei*, *T. maculata*, *T. patagonica*, and *T. matogrossensis*) and two members of the same genus but of different complexes, *T. circummaculata* and *T. dimidiata*. As outgroup, we chose one member of another genus, *Panstrongylus megistus*. We have sequenced both strands of mtDNA fragments of 12S and 16S ribosomal RNA genes (342 and 509 bp, respectively) in two individuals from each of the fourteen species, and of COI protein coding gene (1447 bp) in two individuals from each of nine of the species studied (for *T. sordida*, two different strains were studied, one from Brazil and another from Argentina). Interspecific distances (maximum likelihood, Felsenstein 1981) varied from 0.00 to 15.21% for 12S, 0.20 to 12.41% for 16S, and 0.30 to 20.60% for COI. There was remarkable mtDNA similarity among *T. infestans*, *T. platensis*, and *T. delpontei* that is compatible with karyotype analysis (Panzera et al., 1995). Phylogenetic trees were inferred from aligned sequences by neighbor-joining (Saitou & Nei 1987) and maximum parsimony (Swofford 1993). The 12S, 16S, and COI gene sequences were studied individually and combined as follows: 12S+16S, and 12S+16S+COI. Evolutionary trees were constructed using *P. megistus* as outgroup. As expected, all the phylogenetic trees inferred by analyses of the molecular sequence data paired *T. infestans* with *T. platensis* with good bootstrap value as well as these two species with *T. delpontei*. *T. sordida* and *T. matogrossensis* also cluster together with strong support. Other well supported nodes at the interespecific level, in the trees obtained from the combined data sets, are: (i) the node clustering *T. guasayana*, *T. circummaculata*, *T. rubrovaria*, and *T. patagonica*, (ii) the node connecting the *infestans-platensis-delpontei* clade and the other species of *Triatoma* analyzed (except *T. vitticeps* and *T. dimidiata*), and (iii) the node supporting *T. vitticeps* as the sister taxon of the clade that contains all the other species of *Triatoma* studied (except *T. dimidiata*). The outgroup, *P. megistus*, indicates that the root for the species group studied would be between *T. dimidiata* and the rest of the species. The mtDNA sequence data suggest that *T. dimidiata* would be the earliest separated species of the *Triatoma* taxa examined.

VE-14
ATTACHMENT OF *TRYPANOSOMA RANGELI* TO DIFFERENT PARTS OF THE GUT AND SALIVARY GLANDS OF *RHODNIUS PROLIXUS*

Soares RPP/*, Pimenta PFP*
Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil, *Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil

Trypanosoma rangeli is a parasite of man, domestic and wild animals, and triatomines insects. In the invertebrate host, the main features of its interaction, are the invasion, the multiplication in the hemocoel and the reproduction in the salivary glands. But in order to enter the hemocoel, the parasites must first invade and cross the gut. The parasite attachment to the gut epithelium is the first step to initiate the invasive process. Different degrees of attachment to the gut epithelial cells have already been reported using light and electron microscopy. It appears that the invasion of the hemocoel takes place in the slender midgut and in the posterior part of the wide gut ("stomach"). In the rectum the flagellates cannot be found adhered under division, the way it occurs with *T. cruzi*. Based on these data, the main object of this work was to develop an *in vitro* study to observe the attachment of *T. rangeli* to the

different parts of the insect gut. The following anatomical gut parts were studied in parasite attachment: the wide gut ("stomach"), tree parts of the slender midgut ("anterior", "medium" and "posterior") and the rectum. We also observed the possible attachment of the parasites to the salivary gland. Initially, guts from one week-fed insects were dissected, separated into parts (square of 2 mm) and opened horizontally in order to expose the lumen. The opened gut parts were allowed to interact with *T. rangeli* (H8GS strain) during 20 minutes. The salivary glands were dissected and exposed entirely. After this, the tissues were thoroughly washed in saline and the attached parasites were counted under the microscope. We observed a preferentially massive parasite attachment in the stomach. The adhesion in the slender gut (anterior and posterior) were observed, but in less intensity when compared to the stomach. Few parasites were observed attached to medium gut, rectum and glands. Even though in the literature, adhesion of epimastigotes to the rectum is not observed, we could demonstrate that small number may occur. Our results indicate that the attachment is preferably observed in the stomach followed by the slender gut. Probably in these regions most of the parasites cross the epithelium to reach the hemocoel. Besides that, it is not well known what type of cells can be invaded and their specific location in the gut. But this must await the results for further investigations.

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VE-15
AEDES AEGYPTI PERITROPHIC MATRIX: A CHEMICAL BARRIER TO THE PROSTETIC GROUP HEME

Lemos VP, Gusmão DS, Petretski MDA, Silva JR, Oliveira PL, Jacobs-Lorena M*, Lemos FJA
Laboratório de Biotecnologia, Laboratório de Química e Função de Proteínas e Peptídeos, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, 28015-620 Campos dos Goytacazes, RJ, Brasil
Departamento de Bioquímica Médica, CCS, UFRJ, Rio de Janeiro, RJ, Brasil *Departmente of Genetics, Case Western Reserve University, School of Medicine, Cleveland, USA

The mosquito *Aedes aegypti* is the most important vector of dengue and yellow fever, endemic diseases present in large scale in Latin America and Africa. As hematophagous insects, these mosquitoes need a blood meal to produce eggs. An important event is observed in the adult females mosquitoes in response to the blood meal: the formation of an extracellular matrix containing chitin, proteins and glycoproteins, separating ingested food from the gut epithelial cells. This layer is called peritrophic matrix (PM). Also during digestion a huge amount of the prostetic group heme is released free resulting from proteolysis of ingested red cell hemoglobin. Despite the large number of information available, it is surprising how little is known about the PM composition and function. Our goal is to investigate the physiological role of the PM as a chemical barrier to the potencially toxic heme. By light microscopy the matrix appeared as a light-brown structure when the blood digestion is increased. The ultrastructural observations showed electron-dense inclusions characteristic of heme are binding the PM. We also carried out histochemical and cytochemical studies using diaminobenzidine to localize the heme peroxidase activity in the midgut. Guts were dissected from *A.aegypti* at 10, 11, 12, 16, 18, 20, 24, 30, 36 and 48 hr abm. Our results showed that the heme peroxidase activity is mostly present on the PM. Our future objective is search for a putative PM molecule(s) responsible for the heme sequestration.

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VE-16
ANOMALIES OF *LUTZOMYIA INTERMEDIA* (DIPTERA, PSYCHODIDAE, PHLEBOTOMINAE)

Carlos Brisola Marcondes
Departamento de Microbiologia e Parasitologia, CCB, UFSC, Campus Trindade, 88040-900 Florianópolis, SC, Brasil

Although there are several observations on anomalies in Phlebotomine sandflies, listed by Marcondes (1997), including some in *Lutzomyia neivai* (Taniguchi et al., 1992; Marcondes et al., 1998), none was cited yet in *L. intermedia*.

I observed the following anomalies in *L. intermedia*: a male from Venda Nova do Imigrante (ES) had an extraordinary subapical spine on each style; a female from the same municipality had nine cibarial horizontal teeth; a male from Rio Bonito (RJ) had the third spine of the style on a protuberance and another from the same municipality had a constriction in the 5th palpomere; a male from Governador Valadares (MG) had an extraordinary spine in a style; a male from Itaguaí (MG) had a conical protuberance in a posterior femur and another male from the same municipality had a conical protuberance in one posterior femur and two in the other.

The ratio anomalous insects/ studied insects for this species complex (including also *L. neivai*) (128:10,000) is much higher than those observed in *L. longipalpis* (3:10,000) and in two studies on several species from Venezuela (11:10,000 and 22:10,000). The importance of symmetrical extraordinary spines on the styles in the identification of sandflies is noted and a complete revision of the reports of anomalies was done.

VE-17
BIOLOGICAL CYCLE OF *TRIATOMA ARTHURNEIVAI* LENT & MARTINS, 1940 UNDER LABORATORIAL CONDITIONS

Medeiros MP, Matsumoto EN, Fernandes MZT, Martinez I, Rosa JA
Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Caixa Postal 502, 14801-902 Araraquara, SP, Brasil

T. arthurneivai is distributed in the states of Bahia, Minas Gerais and São Paulo. The specimens used in this study are originated from a colony collected in 04/24/81 in the farm Santa Tereza, in the municipal district of Espírito Santo do Pinhal - SP. Twenty females and eight males recently born and maintained in camera BOD to the temperature of 28ºC, with relative humidity of the air varying between 51% and 85%, in the period of January for December/1997 were separeted. From those specimens it was isolated 14 lots of separate eggs for posture date, in a total of 234 eggs. The observations on time demanded incubation of eggs and on the duration of the nymphs instar was done daily. The obtained data were analyzed statistically by means of the program Instat. From these 234 eggs 140 nymphs were born, 14 nymphs in the 1st instar, 04 nymphs in the 2nd instar, 03 in the 3rd instar, 03 in the 4th instar and 10 in the 5th instar died. Referring to the results of the incubation period and of evolution of the five nymphs instar of *T. arthurneivai*: Incubation - 17,15 days; 1st instar - 18,47 days; 2nd instar - 21,02 days; 3rd instar - 26,82 days; 4th instar - 29,72 days; 5th instar - 49,69 days.

Suppoted by Fapesp.

VE-18
BIOLOGICAL EFFECTS OF ANTI-SANDFLY ANTIBODIES ON THE LEISHMANIASIS VECTOR *LUTZOMYIA LONGIPALPIS* (LUTZ & NEIVA, 1912) (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE)

Vilela ML, Souza NA, Oliveira SMP, Ramalho-Ortigão JM*, Rangel EF, Traub-Cseko YM*
Departamento de Entomologia *Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Visceral leishmaniasis is caused by *Leishmania (L.) chagasi*, and transmitted by the sandfly *Lutzomyia longipalpis* (Lutz & Neiva, 1912), that is widely distributed from Mexico to Argentina. In view of the collateral effects frequently observed in therapy, and the resurgence of new cases, searching for new control methods is a necessity, with special emphasis in a better understanding of the molecular mechanisms of parasite-insect vector interaction.

The digestive system of hematophagous insects, especially the midgut, is an important putative target for antibodies and drugs ingested with blood. From this fact stems the importance of studies that can lead to means of interfering and blocking disease transmission, by affecting biological aspects of the vector.

We have immunized rabbits with tissues, either gut or carcass, of female sandflies fed on sugar or blood, to evaluate the effect of anti-sandfly antibodies on biological parameters of *L. longipalpis*. After the immunizations, female insects born in the insectary were fed on the experimental or control animals. Preliminary results indicate there was no difference in the various groups of sandflies in relation to the first feeding. Death without egg-laying occurred after the second day, with a slight increase until the 7th day, and the greatest number of dead females in the group fed in the rabbits immunized with sugar-fed gut. The highest number of females dead after egg-laying was observed between the 5th and 7th days. The highest number of deaths of females fed in rabbits immunized with the carcasses occurred in insects that laid eggs. A reduction in number of laid eggs was observed in females fed in rabbits immunized with sugar-fed gut.

We are presently determining antibody titers of the immunized rabbits sera, and determining their specificity by Western blots using various insect tissues.

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VE-19
BIOSYSTEMATICS OF THREE BRAZILIAN POPULATIONS OF *PANSTRONGYLUS MEGISTUS* (BURMEISTER, 1835)

Barbosa SE /*, Soares RPP /*, Pires HH, Laranja LS, Melo MD, Pimenta PFP, Margonari C, Dujardin JP**, Catalá S***, Panzera F****, Santoro MM*****, Romanha AJ, Pereira MH*, Diotaiuti L
Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002, Belo Horizonte, MG, Brasil *Departamento de Parasitologia, Universidade Federal de Minas Gerais, Caixa Postal 2486, 31270-901, Belo Horizonte, MG, Brasil **Instituto Boliviano de Biologia de Altura Casilla de Correos 641, La Paz, Bolivia ***FCEFN, UNC Velez Sarsfield 299, 5000 Córdoba, Argentina ****Facultad de Ciencias, Universidad de la

Republica, Tristan Narvaja 1674, 11200 Montevideo, Uruguay *****Departamento de Bioquímica, Universidade Federal de Minas Gerais, Caixa Postal 2486, 31270-901, Belo Horizonte, MG, Brasil

The triatomine *P. megistus* is the Brazilian species that exhibits the greatest capacity to invade and colonize houses (domiciliation) and is presently considered to have the greatest epidemiological importance in Brazil, except in the Northeast region. However this species exhibits variations in its domiciliation capacity, so that its epidemiological importance varies among different regions. In the present study biological, morphological, biochemical and genetic parameters were examined for three Brazilian populations of *P. megistus* (Bahia-BA, Minas Gerais-MG and Santa Catarina-SC), each of which showed a distinct pattern of behaviour. The results showed discernible differences in the biology of the three populations, in relation to the time of eclosion of the nymphs, development time from 1st instar to adult insect, mortality and mean number of blood meals, although these differences were not statistically significant. First instars always showed a greater capacity for blood meal ingestion than later instars, the number of meals however increasing with developmental stage. The size and pattern of the antennal sensilla differed among the three populations, this being most marked for SC in relation to those of BA and MG. Differences were observed among the populations with respect to the weight and dimensions of the eggs, although not for the morphology of the exochorion. The morphology of the masculine genitalia could not be used to characterize the populations, nor was it possible for them to be distinguished by cytogenetic studies or based on comparison of a-GPD activities. PGM was the only enzyme that showed polymorphism, two patterns being observed of which both were present in the BA and MG populations and only one in that from SC . Phenetic analysis of the electrophoretic pattern of the saliva allowed the populations to be placed in two groups including insects from BA or SC, with those of MG occupying an intermediate position, albeit closer to SC. Based on the results presented, the hypothesis of genetic differences to explain the behavioural variation shown by this species in different regions of Brazil can be rejected, a clearest distinction being seen for the population de SC in relation to those of MG and BA.

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VE-20
CHARACTERIZATION OF THE UROTERGITE PROCESS IN *RHODNIUS NASUTUS* STAL, 1859 AND *RHODNIUS ROBUSTUS* LARROUSSE,1927 THROUGH SCANNING ELECTRON MICROSCOPY AND OPTIC MICROSCOPY

Barata JMS, Rosa JA*, Urbinatti PR, Ceretti JR W, Andrade RMG**, Marassá AM**
Faculdade de Saúde Pública, USP, São Paulo, SP, Brasil *Departamento de Parasitologia, UNESP, Araraquara, SP, Brasil **Instituto Butantan, São Paulo, SP, Brasil

The Urotergite Process was characterized o*n?odnius* Genera to the species *R.prolixus* and *R. neglectus* (Barata et al,1994,1996a) and has been showed as a significant taxonomic character.With the objective to consolidate this character in *Rhodnius* Genera, this study will be extended to the species *R.robustus* and *R.nasutus*.

R.robustus Larrousse,1927, on dorsal view, presents th I Urotergite on a slightly higher level than the II Urotergite;has a triangular shape with a rhomboid apex, differing from *R.prolixus* (Barata et al , 1996a); grooves irregularly displayed are present,and they are more evident that the ones in *R.prolixus*; there was no downward sloping areas on the lateral fringes and on the central area; the apex of the triangular plate as in *R. prolixus* and in *R. neglectus* is surrounded by a visibly wrinkled colored area.The bristles of the triangular plate are sparcely distributed and are bigger than the ones of *R. prolixus*.

The II Urotergite,as in *R. prolixus* has no bristles;the circular area is well defined by a pair of concentric grooves,in whose interior pleats are observed,their apex is divergent and they are mainly displayed in the central area;the underlying wrinkles are irregularly distributed , not determining a pattern as in the other species.

R.nasutus Stal,1859, on dorsal view presents the I Urotergite on a higher level than the II Urotergite ; has a sharped pointed apex as in *R.prolixus* (Barata et al,1996a) ,presents the shape of an obtuse angle;not so prominent vertical stout grooves are present,and they are contiguous to the ones of the II Urotergite;the apex border of the I Urotergite including its apex is surrounded by a wrinkly colored area as it has been seen in the other species of *Rhodnius*.

The II Urotergite,as in *R.prolixus* presents a circular area characterized by ramified wrinkled grooves;in the median portion of the circular area not so prominent parallel horizontal grooves can be seen.

VE-21
CHARACTERIZATION OF THE UROTERGITE PROCESS IN *TRIATOMA BRASILIENSIS* NEIVA, 1911 AND *TRIATOMA RUBROVARIA* NEIVA, 1913 THROGH SCANNING ELECTRON MICROSCOPY AND OPTIC MICROSCOPY

Barata JMS, Rosa JA*, Urbinatti PR, Ceretti Jr W, Andrade RMG de**, Marassá AM**
Faculdade de Saúde Pública, USP, São Paulo, SP, Brasil *Departamento de Parasitologia, Unesp, Araraquara, SP, Brasil **Instituto Butantan, São Paulo, SP, Brasil

With the aim to consolidate the utilization of the Urotergite Process as a taxonomic character in Triatominae in general and *Triatoma* in particular,we are describing this structure to *T brasiliensis* and to *T.rubrovaria*.

T .brasiliensis Neiva,1911 on dorsal view presents the I urotergite on a higher level than the II Urotergite; it has a rhomboid apex as in *T sordida* (Barata et al,1996a,1196b) which is downward sloping and is almost on the same level of the II Urotergite,being lodged in the center of the circular area. As in *T.infestans* and in *T.sordida* (Barata et al,1996b) many bristles can be seen and they are mainly distributed in the median portion of the I Urotergite. The II Urotergite is smooth and presents a concave shape on its circular area as in *T. infestans* and *T. sordida* (Barata et al,1996a, 1996c) .The circular area presents a smooth surface,without ornamentation,where grooves and raised lines can be rarely seen. *T.rubrovaria* Neiva,1913 on dorsal view,presents the I Urotergite on a higher level than the II Urotergite (Barata et al,1994,1996a,1996b,1996c); its apex is pointed as in *T. infestans* (Barata et al,1996b) though in the sample of Santana do Livramento (RN,CTA 104) is rhomboid as in *T.sordida* (Barata et al,1996b) and presents a downward pronounced slope as in *T.brasiliensis*. The triangular plate presents a trapezium shape as in *T.infestans* (Barata et al,1996b);the wrinkles are concentrated on the lateral fringes and on the apex center of the triangular plate. The bristles of the triangular plate are extremely long,differing from the other species of *Triatoma* which were described. The II Urotergite presents in its circular area,a pair of divergent vertical grooves as the ones in *T.sordida* (Barata et al,1996b) ;on the hind region of the circular area,parallel horizontal grooves are observed in all the extention of the rectangular plate,as in *T.infestans* and *T.sordida*.

VE-22
COMPARATIVE MORPHOLOGIC STUDY OF THE SALIVAR COMPLEX OF *TRIATOMA PROTRACTA* (UHLER, 1854) AND *RHODNIUS PICTIPES* STAL, 1872 (HEMIPTERA, REDUVIIDAE)

Santos CM, Jurberg J, Galvão C, Carcavallo RU
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Studies of salivary complexes of *Triatoma protracta* (Uhler, 1854) and *Rhodnius pictipes* (Stal, 1872) were performed looking for new tools for both, to explain the concept of species in Triatominae, in relation to morphological characters, and to get a new filogenetic approach to the Triatominae taxonomy. Ten fifth instar nymphs of each species were placed and climatized in different crystallizers kept in a stove BOD at 28ºC and 80% of RH, to attain the adult condition. During this period, nymphs were fed twice a week with pigeon blood, untill their ecdysis. Six days after moulting the salivary complex was taken off and placed in saline PBS. Drawings were done with camera lucida. Results show that specimens of *T. protracta* present a pair of principal glands (GL P) , unilobed, reniform and whitish with a structure named hilum (HI) , emerging from there two canals (Can A and Can P) oriented to head. The accesory gland (GL A) is a spheric and small one with an accesory canal oriented to the principal gland. In *R. pictipes* the principal gland (GL P) is unilabed, pear-shapped, and colored of intense red; the accesory gland (GL A) is spheroid, small if compared to the principal gland and translucent. It does not present canals, neither accesory nor principal and the accesory gland opens directly into the principal gland. However, there is a new structure, the protuberance (PROT).Considering these results and comparing to other previous publications (Santos at al., 1997; Lent & Jurberg, 1965) we could think that generic differences found in the salivary structures coincide with significant differences in tha phalic structures, with the only exception of *R. pictipes* where the salivary complex is similar to those of other *Rhodnius* species, while the phalic structures are different. We consider that the "salivar gland" parameter is useful in taxonomy for differentiation of genera.

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VE-23
COMPARATIVE OBSERVATIONS ON THE DEVELOPMENT OF *TRIATOMA INFESTANS* KLUG, 1834 (HEMIPTERA - REDUVIIDAE) REARED IN RATS AND DEFIBRINATED BLOOD

Marassá AM, Prezoto BC*, Caetano LM
Laboratório de Entomologia *Laboratório de Farmacologia, Instituto Butantan, São Paulo, SP, Brasil

Concerning the importance of *Triatoma infestans* in the Epidemiology of Chagas' Disease and due to their interest to Public Health, comparative observations were made on its development with reference its utilization in Xenodiagnostic and with the attempt to compare different laboratory breeding methods, two groups from 108 hatched nimphs were formed and were fed respectively on defibrinated blood from rats that were injected with a dose of venom extract from Lonomia obliqua caterpillary that produces incoagulability (Group - G1) and comparatively in rats (Group - G2).Specimens were mantained under the following conditions: 26º - 28º C and 60-80% of relative humidity. As a result, treatment with the defibrinated blood was more efficient in the 1st , 2 nd and 5th instar larvae

in the Group - G1 ($p < 0,001$) according to Mann - Whitney Test comparing with the Group - G2 (that was fed in rats).Concidering the larval development (from 1 st - instar larvae to adult stage) no significative difference was found between Group G1 and Group G2 and teh mortality rate was similar (15% in Group G1 and 10% in Group G2).

VE-24
COMPARATIVE STUDY OF THE EXCRETION PRODUCTS FROM THE DIGESTED BLOOD MEAL OF NORMAL AND INFECTED MOSQUITOS WITH *PLASMODIUM GALLINACEUM*

Carvalho JC*, Braga EM, Macêdo ME*, Pimenta PFP*
Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade Federal de Minas Gerais, *Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, Belo Horizonte, MG, Brasil, Fax(031) 295-3115, (pimenta@netra.cpqrr.fiocruz.br)

The objective of the present work is to detect possible differences in the digestive process of blood meal of *Aedes aegypti* infected or not with *Plasmodium gallinaceum*, through the electrophoretic analysis of its excretion products. The mosquitoes were experimentally infected with *P. gallinaceum* through blood feeding in infected chickens. The control group was infected with normal blood. The two groups of mosquitoes were maintained for 12 days with a diet constituted of 10% glucose. Later on, mosquitoes from both groups were re-fed with normal chicken blood and the excretion products collected in filter papers. After elution in PBS plus 0.05% Tween solution, the excreted products were analyzed in polyacrilamide gel (SDS-PAGE) and stained with silver. Our preliminary results showed that five protein bands were comparatively identifiable between the two mosquito groups (PM 150, 98, 66, 30 and 25 kDa). But, two proteins (PM 128 and 120 kDa) were weakly visualized in the samples obtained from the mosquitoes infected with the *P. gallinaceum.* Further studies are necessary in order to confirm our results as well as to characterize the different types of proteins present in both groups.

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VE-25
COMPARISON OF ANOPHELES DARLINGI POPULATIONS BASED ON ITS2 DNA SEQUENCES

Malafronte RS, Marrelli MT, Marinotti O
Instituto de Medicina Tropical de São Paulo, Lab.Protozoologia, Universidade de São Paulo, Av. Dr. Eneas de Carvalho Aguiar, 470, 05403-000 São Paulo, SP, Brasil

Specimens of *Anopheles darling,*the major vector of malaria in Brazil, were captures in the states os São Paulo (Dourado), Bahia (Itabela), Rondônia (Porto Velho), Roraima(Boa Vista) and Acre (Plácido de Castro). The distinction os fibling species of insects is of critical importance, since different members in a Complex could exhibit differences ecology, vectorial capacity and response to control measures. DNA sequence analsys and particulary that os the ITS2 region of rDNA cistron has provided diagnostic charcteres in some groups of sibling species, becoming a general tool for taxonomic and phylogenetic studies. The second internal transcribed spacer (ITS2) of nuclear ribosomal DNA were sequenced and compared among these anophelines. Conserved primers of the 5.8S and 28S regions were used for PCR, and amplifield products were cloned and sequenced. The lengths os ITS2 of all mosquitoes captures were about 450 nucleotides, with about 57% GC content. We did not find any ITS2 polymorphism sequence among the mosquitoes captured in Amazon region (porto Velho, Boa Vista and Plácido de Castro) or Northeast (Itabela), howere several differences in the ITS2 sequence of mosquitoes captured in Dourado were observed. Using ITS2 as a tool, we could not differentiate intraspecific variaton in *Anopheles darlingi* mosquitoes.

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VE-26
CONNEXIVUM STUDY OF *TRIATOMA RUBROVARIA* (BLANCHARD, 1843) (HEMIPTERA, REDUVIIDAE) BY ELECTRON MICROSCOPE AND MICROANALYSIS

Rosa JA, Matsumoto EN, Cilense M*, Portillo J**, Barata JMS***, Benedetti AV*
Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Caixa Postal 502, 14801-902 Araraquara, SP, Brasil *Instituto de Química, Universidade Estadual Paulista, Caixa Postal 355, 14801-970 Araraquara, SP, Brasil ** Universidade de Barcelona, Barcelona, 08028, Espanha *** Faculdade de Saúde Pública, Universidade de São Paulo, 01246-900 São Paulo, SP, Brasil

Triatoma rubrovaria is found in Uruguay, Argentina (Missiones, Corrientes and Entre Rios) and Brazil (Rio Grande do Sul), according to Lent & Wygodzinsky (1979), Carcavallo et alii (1987, 1988) and Rosa (1995). This species is able of holding Chagas'disease in wild environment and is highly susceptible to *Trypanosoma cruzi* (Silva, 1985 and Perlowagora-Szumlewicz, 1988). The connexivum of Triatominae is a structure that is often used in taxonomy and needs a more detailed analysis. Analysis results of *T. rubrovaria* connexivum made by spectrometry and nuclear magnetic ressonance were previously related (Matsumoto et alii, 1996, 1997). For the present research connexivum black and red portions of *T. rubrovaria* adults were separated and pulverized in a grail of agate and isopropyl alcohol. The pulverized sample was deposited on a miniguid of copper and then submitted to a general surface microanalysis using and EDX system from PGT model OPP 014-3038 coupled to a transmission electron microscope Philips CM200. EDX analysis showed in both red and black portions of the connexivum titanium, silicon, oxygen and iron as the majority element. In order to distinguish the structure responsable for the red and blak colors, the samples were examined by TEM and the respective electron diffraction patternss were obtained. The results revealed: 1) red portions are characterized by centered body cubic cristals that were iron oxide (Fe_2O_3) hematite type. 2) black portions are composed by cristals that are iron oxide hematite type, yet,mixed with other iron oxides richer in oxygen. In both samples was detected a small amount of titanium. The observation of nymph instar, using the scanning electron microscope JEOL model JSM-T330A, demonstrated that the connexivum is built up from 2nd instar one. In adults, 3 kinds of bristles were found once that in the 5th instar only two kinds were noticed.

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VE-27
DETECTION BY CONFOCAL OF AN INTERNAL THORACIC STRUCTURE IN *ANOPHELES ALBITARSIS* LATE EMBRYOS

Valle D, Vale BS*, Manso PPA**, Lenzi HL*, Pelajo-Machado M*, Caputo LFG*, Monnerat AT, Rosa-Freitas MG, Lima JBP**
Departamento de Bioquímica e Biologia Molecular *Departamento de Patologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil **US Army Medical Research Unit, Instituto de Biologia do Exército, Rio de Janeiro, RJ, Brasil

In order to collaborate with the strategy, under development, of construction of genetically modified malaria vectors refractory to the *Plasmodium*, a systematic analysis of neotropical *Anopheles* embryo development is being carried on.

Under constant conditions (26^{o}C and 80% r. h.) the whole *Anopheles albitarsis* embryogenesis process takes 42 to 44 hours to be accomplished. Clarified embryos are being analysed through LSM at 2 hours intervals in order to detect major morphogenetic movemens and to describe embryo morphology. Confocal microscopy analysis was done with a Zeiss LSM 410 microscope, using a He/Ne laser beam (543nm) and a LP 570 filter. With this set up *Anopheles* eggs become fluorescent when stimulated by the laser, emitting light with wave-length longer than 570 nm.

During the morphological analysis of mosquito embryogenesis process, a pair of internal thoracic structures was detected in late embryos, arising during the dorsal closure movement and persisting until the end of embryogenesis. These structures, whose nature is unknown, are roughly spherical, have a diameter of 20-30 mm and are comprised of an unique array of cells surrounding an apparent empty lumen. Two pairs of filaments project from the basis of these structures towards the abdominal region.

We aim at performing histological sections and immunohistochemical assays on permeabilized embryos in order to unravel its structure and predict its funcion.

Supported by Fiocruz CNPq.

VE-28
DEVELOPMENT OF *LEISHMANIA BRAZILIENSIS* AND *L. AMAZONENESIS* INFECTIONS IN THE SANDFLY *LUTZOMYIA MIGONEI*

Nieves E, Pimenta PFP*
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima, 1715, Belo Horizonte, MG, Brasil, Fax: (031) 295-3115, (pimenta@netra.cpqrr.fiocruz.br), (nevelsa@netra.cpqrr.fiocruz.br)

We are presenting the results of a laboratory study of the behavior of *Leishmania braziliensis* and *L. amazonenesis* infecting the sandfly *Lu. migonei*. This study is to evaluate the insect vector potential and to clarify the mechanism of parasite transmission. Batches of 200 females of 3-4 days old of *Lu. migonei* from a closed laboratory colony, were infected with *L. braziliensis* and *L. amazonenesis* amastigotes. The flies were dissected and observed for *Leishmania* infections from one to ten days after feeding. Parasite density and distribution were observed in fresh dissected guts. Then, the guts were homogenized and the released parasites were counted in a haemocytometer. The

released parasites were also fixed and stained by Giemsa. Infectivity at the vertebrate host was determined by the inoculation of the hamsters with gut contents from infected flies. The results show the ability of the *Lu. migonei* to harbour both the *L. braziliensis* and the *L. amazonenesis*. The infection's development was peripylaria for *L. braziliensis* a natural host-parasite association, with parasite colonization of the hindgut. It was also frequent to observe small number of parasites attached to the pylorus. In the case of the *L. amazonensis* infection, an unnatural host-parasite association, the development was suprapylaria with parasites restricted to the abdominal midgut. The infection of the Malpighian tubules was exclusively observed with *L. amazonensis*. Five promastigote forms were observed: procyclic, nectomonad, haptomona and metacyclic. There was a clear difference in the pattern of appearance of the developmental forms, but the metacyclics even in the late infections were small in number. Infected flies were able to transmit both *Leishmania sp* to the hamsters, but with a delay for the *L. amazonenis*. The *Lu. migonei* has not yet been implicated in the transmission of *L. amazonensis*, but both the sandfly and parasite coexist in some geographic regions. This study indicate that *Lu. migonei* is a suitable vector for *L. amazonensis* in the laboratory and confirms the role of the fly as the potential vector for *L. braziliensis*.

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VE-29
DIMENSIONAL PARAMETERS OF THE ECOTOPES, NICHE AND NATURAL INFECTION OF SYMPATRIC TRIATOMINES AT JUAZEIRO, BAHIA, BRAZIL

Marchon-Silva V, Diotaiuti L*, Almeida JR**, Costa J
Coleção Entomológica, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Centro de Pesquisa René Rachou, Fiocruz, Belo Horizonte, MG, Brasil *Cope/UFRJ, PANEEA/ Uerj, Rio de Janeiro, Brasil

Surveys of the geographic distribution of triatomines showed that *Triatoma brasiliensis* (*Tb*), *T. sordida* (*Ts*), *T. pseudomaculata* (*Tp*) and *Rhodnius neglectus* (*Rn*) occurred sympatrically at Bahia, Maranhão, Minas Gerais, Piauí and Tocantins states (Silveira *et. al.*, 1984). Field collections carried out at Juazeiro in September 1996 and April 1998 revealed that these species may occur in the same habitat. In order to detect strategies of the inter-specific competition and to collect data of vetorial status of the species mentioned, exhaustive captures were carried out in domiciliar and peridomiciliar ecotopes at Junco and Aldeia, Juazeiro (BA). Preliminary data about the natural and artificial ecotopes where the triatominae bugs were captured, population density and natural infection are presented. These analyses will allow more precise procedures for vetorial control. In both captures a total (T) of 710 triatomines were captured: 21 in intradomicile (76% *Tb*, 24% *Ts*) and 689 at peridomicile (60,8% *Tb*, 27% *Ts*, 1% *Tp*, 10,6% *Rn* and 0,6% nymphs not identified). From the 40 domiciles examined in the surroundings of Junco (19) and Aldeia (21), 30 of them (75%) were infested by more than one species. At Junco, the percentual relation among the species captured in 1998 (T-152 specimens: 95% *Tb*, 1% *Ts*, 4% *Tp*, 0% *Rn*) differed greatly from the one recorded in 1996 (T-70: 39% *Tb*, 4% *Ts*, 40% *Tp*, 17% *Rn*). However at Aldeia, the percentual relation among the species captured in 1998 (T-248 specimens: 57% *Tb*, 42% *Ts* , 1% *Tp*, 0% *Rn*) was similar to that recorded in 1996 (T-240: 34% *Tb*,27% *Ts*, 1% *Tp*, 23% *Rn* e 15% nymphs not identified). About the ecotopes preference, all of these four species observed in 1996 were found in greater percentage in chicken coops. However, in 1998, the predominance of certain species to a specific ecotope was recorded: 129 (40%) specimens of *Tb* were captured in goat pen, 51 (41%) of *Ts* on the shingles of roofs and 2 of *Tp* in straw. *Tb* was the most eclectic species in relation to the different ecotopes observed. When the presence of *Rn* was recorded, it occurred always in higher densities than the *Ts* and *Tp*. The analyses of the natural infection rate by *T. cruzi*, from a total of 310 specimens examined and captured in April 1998, positivity for only one specimen of *Ts* from Aldeia was observed.

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VE-30
ECO-EPIDEMIOLOGICAL PARAMETERS IN THE DESIGN OF A DESCENTRALIZED CONTROL STRATEGY FOR THE LEISHMANIASES IN BRAZIL'S NORTHEAST REGION

Andrade PP
Departamento de Genética, Universidade Federal de Pernambuco, Av. Moraes Rego s/n, 50670-901, Recife, PE, Brasil

Planning decentralized control actions for leishmaniases, in response to the new guidelines of the Unified Health System (SUS), depends upon a deep understanding of local peculiarities concerning both the vector and the reservoirs, as well as climate and soil, anthropic pressure upon the environment, suburban characteristics of the target cities, etc. Based on field work developed by our group, between 1995 and 1998, in the Pernambuco districts of Petrolina, Ouricuri, Exu, Tuparetama and Itamaracá, as well as in the metropolitan area of Recife, we determined a set of eco-epidemiological parameters that may be used as guidelines for those health officers in charge of planning and executing leishmaniasis control actions at the district level. Most of these parameters may be extended for other

Northeast states, specially those which have a clear climatic transition, from wet coastal lowlands to a dry hinterland. *L. longipalpis* (and, hence, kala-azar) is to be expected in a coastal fringe approx. 15 km. wide, where annual rainfall is > 1,500 mm and the soil is largely composed of sand. In this area no known vectors of cutaneous leishmaniasis have been consistently reported. Just after this coastal area, soils tend to be of low drainage, while rainfall indexes are still high. In these areas *L. longipalpis* is very rarely found, while *L. intermedia* and *L. whitmani* are frequently reported. These areas are endemic for cutaneous leishmaniasis. Following the slope of the Borborema plateau, the climate quickly changes and rainfall indexes are between 800 and 1,500 mm. Those areas are endemic for both visceral and cutaneous leishmaniasis, with *L. intermedia* being the major vector for the cutaneous form. All the dry hinterland is endemic for kala-azar, except for those districts (and even so, only in certain areas) were rainfall indexes may be > 600 mm (at the south piedmonts of mountain ridges) and were *L. intermedia* and *L. longipalpis* are sympatric. Additionally, *L. longipalpis* is consistently found in the very urban limits of the cities in dry areas, whenever the original bush ("caatinga") still exits, limiting kala-azar cases to a city fringe perimeter of ca. 200 m wide. Factors determining peri-urban kala-azar in coastal cities are less clear.

VE-31
ECO-EPIDEMIOLOGY OF CHAGAS' DISEASE IN PERNAMBUCO: I. PRESENT ROLE OF WILD TRIATOMINES IN THE TRANSMISSION OF *TRYPANOSOMA CRUZI*

Torquato GN, Santos CCD, Mendonça MB, Carvalho SMS, Kido EA, Santos ESC, Andrade PP
Departamento de Genética, UFPE, Av. Moraes Rego s/n, Recife, 50732-970, PE, Brasil

Since *Triatoma infestans*, considered to be the only vector for Chagas' disease, was virtually eradicated from most endemic areas in Brazil, WHO assumed that the vectorial transmission of *T. cruzi* was interrupted. However, many wild triatomine species have been consistently reported in Pernambuco State, both at the peri-domicile and indoors. In order to investigate the present role of wild triatomines in the transmission of Chagas' disease in this state, 7 endemic areas were visited, belonging to "Agreste" (the counties of Iguaraci, Garanhuns and Tuparetama) and "Sertão" (Afrânio, Petrolina, Salgueiro and Santa Maria da Boa Vista). A total of 409 bugs (*T. braziliensis* and *T. pseudomaculata*) was collected, 30 indoors (12 nymphs); 58 triatomines captured at the peri-domicile were infected, as well as 10 from indoors. Stools were observed under microscopy and positive samples were inoculated in newborn mice. Parasitemia was investigated every third day after the first week. Infected mice were sacrificed under anaesthesia and the blood seeded in LIT medium at 26ºC. Sixteen presumptive *T. cruzi* strains were isolated, 13 being originated from peri-domicile triatomines and 3 from indoors. Analysis of the duration of the prepatent period of the murine infection and the differentiation in culture to epimastigote forms showed that the isolates could be grouped in three clusters: those with a short prepatent period (6 to 14 days) and early differentiation (10 to 21 days); those also with short prepatent period and delayed differentiation (24 to 45 days) and those with a long prepatent period (> 21 day). The development of the infection in mice could not be correlated to the insect instar or the geographical origin of the isolate. The polar characteristics of the clusters point towards the existence of at least two distinct *T. cruzi* groups in Pernambuco, possibly equivalent to the biodemes defined by Andrade et al. (Rev. Bras. Med. Trop. 1997; 30(1): 27-35), implying the coexistence of wild and human *T. cruzi* strains in triatomines.

VE-32
EPIDEMIOLOGY OF AMERICAN CUTANEOUS LEISHMANIASIS (ACL) IN THE STATE OF SÃO PAULO. SANDFLIES COLONIZATION IN ENDEMIC ENVIRONMENT, NATURAL INFECTION BY PROMASTIGOTES AND SPRGAD OF *LUTZOMYIA LONGIPALPIS*

Taniguchi HH, Siqueira ML, Elias CR, Larosa R, Tolezano JE
Seção de Parasitoses Sistêmicas, Instituto Adolfo Lutz, Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brasil

In two ACL endemic regions in the State of São Paulo, Itupeva City in southeast and Ilhabela City in north shore, we did investigations aiming the knowledge about preferences of phlebotomines to colonize forested or modified environment and, the natural infection by *Leishmania* sp. In the forested environment predominated : *Lu. firmatoi, Lu. pessoai, Lu. fischeri, Lu. migonei, Lu. alphabetica* and *Lu. whitmani* in Itupeva, while in Ilhabela were: *Lu. intermedia, Lu. shannoni, Lu. edwardsi, Lu. monticola* and *Lu. migonei*. In the modified environment the more frequent species were *Lu. fischeri, Lu. whitmani, Lu. intermedia, Lu. migonei* and the surprising *Lu. longipalpis* that represented 17% of the total collect there, while in Ilhabela were *Lu. intermedia, Lu. migonei* and also found, in low density, *Lu. longipalpis*. Until now we have recognized sandflies species that have more or less eclectic preference about environment of natural colonization having those that colonize with same intensity the two envionment (Itupeva – *Lu. fischeri, Lu. whitmani* and *Lu. migonei*; Ilhabela – *Lu. intermedia* and *Lu. migonei*). Others have presented a clear preference by the forested areas (Itupeva – *Lu. firmatoi* and *Lu. alphabetica*; Ilhabela – *Lu. shannoni* and *Lu. monticola*) or least in modified environment (Itupeva – *Lu .intermedia* and *Lu. longipalpis*). In Ilhabela *Lu. intermedia* were almost absolute (92,9%) that can represent a more frequent contact between sandflies and or dogs and consequently a great risk to the transmission of *L.(V.) brazilensis* the recognized agent of human and

canine authocthonous cases in São Paulo. In Itupeva, *Lu .fischeri, Lu. whitmani* and *Lu. migonei* divided with *Lu. intermedia* the modified environment in human peridomiciliary level. Preoccupyind is the concomitant presence of *Lu. longipalpis* in the two regions, only in this environment being in high density in Itupeva. Either from Ilhabela and Itupeva modified environment we found one *Lu. intermedia* with promastigote forms in the gut.

VE-33
ESTABELECIMENTO E MANUTENÇÃO DE COLÔNIA DE *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) (LUTZ & NEIVA, 1912) COM DIFERENTES DIETAS ALIMENTARES

Leandro MJF, Gonçalves CA, D'Almeida JM*, Mello RP, Brazil RP**, Oliveira SMP
Departamento de Entomologia * Departamento de Biologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Laboratório de Leishmaniose, Centro de Pesquisa Renée Rachou, Fiocruz, Belo Horizonte, MG, Brasil

Laboratory colonies of sandflies may be used in various types of research in leishmaniasis, such as xenodiagnosisin infected humans or animals experimental infections, the systematic of the parasites, the biology and genetics of the vectors. Such experiments may require a large number of sandflies and, therefore, the improvement of rearing techniques aiming at mass production with a reduced amount of labor is, obviously, important.

There are many especies from genus *Lutzomyia* (Diptera: Psychodidae: Phlebotominae) considered vectors of leishmaniasis and beyond them *Lutzomyia. longipalpis.*

On the laboratory the colony is maintained according to Modi & Tesh with some modifications.

After the oviposition the eggs are counted and transported to different boxes. After the eclosion were tested the following feeds: decomposed organic matter, vegetable roots, beer yeast, "vitormonio", fish fish food, shrimp fish food and a mix of them all utilized as standard also offered on the maintenance of the colony.

Until now among the different feeds the standard has presented the shortest evolutive cycle followed by the fish fish food. On the other hand the vegetable roots have presented the longest evolutive cycle followed by the decomposed organic matter. The other feeds have presented no significative differences but the shrimp fish food presented the major number of adults followed by the fish fish food.

VE-34
ESTABLISHMENT OF AN AXENIC CLOSED COLONY OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE), VECTOR OF *PLASMODIUM GALLINACEUM*

Macêdo ME, Carvalho JC, Nicoli JF*, Vieira EC**, Pimenta PFP
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, Belo Horizonte, MG, Brasil, Fax(031) 295-3115 (esther@netra.cpqrr.fiocruz.br), (pimenta@netra.cpqrr.fiocruz.br) *Departamento de Microbiologia, **Departamento de Bioquímica, Instituto de Ciências Biomédicas, UFMG, Belo Horizonte, MG, Brasil

There is no too much knowledge about the relationship among the normal microbiotica present in the midgut of mosquitos with the *Plasmodium* parasites that invade the organ. In order to understand the possible role of this microbiotica in the interaction with the parasites, we tried to obtain a population of axenic mosquitoes. In this case, axenic *Aedes aegypti* mosquitoes which can be infected with *P. gallinaceum.* Firstly, in order to obtain axenic larvae, we sterilized eggs originated from a closed colony kept in the Entomology Section of the "Fundação Nacional de Saúde", located in Belo Horizonte city, Minas Gerais State, Brazil. The sterilization process was done with the eggs inside a 25x150 mm glass tubes containing distilled water mixed with 2% clorox for 10 minutes. The sterilized eggs were washed 3 times with sterilized distilled water and then transferred to glass tubes containing distilled water and fish food (Tetra Gold Medal - Tetra Werke, Germany) which also were sterilized. Control eggs were put in the same condition without any of the sterilization process. The time for the egg hatchings was similar in both groups (control and sterilized eggs), two hours. In the control group, we observed the first pupae after five days. The adult emergence of the mosquitos started on the 6th day following through the 15th day. On the other hand, there was a considerable delay in the development of the axenic mosquitoes. It took twenty days for the first pupae to appear and the first adult (a male) only four days after, different from the controls ones that appeared on the first day after the pupae. We observed that in the axenic population the 4th larvae stage does not transform into pupae and several of them die. We ensured that we obtained axenic larvae by adding samples of the larvae growing medium in Brain Heart Infusion e Thioglycollate Broth. After several days of incubation at 22^{o}C and 37^{o}C there were no observations of any microorganisms. In conclusion, we obtained *Ae. Aegypti* larvae in axenic conditions, but the passage to the adult phase is presenting some problems, which probably is due to the difference of the growing conditions of the two groups, or because of the absence of the microbiotica.

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VE-35
FEEDING AND DEFECATION PATTERNS OF *TRIATOMA MELANOSOMA* MARTÍNEZ, OLMEDO & CARCAVALLO, 1987 (HEMIPTERA, REDUVIIDAE) UNDER LABORATORY CONDITIONS

Galvão C, Rocha DS, Carcavallo RU, Jurberg J, Cunha V
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Martínez, Olmedo & Carcavallo described in 1987 a new Argentinian subspecies, *Triatoma infestans melanosoma.* The original description was based on completely black specimens collected on the surroundings of human rural dwellings, at the province of Misiones, on the extreme NE of Argentina. This species was reared in several insectaries of Argentina and Brasil showing a complete stability of the phenotypical color pattern through several generations; this was one of the reasons taken into account by Lent et al. (1994) to change the taxonomic status to the specific one, becoming *Triatoma melanosoma.* Its affinity with the most important Chagas' disease vector, *T. infestans*, was the principal reason to evaluate the potential vector capacity in the laboratory, searching the feeding and the deffecation patterns of 30 specimens of each developmental stage. Insects used for this study are from the colony maintained at the insectary of the National and International Reference Laboratory on Triatominae Taxonomy. They were fed individually on pigeon (*Columba livia*) and the feeding source was offered during 10 minutes, controlled with a chronometer. After feeding, specimens were observed during other 10 minutes to determine the defecation time. *T. melanosoma* has demonstrated a great voraciousness because 65% of insects has accepted to start feeding in 1 minute, completing the suction activity between 5 and 15 minutes in 87 % of the observations in 1st instar nymphs. For 2nd and 3rd stages the proportion was 86 % during that time, while in 4th and 5th instar nymphs the percentage was 74 and 69 respectively during the same period. The maximum time for feeding was 30 minutes for 5th instar nymphs. The percentage of defecations imediately after feeding was high, more than 70 % in stages 1st, 2nd and 3rd, decreasing in stages 4th and 5th with only 45% of inmediate defecations. Results show the high potential vector capacicity of this species.

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VE-36
FEEDING BEHAVIOUR OF TRIATOMINES ON DIFFERENT HOSTS

Guarneri A, Diotaiuti L, Gontijo N*, Gontijo A**, Pereira MH*
Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil *Departamento de Parasitologia, ICB, UFMG, Caixa Postal 2486, 31270-901 Belo Horizonte, MG **Coltec, UFMG, Caixa Postal 2486, Belo Horizonte, MG, Brasil

The ingestion of blood by triatomines is associated with certain morphological and physiological adaptations, including mouthparts adapted to penetrate the skin of the host, locate blood vessels and maintain blood flow during feeding. Several studies have investigated feeding behaviour of triatomines using artificial feeders. In the present study we looked at several aspects of the feeding behaviour of *Triatoma infestans*, *Triatoma brasiliensis* and *Triatoma pseudomaculata* fed on pigeons and mice. Fifth instar nymphs that had fasted for a mean of 30 days were used in the study. The method used was based on the technique of Friend & Smith (1978) and permitted us to study feeding behaviour by monitoring the electrical activity of the cibarial pump, using eletrodes inserted into head of the triatomine and the body of the host. The number of probes, time of contact (min), increase in weight during feeding (mg/min), increase in total weight (mg) and rate of blood ingestion were measured in *T. infestans*, *T. brasiliensis* and *T. pseudomaculata* fed on pigeons (pi) and mice (mi). All insects that at least doubled their initial weight were included in the analysis. Total weight gains of 225±95 mg (pi) and 195±72 mg (mi) were recorded for *T. infestans*, 264±107 mg (pi) and 267±100 mg (mi) for *T. brasiliensis;* and 157±51 mg (pi) and 231±287 mg (mi) for *T. pseudomaculata*. The mean contact times were 8,8±2,6 min (pi) and 14,2±6,4 min (mi) for *T. infestans;* 20,2±9,6 min (pi) e 30,8±18,2 min (mi) for *T. brasiliensis* and 34,5±11,6 min (pi) and 38,5±16,4 min for *T. pseudomaculata*. The mean rates of engorgement were 28±5,8 mg/min (pi) and 21,3±5,5 mg/min (mi) for *T. infestans;* 17,1±6 mg/min (pi) and 12,6±6,2 mg/min (mi) for *T. brasiliensis* and 5,2±1,9 mg/min (pi) and 3,9±1,4 mg/min (mi) for *T. pseudomaculata*. Statistically significant differences in weight gain were not seen for any of the species studied when feedings on the two host species were compared. Engorgement of the three species was always more rapid in pigeons than in mice, although this difference was not statistically significant in *T. pseudomaculata*. Feeding rates were also different among the species, with *T. infestans* showing the highest values, followed by *T. brasiliensis* and *T. pseudomaculata*. Times taken to locate a blood vessel after probing again showed lower values in the groups fed on pigeons. No differences were seen among the species with respect to the number of probes or interruptions during feeding. These results demonstrate the greater feeding efficiency of *T. infestans* in relation to the other two species, this species showing a higher mean rate of engorgement and shorter time of contact with the hosts.

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VE-37
FIELD EVALUATION OF THE LETHAL OVITRAP SYSTEM FOR CONTROL OF *AEDES* MOSQUITOES

Portal IF, Mello RCG***, Galardo AKR***, Soares SS***, Braga IA**, Lima JBP, Quintana M, Perich MJ*
USA Medical Research Unit, Brazil, Walter Reed Institute of Research, Instituto de Biologia do Exercito, Rua Francisco Manoel 102, 20911-270 Rio de Janeiro, RJ, Brasil *Department of Entomology, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA **Fundação Nacional de Saúde, Gerência Técnica de Entom. e Pesquisa Operacional, SAS Q4 BL N S/711, Brasília, DF, Brasil ***Fundação Nacional de Saúde, Nucleo de Entomologia do Rio de Janeiro, Av. Brasil 4036/109, Rio de Janeiro, RJ, Brasil

A field test of the ovitrap was performed from Feb. to May 1998 at four sites in Baixada Fluminense within the Rio de Janeiro area of Brazil. At each site, 30 houses were selected for participation in the study. Sixty received the lethal ovitrap treatment and an additional 60 houses were selected to serve as untreated controls. Each treated house had 10 lethal ovitraps with strips impregnated with insecticide (deltamethrin 0.9mg per strip), five traps were placed inside the house and 5 traps outside the house. Each week 10 houses were examined, and the adult mosquitoes were collected. Prior to placing the lethal ovitraps at each house (both the treated and control), samples were taken of all mosquitoes found in the houses. Mosquitoes were collected for a ten minute period, using flashlights and battery operated aspirators. All ovitraps were checked monthly, during which time, the strip and the hay water solution were replaced. In addition, larvae and pupae found in the ovitraps were collected and identified to determine the effect of lethal ovitraps on natural *Aedes aegypti* and *Ae. Albopictus* populations. Results from this test indicate that the lethal ovitraps significantly reduced the number of dengue vectors within the treated homes compared to the untreated control homes.

VE-38
GEOGRAPHICAL DISTRIBUTION OF AMERICAN TRYPANOSOMIASIS VECTORS IN THE SANITARY DISTRICTS OF THE STATE OF GOIÁS, IN THE YEARS 1996/1997

Isac E*, Santos AH*, Silva EA, Camargo MF, Silva JL, Oliveira AWS
Fundação Nacional de Saúde *Universidade Federal de Goiás, Goiânia, GO, Brasil

The planning of control action is based on the infestation indices and the geographical distribution of the vectors. The objective of this study is to get to know the current situation in the sanitary districts(SDs), as a way of giving priority to the measures that will be taken in order to control the vectorial transmission of Chagas disease, in the State of Goiás.

The triatominical survey was carried out in and around the 139 municipalities which form the four SDs of the State of Goiás.

A total of 8,802 triatominoes were caught during the period, of which 70,34% were in the SD of Formosa, 18,05% in the SD of Jataí, 5,86% in the SD of Ceres and 5,73% in the SD of Morrinhos. The species with greater infestation frequency was the *T. sordida* (88,24%), followed by the other species of synantropicals.

These results demonstrate that triatomino control action should be intensified in all the SDs, but especially where *T. sordida* was found as this was the more widespread.

VE-39
GEOGRAPHICAL DISTRIBUTION OF PHLEBOTOMINE (DIPTERA: PSICHODIDAE) IN THE STATE OF RIO GRANDE DO NORTE

Ximenes MFFM, Castellón EG*****, Souza MF, Freitas RA*****, Wilson M***, Pearson R**** , Jeronimo SMB**
Departamento de Microbiologia e Parasitologia **Departamento de Bioquímica, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil ***Department of Medicine, University of Iowa, ****Department of Medicine, University of Virginia, USA *****Instituto Nacional de Pesquisa da Amazônia, Manaus, AM, Brasil

The biology and ecology of sandflies involved in the transmission of visceral and cutaneous leishmaniasis in Brazil have been the focus of several studies, but little is known about the species and distribution of sandflies in the state of Rio Grande do Norte, where both cutaneous and visceral leishmaniasis are endemic. The objective of the present study was to determine the geographical distribution and frequency of sandflies in Rio Grande do Norte that could transmit *Leishmania* species to humans. Sandflies were captured using CDC light traps in 30 municipalities distributed throughout the 8 geographic zones of the state. Eleven *Lutzomyia* species were captured. *L. longipalpis* was the most prevalent species and accounted for 85.59% of the sandflies captured. The remainder were the

following: *L. evandroi, L.oswaldoi, L. sallesi, L. migonei, L. lenti, L. goiana, L. intermedia, L.samueli, L. capixaba and L. walkeri*. *L. longipalpis* was captured in 93%of the municipalities and was distributed across all geographical areas of the state independent of climate and topography. Other *Lutzomyia* species these variables seem to be important. *L. longipalpis* was identified in all municipalities where human visceral leishmaniasis has been reported. *L. intermedia* and *L. migonei* were captured in the municipalities of Luís Gomes, São Miguel, and Coronel João Pessoa where cases of mucosal and cutaneous leishmaniasis have been reported, as well as Extremoz. Both species have been associated with transmission of *L. braziliensis* in other Brazilian states. The sandfly breeding sites, the main animal reservoirs and the variables that affect the transmission of *Leishmania* species to humans in these regions have yet to be determined.

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VE-41
IDENTIFICATION OF THREE ISOFORMS OF VITELLIN IN *RHODNIUS PROLIXUS* AND THEIR POSSIBLE SITE OF SYNTHESIS

Salerno AP, Melo ACA/** , Petretski MDA*, Masuda H
Departamento de Bioquímica Médica, ICB, CCS,UFRJ, Rio de Janeiro, RJ, Brasil *Laboratório.de Química e Função de Proteínas, CBB, UENF, Campos, RJ, Brasil **Departamento de Patologia, CCB, UFPA , Belém, PA, Basil

Vitellogenins (VG) are high molecular weight phospholipoglycoproteins. They are taken up from hemolymph by the oocytes, now VG is named vitellin (VT). VT, the major yolk protein of *Rhodnius* oocytes was purified by means of ion exchange chromatography DEAE TOYO-PEARL. Three VT isoforms were identified and named VT_1, VT_2, and VT_3. Since VG is the known precursor of vitellin, we purified VG by DEAE column, in order to compare with VT isoforms. Only one VG form was identified and resembled to VT_3 in its elution profile. We injected metabolic labeled ^{32}P-VG into the hemocoel of vitellogenic females and the oocytes were analyzed by ion exchange chromatography. Chorionated oocytes, 48 hours after injection, showed two peaks of radioactivity on DEAE column. The first peak corresponding to the elution of VT_1 and the second corresponding to VT_3. Oocytes analyzed 24 hours after injection were divided in two groups: (1) non-chorionated oocytes less than 1mm in lenghts showed only one peak of radioactivity associated with the fraction that corresponded to $VT_1$and (2) non-chorionated oocytes bigger than 1 mm in lengths where we observed VT_1, VT_2 and VT_3 but only a single peak of radioactivity which comigrate with VT_1. Recently, Melo et al. (1998) showed that besides fat body, the follicles cells are also a site of synthesis of VG. In order to investigate which of the VT isoform is that synthesized in the follicular epithelial cells, we incubated them in culture medium enriched with [^{32}Pi] or [^{35}S]- methionine and separated the secreted proteins on a DEAE column. We observed that the major radioactive peak labeled corresponds to VG. Taken together these results suggest that VG taken up by the oocytes suffer a post-endocytotic modification forming VT_1 and possibly VT_2 correspond to the isoform synthesized by follicles cells, but this still requires to be proved.

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VE-42
INFECTION OF FED AND STARVED *RHODNIUS PROLIXUS* (TRIATOMINAE) BY *BEAUVERIA BASSIANA* (HYPHOMYCETES)

Christian Luz
Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brasil, e-mail: wolf@ipe.ufg.br

Rhodnius prolixus is a serious vector of Chagas disease in Colombia and Venezuela. Hyphmycete fungi which invade hosts via the integument are good potential candidates for microbial control of sucking arthropods, and have been reported to be pathogenic to *R. prolixus* and other triatomine species. Abiotic and biotic factors can interfere with the host-pathogen relationship. Nutritive status may affect triatomine susceptibility to infection with fungi and have an important impact on effectiveness of biological control. Susceptibility of *R. prolixus* first instar nymphs to infection by *Beauveria bassiana* was studied on fed and on starved individuals at different points of nymphal interphase. Insects were found to be less susceptible to fungal infection when treated one day before molting. Recently molted, fungus-treated second nymphs, were as susceptible to infection as unfed or fed, but not molting first instar nymphs. Prolonged starvation had no effect on insect susceptibility to the fungus.

VE-43
MAINTENANCE THROUGH FIFTY GENERATION OF *ANOPHELES AQUASALIS* AND *AN. ALBITARSIS* (DIPTERA: CULICIDAE) USING FREE MATING IN LABORATORY CONDITIONS: EVALUATION OF THREE DIFFERENT FISH FOOD FORMULATIONS FOR LARVAE DEVELOPMENT

Da Cunha MP, Pereira ERR, Manso PPA, Quintana M, Lima JBP
US Army Medical Research Unit, Instituto de Biologia do Exército, Rua Francisco Manuel 102, Rio de Janeiro, RJ, Brasil

In the present study we evaluated three fish food products (Tetramin, Tetramin L and Tetraruby) for maintenance of Neotropical *Anopheles* colonies. During the trial larvae mosquitoes of the species *Anopheles albitarsis* sensu stricto Linch Arribalzaga) and *Anopheles aquasalis* (Curry 1932), were feed with different combinations of the products and allowed to mature and mate. We then evaluated each group for percentage of adult females inseminated the average number of eggs produced, percent of eggs hatched and survivability of larvae and adults. Our finding suggest the *Anopheles albitarsis* reared on a combination of Tetramin plus Tetramin L performed the best on the evaluation criteria, while *Anopheles aquasalis* did the best when feed a mixture of Tetramin plus Tetraruby.

VE-44
MICROCLIMATIC PROPERTIES OF DOMESTIC AND PERIDOMESTIC TRIATOMINE HABITATS IN NORTHERN ARGENTINA

Lazzari CR, Gürtler RE, Canale D*, De Marco R, Lorenzo MG
Departamento de Cs. Biológicas, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina *Servicio Nacional de Chagas, Córdoba, Argentina, e-mail: lazzari@bg.fcen.uba.ar

Several attempts have been made to find a reliable climatic indicator of the distribution of triatomines, in particular to predict changes originated in regional or global climate modifications. Given the particularities of the places that bugs inhabit, this focus should be complemented with two additional approaches. First, what microenvironmental conditions bugs actually experience in the nature and, second, the conditions that insects actively prefer should be established. Concerning the first, we measured the microclimatic conditions inside structures that *T. infestans* usually occupies in an endemic region of Argentina (Amamá, Prov. Santiago del Estero), during December 1997. Sensors associated to data-loggers simultaneously recorded every 12 min during 15 days, the temperature and relative humidity (RH) in a, a house veranda (i.e., external reference); b, bedroom thatched roof (interior); c, corral roofing (a mixture of earth and thatch); d, within a crack in a brick wall and e, inside a hollow branch of a tree located inside the corral enclosure. In the refuges (i.e., b, c, d and e) both variables suffered damping and delay in reaching extreme values, but in a different way. With the exception of the hollow branch, no statically significant difference occurs among the average temperature occurred outside and in the different structures (range 20°-40°C, one-way ANOVA followed by Tukey test). In contrast, a marked asymmetry occurred in the dynamics of RH changes. Every increase in external RH was accompanied with certain delay by the RH inside refuges, which reached maximal values rather lower than the external RHs. In contrast, decreases in the local RH were followed almost immediately by RH inside refuges, which reached values relatively closer to the external minima. This phenomenon results in that, with the exception of the hollow branch, all other potential refuges maintained on average, statistically significant lower RHs than the external environment (range 22-100%). Although surprising, these results are remarkably consistent with the spontaneous preference of *T. infestans* (the main vector in that area) for dry environments, as determined in experimental trials, and the complex pattern of thermopreference exhibited by this species.

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VE-45
MOLECULAR STUDIES OF GEOGRAPHICAL POPULATIONS OF *LUTZOMYIA WHITMANI* (DIPTERA, PSYCHODIDADE, PHLEBOTOMINAE) BY RAPD-PCR

Margonari C, Fortes Dias CL*, Dias ES
Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, Belo Horizonte, MG, Brasil *Fundação Ezequiel Dias, 30510-010 Belo Horizonte, MG, Brasil

Phlebotominae or sand flies are proven vectors of both visceral and cutaneous leishmaniases. *Lutzomyia whitmani* is a sand fly species belonging to the subgenus *Nyssomyia*, that has been described as prevalent in certain loci of leishmaniases where the transmission is evident. However. populations of *L. whitmani* from geographical regions

show subtle variations in morphology, suggesting the existence of a complex of species.The exact specific identification is fundamental in epidemiological studies as behavioral and/or physiological differences may interfer with the vectorial potentiality of a given population. A complementary tool for individual identification comprises DNA analysis due to its high specificity. The purpose of this work is to evaluate the taxonomic status of *L. whitmani* by analysing different geographical populations using RAPD-PCR. Genomic DNA was extracted individually from fifteen males and fifteen females from four different Brazilian localities: Ilhéus-BA, Corte de Pedra-BA, Baturité-CE and Martinho Campos-MG. The samples were submitted to PCR amplification with a series of twenty random primers. After DNA fingerprinting analysis on PAGE silver-stained, two of them were selected as good for specimen discrimination. By now, individual fingerprintings are been analysed to determine intra and inter-specific polymorphisms in two out of the four populations under study.

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VE-46
MORPHOLOGIC STUDY OF WINGS OF *TRIATOMA JURBERGI* CARCAVALLO, GALVÃO & LENT, 1998 (HEMIPTERA: REDUVIIDAE: TRIATOMINAE) SEEN BY SCANNING ELECTRON MICROSCOPY (SEM)

Bulhões DM, Jurberg J, Carcavallo RU, Vale BS*
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos *Departamento de Patologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Triatoma jurbergi is the 113th Triatominae species described in the Americas. It was previously considered as *T. guazu* Lent & Wygodzinsky, 1979, but after being correctly morphologically studied in adults of both sexes and nymhs from two samples reared at the LNIRTT Insectary, it was concluded that they are two different and taxonomicaly easy to separate species (Carcavallo, Galvão & Lent, l998). Looking for new tools to facilitate the distinction and differentiation between species of the genus *Triatoma* Laporte, 1832, we studied the fore wings of males and females of *T. jurbergi*, both right and left wings, and internal and external surfaces of the hemelytron. For these studies we used an scanning electron microscope, using different magnifications. It was possible, in this way, to find some interesting structures for morphologic or taxonomic puposes. Specimens used in this study were reared in the Insectary of the National and International Reference Laboratory for Triatominae Taxonomy of the Dpt. of Entomology, Institute Oswaldo Cruz. In the ventral side of the chorium there exists an interesting structure, the coaptor system, formed by something remaining a comb, the ctenidium, integrated by 26 teeth-like spines, somewhat separated at the apex; they cover a depression where the costal vein of the hind wing takes place and having in front a rugose protuberance. On the dorsal surface, the base of wing is rugose with two different structures: long and acuminated spines, and short and rounded tubercle-like saliences, with a few setae; the tegument has rounded projections. Near the costal vein there are perfilated long setae.

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VE-47
MORPHOLOGICAL STUDY OF THE THORAX OF THE FIVE INSTAR NYMPHS OF *TRIATOMA ATHURNEIVAI* LENT & MARTINS, 1940

Rosa JA, Medeiros MP, Rodrigues VLCC*, Cilense M**
Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Caixa Postal 502, 14801-902 Araraquara, SP, Brasil *Superintendência de Controle de Endemias (SUCEN), Mogi-Guaçú, SP, Brasil **Instituto de Química, Universidade Estadual Paulista, Caixa Postal 355, 14801-970 Araraquara, SP, Brasil

T. arthurneivai is a type of Triatominae, that can be found distributed in the states of Bahia, Minas Gerais and São Paulo. It was used in this study copies servants in laboratory picked from a colony collected in 04/24/81 in the farm Santa Teresa, in the municipal district of Espírito Santo of Pinhal - SP. The study of the number of pleural faces were driven in scanning electron microscope JEOL model JSM-T330A. The 2nd nymph instar of *T. arthurneivai* is distinguished from the 1st nymph instar through the tubercles presence and hairless areas in the pro, meso and metatorax. The distinction of the 2nd and 3rd instar are made by the beginning of the formation of the alares fins in the nymphs of 3rd instar. The nymphs of 4th are characterized by the expansions of the alares fins and for the partial projection of the previous alar fin on the posterior. relationship to the nymphs of 5th instar, the main distinction is for that the previous alar fin to cover again almost that totally the posterior, so that it is noticed only vestiges of the internal lateral portions and of the points of the posterior alares fins. In the lateral faces two (1+1) openings were observed in the medial portion of the 1st pleurito, close to the limit with the 2nd pleurito. It felt that such openings are glandular for they are similar to the openings of the glands of Brindley. It wasn't found in the literature consulted references about these openings.

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VE-48
MORPHOLOGY OF PHEROMONE GLAND PORES IN MALE *LUTZOMYIA CRUZI* INSECTS (DIPTERA: PSYCHODIDAE)

Spiegel CN, Brazil RP* , Santos SO** , Soares MJ
Lab. Biologia Celular de Microrganismos, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, 21045-900 Rio de Janeiro, RJ, Brasil *Lab. de Leishmanioses, Centro de Pesquisas Reneé Rachou, Fiocruz, 30190-000 Belo Horizonte, MG, Brasil **Fundação Nacional de Saúde, Campo Grande, MS, Brasil

Sex pheromones are widespread in insects and appear as an interesting option for biological control to vectors of some human diseases, such as leishmaniasis. In this sense, development of pheromone traps can be considered as an alternative to inseticides. Several *Lutzomyia* species have been described to possess a sex pheromone disseminating structure (Ward *et al*. Bull. Entomol. Res., 83: 437-445, 1993), among them *L. longipalpis*, vector of *Leishmania chagasi*, the causative agent of visceral leishmaniasis in the New World. The presence of pheromone glands is associated to pale tergal spots on some abdominal segments. The chemical nature of these pheromones has been broadly classified as farnasene/homofarnasene-like and dipertenoid-like, with the function of attracting flies to common mating and feeding sites.

Pheromone glands in insects are invariably associated with cuticular pores, which allow transport of the pheromone from the gland to the cuticle surface. The cuticle may be adorned with a variety of architectural modifications, allowing a greater surface area for pheromone evaporation. In this study, we have analyzed the external morphology of the pheromone glands of *Lutzomyia cruzi* male insects, a phlebotomine species closely related to *L. longipalpis*, which has been incriminated as a vector of visceral leishmaniasis in Mato Grosso State, Brazil.

Examination of the insects under the stereomicroscope showed that the pheromone openings are restricted to a pair of pale spots at the fourth male tergites, where macrotrichia are absent. The fine architecture of the gland openings was further analyzed by scanning electron microscopy. The openings present a central pore of about 200 nm and at least two different types could be observed: the first one appears as round elevations of the cuticle, while the second one presents a segmented halo around the central opening. Further studies of the gland morphology are being carried out by scanning and transmission electron microscopy.

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VE-49
MORPHOMETRIC ANALYSIS OF COLOMBIAN POPULATIONS OF *RHODNIUS PALLESCENS*

Caro-Riaño H, Mejia E, Moreno J, Jaramillo N
Departamento de Biología, Universidad de Antioquia. A.A. 1226. Medellín, Colombia

In order to analyze the genetic relationship between sylvatic and domestic populations of *R. pallescens*, 22 morphometers of the head, thorax and wings from 270 adult insects taken from laboratory colonies were studied. Thus, the variability caused by the populations original environment was eliminated, permitting only the products of genetic variation to be observed.

Four sylvatic populations from both the North Coast and the Central West of Colombia, and an additional domestic population from the Central region of Panamá were used. Another domestic colony of *Rhodnius prolixus* originating from the Colombian Eastern Plains region was used as an external group. The geographical distances of the places where the insects were obtained are longer than 50 km and have different ecological characteristics.

The statistical analysis was carried out on males and females independently, and a significant sexual dimorphism detected by means of ANOVA was also taken into account. From the data transformed to natural logarithms, a Principal Components Analysis (PCA) was performed. The coefficients of PCA (size and shape orthogonal factors) were used to pursue a Canonical Discriminant Analysis (CDA) which allows the differentiation of natural groups from the studied places.

The results showed a total morphological separation in both studied species; however, no significant *R. pallescens* inter-colonies differences were detected.

The colony originating from the Central West of the country showed a light tendency towards separation, different from the colonies coming from the North Coast which overlapped with the one coming from Panamá. The Colombian Central West region ecological characteristics are very different from both the North Colombian North Coast and the Central Region of Panamá. These results were confirmed by analyzing a UPGMA phenogram of Mahalanobis distances from CDA.

These results are of great importance since the *R. pallescens* Colombian colonies are genetically very close to the Panamanian, and is already domiciled. In addition, all of them are placed in altered ecosystems due to human action in the sylvatic niche. Man has been cutting down the palms to use the land for livestock. Therefore, the natural reservoirs are being eliminated. This proposes the risk that Colombian *R. pallescens* will also domicile.

VE-50
MORPHOMETRIC STUDY OF NYMPHS OF *TRIATOMA GUAZU* LENT & WYGODZINSKY, 1979

Silva MBA, Jurberg J, Galvão C, Silva LF
Departamento de Entomologia, Laboratorio Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Jeneiro, RJ, Brasil

Nymphs and eggs of this species were analysed to improve its taxonomic and morphologic concept. The original strain was collected in Barra da Garças, Estado de Mato Grosso and adapted to be reared under laboratory conditions at the Insectary pf the National and International Reference Laboratory on Triatominae Taxonomy, Department of Entomology, Institute Oswaldo Cruz. For morphometric studies were used 10 eggs and 10 nymphs of each developmental instar. Results.

	Lenght of head			Width of ocular region			Posocular region		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
instar I	1,309	1,124	1,214	0,616	0,539	0,587	0,354	0,231	0,299
instar II	1,848	1,617	1,729	0,847	0,755	0,785	0,462	0,308	0,389
instar III	1,303	0,999	1,105	0,545	0,455	0,485	0,333	0,242	0,297
instar IV	1,606	1,182	1,500	0,666	0,515	0,612	0,454	0,303	0,385
instar V	2,363	1,999	2,127	0,909	0,818	0,845	0,545	0,454	0,491

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VE-51
MOSQUITO EMBRYOS AND EGGS: POLARITY AND TERMINOLOGY OF CHORIONIC LAYERS

Valle D, Monnerat AT, Soares MJ*, Rosa-Freitas MG, Pelajo-Machado M***, Vale BS***, Lenzi HL***, Galler R, Lima JBP**
Departamento de Bioquímica e Biologia Molecular *Departamento de Ultraestrutura e Biologia Celular **US Army Medical Research Unit, Instituto de Biologia do Exército, RJ, Brasil ***Departamento de Patologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

The development of genetically modified vectors refractory to parasites is seen as a promising strategy in the future control of endemic diseases such as malaria. Nevertheless, knowledge on mosquito embryogenesis, a prerequisite to the establishment of transgenic individuals, is still incipient.

We have studied some aspects of neotropical malaria vectors embryogeny. Eggs from *Anopheles* (*Nyssorhynchus*) *albitarsis* and *Anopheles* (*Nyssorhynchus*) *aquasalis* were analyzed by Laser Scanning Confocal and Scanning Electron microscopies and compared to those of *Drosophila melanogaster*. We verified basic conflicting data such as mosquito egg polarity and ultrastructure of eggshell layers. A 180^{o} rotation movement of the mosquito embryo along its longitudinal axis, a phenomenon not conserved among all Diptera, was confirmed. As a consequence of this movement, that does not occur in *Drosophila*, the mosquito larval ventral side faces upwards at hatching, bordering the flattened dorsal side of the egg. This early event is not taken into account by several present groups, leading to a non-consensual assignment of egg dorsal and ventral poles.

The structure of *Anopheles* eggshell was also examined. Scanning Electron Microscopy was used to tentatively solve some inconsistencies of the literature about structural aspects of mosquito eggshell layers. Based on the zoological convention used to define embryo membranes, we consider the existence of two chorion layers in mosquito embryos, a smooth inner chorion layer, or endochorion and a compound exochorion, or outer chorion layer, this later assembled by a thin basal lamellar sheet and external tubercles. The basal lamellate sheet of the exochorion, contiguous to the endochorion, and the tubercles arising from it are common to both dorsal and ventral sides of mosquito egg. However, the ventral side is more uniform, exhibiting an array known as plastron. Dorsal tubercles and the dorsal side of floats and frill appear to plunge directly and individually into the basal lamellar sheet of the exochorion. In contrast, at the ventral side all these structures seem to be continuous on both *Anopheles* species analysed.

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VE-52
NATURAL INFECTION OF *LUTZOMYIA PESSOAI* AND *LU. MISIONENSIS* BY *LEISHMANIA BRAZILIENSIS S.L.* IN RIO GRANDE DO SUL, BRAZIL

Silva OS, Grunewald J
Institute of Tropical Medicine of the University, Wilhelmstrasse 27, D-72074 Tuebingen, Germany (e-mail: onilda.silva@student.uni-tuebingen.de)

American Cutaneous Leishmaniasis (ACL) is endemic in Rio Grande do Sul. As reports of nearby hospitals suggest, the "Parque Estadual do Turvo" seems to serve as a reservoir for the disease.

During three months (November 1996 to February 1997) 2,228 sandflies were collected in this place (10 *Lutzomyia* spp. and 2 *Brumptomyia* spp.) PCR probes were applied in 920 females which belong to species *Lutzomyia migonei*, *Lu. pessoai*, *Lu. fischeri*, *Lu. misionensis*, *Lu. lanei*, *Lu. neivai*, *Lu. shannoni*, and *Lu. monticola* to verify the natural infection by *Le. braziliensis s.l* the causative agente of ACL.

Two oligonucleotide primes (GAACGGGGTTTCTGTATGC and TACTCCCCGACATGCCTCTG) were employed to amplify the conserved region of minicircle kinetoplast. After the morphometric identification, the females were immersed individually in 50l lysis buffer containing Proteinase K and incubated at 65 °C for three hours for DNA extraction. The PCR master mix were prepared with 5U/µl of Taq Polimerase, 2,5 mM from each of dATP, dCTP, dTTP, dGTP, 70 mM Tris-HCl 0,5M (pH 8,8), 17 mM $(NH_4)_2SO_4$, 0,1 mM 2-Mercaptoethanol, 2mM $MgCl_2$, 1 µM from of each oligonucleotide. For the amplification 5 µl of DNA from a pool of 10 females was added to 15 µl of the above-described solution. Amplified products were analysed by eletrophoresis agarose gel (3,5%), stained with ethidium bromide and visualized under UV light.

In two *Lu. pessoai* and one *Lu. misionensis* female *Le. braziliensis* s.l. was proved by identification of the 70 bp of *Le. braziliensis* DNA. *Lu. pessoai* is a known vector of *Le. braziliensis* in other endemic areas of north-eastern and south-eastern Brazil, where it can be found in and near houses. However, *Lu. misionensis* was never been found carying natural infection of *Le. braziliensis* before.

VE-53
OBSERVATIONS ON *RHODNIUS PROLIXUS* STAL, 1859 FENOTYPE BY CAPTIVITY INFLUENCE (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

Galíndez Girón I, Torres E, Gonzalez R, Lizano E, Barazarte R, Marquez J, Matos E
Universidad de Los Andes, Núcleo "Rafael Rangel", Laboratorio "Biología de Triatominae", La Concepción, Trujillo, Venezuela. Fax: (72)362177, e-mail: glourdes@cantv.net.

The purpose of this study was to analyze the flexibility of *R. prolixus* fenotype by captivity influence by means of Multivariate Analysis. The samples consisted in two groups of insects. A group was selected from a laboratory collection prepared with intradomiciliar insects captured in 1970 in Caballito locality, Lara, Venezuela (ubication unknown). The second group was selected from laboratory colony, descendent from those, in 1995. Twenty-two quantitatives variables were measured on 30 male specimens and the same number of females of the both two samples. Phenotypic variability was determined employing the Principal Components Analysis and the 100% of individuals were properly classified by means of the Euclidean Distances using 30 Nearest Neighbors Squared Distance Function. The results suggest that rearing or microhabitats conditions leads to variations of the phenotype of *R. prolixus*.

VE-54
OBSERVATIONS ON THE BIOLOGY OF PHLEBOTOMINE SANDFLIES COLLECTED IN THE EASTERN ATLANTIC PLUVIAL FOREST IN PARANÁ STATE, BRAZIL (DIPTERA, PSYCHODIDAE, PHLEBOTOMINAE)

CB Marcondes, Santos-Neto LG*, A Lozovei L**
Departamento Microb. Paras., CCB, UFSC, Campus Trindade, 88040-900 Florianópolis, SC, Brasil *Departamento de Zoologia, SCB, UFPR, Centro Politécnico, Curitiba, PR, Brasil - Graduation student (Entomology) **Departamento de Patologia Básica, SCB, UFPR, Centro Politécnico, Curitiba, PR

Phlebotomine sandflies were collected by CDC-like light traps in Atlantic primary forest in the nature reserve of IAPAR, at the Municipality of Morretes, State of Paraná (25º 28' 37" S, 48º 50' 04" W, 10 m a. s. l.), one night/month, from April 1995 to March 1996. Samples were obtained in the soil and in the canopy levels, and the insects of each two-hour period were separated.

Lutzomyia (Psychodopygus) ayrozai (1510 insects) and *Lu. (P.) geniculata* (779) were the most common species, besides *Lu. fischeri* (354), *Lu. pascalei* (55), *Lu. shannoni* (44), *Lu. lanei*, *Lu. monticola*, *Lu. migonei* and *Brumptomyia nitzulescui*, in a total of 3106 specimens.

The study of the seasonal fluctuations showed a lower number of insects in the drier and colder months. The study of the variations of collected quantities during the night in the seasons showed different peak periods for species, depending on the level and on the season. The study of the fluctuations of the insects of each species could be useful to understand their biology and possible epidemiological role.

Man-biting and occurrence in foci of dermal leishmaniasis were previously observed in some of the species (*L. ayrozai*, *L. fischeri* and *L. shannoni*). The finding of *Lu. geniculata* at Morretes is the first for the State of Paraná. Our samples did not include some species collected by Gomes & Galati (1987) and Gomes et al. (1989) in primary forest of Ribeira River Valley, in the South of São Paulo, mostly *Lu. intermedia s. l.*, possibly due to the latitude and/ or to some unknown ecological difference between the areas. The absence of *L. neivai*, which is common in deforested areas in the East of Santa Catarina, could probably be atributed to the primary vegetation in the area of Morretes.

Leishmania enrietti and *Le. braziliensis* were recently isolated, respectively, from rodents of São Paulo and Paraná States (Machado et al., 1994; Thomaz-Soccol et al, 1996), and from a human patient of the north-eastern region of Santa Catarina (Steindel et al., 1998). These localities are situated near the Morretes region. It is possible that some *Leishmania* are isolated from this region in the future, and the knowledge of the sandfly fauna is very important.

VE-55
ON THE IDENTITY OF *LEISHMANIA HERRERI* AND ENIGMATIC *ENDOTRYPANUM* PARASITES INFECTING SLOTHS AND PHLEBOTOMINE SANFLIES FROM SYLVAN AREAS OF THE NEOTROPICS

Franco AMR, Moreira CFS, Santamgelo A, Medina-Acosta E, Schjizman A, Grimaldi Jr.G
Departamento de Imunologia, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Universidade Estadual do Norte Fluminense, Campos, RJ, Brasil **Instituto de Investigaciones en Ingenieria Genética y Biologia Molecular (Ingebi), Buenos Aires, Argentina

Leishmania herreri was originally isolated from two (*Choloepus hoffmanni*) and three-toed (*Bradypus griseus*) sloths, and sandflies (*Lutzomyia trapidoi, Lu. ylephiletor* and *Lu. shannoni*) in Costa Rica (Zeledon et al. 1979, *J Parasitol 65*: 273)). Recent phylogenetic studies have demonstrated that *L.* (*L.*) *herreri* and *L.* (*L*). *hertigi*, as well as two other enigmatic new leishmanial species, *L.* (*V.*) *colombiensis* and *L* .(*V.*) *equatorensis*, claimed to be genetically closer to *Endotrypanum* than to the other *leishmania* species (Noyes *et al.* 1996, *Mol Biochem Parasitol 80*: 119; Cupolillo et al. 1998, *Mem Inst Oswaldo Cruz 93*: In Press).

Taxonomic studies of *Endotrypanum* isolates from the Americas indicate genetic diversity among these microorganisms (Lopes et al. 1990, *Mol Biochem Parasitol 40:* 151). As a result of using numerical zymotaxonomy for classifying these organisms (Franco et al. 1996, *Parasitology 113*: 39), the taxonomic horizon of *Endotrypanum* spp. has been widened. Here we have used the following criteria (*i*) isoenzyme electrophoresis, (*ii*) reactivity patterns with specific monoclonal antibodies, (*iii*) restriction-endonuclease fragment patterns of kinetoplast DNA (k-DNA), (*iv*) measurement of the sialidase activity, and (*v*) analyses of phylogenetically informative nuclear DNA sequences using Touch-Down PCR with primers from kinetoplastid housekeeping genes (ribosomal P proteins and repetitive sequences, SIRE) to examine relationships among *Endotrypanum* (N= 22 strains, comprising the major taxa of this genus) and three *L.* (*L.*) *herreri* strains (ISHA/CR/74/LV341 and IYLE/CR/74/LV342, both isolated from sand flies, and MCHO/CR/74/LV344, isolated from sloth), to see whether these approaches could be used to differentiate further among these parasites. The results revealed differences among the leishmanial strains analyzed, but showing closed genetic similarity between strain LV342 and some *Endotrypanum* parasites representative of the most recent group (group A, see in Franco et al. *loc. cit.*). However, leishmanial strains LV341 and LV344 have different characteristics when compared with all *Endotrypanum* stocks analyzed. In conclusion, the results confirm previous studies (Noyes et al. *loc. cit.*; Cupolillo et al. 1998, see accompanying abstract) considering *L. herreri* as an heterogenous group of parasites showing distinct levels of genetic similarities with some *Endotrypanum* parasites. Comparative phylogenetic reconstruction studies are in progress to address the evolutionary links among these related kinetoplastid lineages.

VE-56
PHYSIOLOGICAL ROLE FOR HEMOZOIN SYNTHESIS IN *RHODNIUS PROLIXUS*

Oliveira MF, Timm BL, Sorgine MHF, Oliveira PL
Departamento de Bioquímica Médica, CCS, UFRJ, Rio deJaneiro, Brasil

Blood sucking arthropods use hemoglobin as the major food source and its digestion releases a great amount of its prosthetic group heme. Free heme molecules are toxic and promote oxidation of a variety of biomolecules such as lipids, proteins and carbohydrates. Furthermore, at milimolar range, heme is able to associate to phospholipid bilayers causing alterations in its structure, resulting in cell lysis. Many systems were developed through the course of evolution to avoid the deleterious effects of heme. In *Plasmodium* parasites, the main defense against heme toxicity is its sequestration into a insoluble brown pigment named malaria pigment or hemozoin.

In a previous work we isolated and identified hemozoin in the blood sucking bug *Rhodnius prolixus*. In this work we verified some of the physiological aspects of the hemozoin synthesis in *R. prolixus*. Chloroquine, a 4-aminoquinoline antimalarial drug, inhibited the heme polymerisation reaction induced by perimicrovillar membranes *in vitro*. Furthermore, when adult insects were fed with rabbit blood suplemented with different concentrations of chloroquine, there was an inhibition of hemozoin synthesis *in vivo* in a dose-dependent way. At 5 mM, Chloroquine inhibited more than 95 % the hemozoin production inside of the midgut. The production of thiobarbituric acid reactive substances (TBARS) induced by hemin (Hm), b-hematin (bH) and *Rhodnius* hemozoin (rHz) was analysed using two different subtrates for oxidation: linolenic acid and 2-deoxyribose. Hemin was able to yield high amounts of TBARS in both substrates, while ?H and rHz produced expressively less TBARS than hemin. However, when bH and rHz were depolymerized with the addition of NaOH 0,1N, the levels of TBARS production returned to those of hemin. These results strongly suggest that hemozoin synthesis in the midgut of *Rhodnius prolixus* represent an efficient way to avoid heme toxicity.

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VE-57
PRELIMINARY ANALYSIS OF EXTERNAL MORPHOLOGY OF *RHODNIUS BRETHESI* MATTA, 1919 BY SCANNING MICROSCOPY (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

Ferro ZPA, Junqueira ACV*, Moreira CJC*, Spata LM*, Silva LM**, Gonçalves TCM
Departamento de Entomologia, *Departamento de Medicina Tropical, **Departamento Ultra-Estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Rhodnius brethesi is a sylvatic species which has been described in "piassaba palms"(*Leopoldina piassaba* Wallace) from Brazil (Amazonas and Pará) and Venezuela. It takes blood in animals seeking shelters and has also report attacking humans during harvesting palm fiber (Lent & Wygodzinsky, 1979). Previous taxonomic studies regarding this species were based on optical microscopy (Matta, 1919; Lent & Jurberg, 1969). In the present study the scanning electron microscopy was used to demonstrate ultraestructural aspects of external morphology with taxonomic purpose. Specimens of *R. brethesi* were collected in Barcelos district, located on the right bank of "Rio Negro", on the northern part of the State of Amazon, Brazil (Coura et al., 1994) and subsequently maintened in the bug colony at the Tropical Medicine Department, Oswaldo Cruz Fundation. The insects were placed individually into Borrel flasks and submitted to starvation until death. Micrographs were performed in a Digital Scanning Microscope (DSM-940, Zeiss). The head, thorax and legs of the first and fifth instar nymphs and adult specimens were analysed. Concerning to morphological characters in the head, it could be observed a granulose cuticle, a trichobotria on the second antennal segment, labrum with star-like structures. The thorax shows a stidulatory sulcus with horizontal parallel bars. Other structures are being analysed in the same instars as well as other instar nymphs.

VE-58
RESEARCH ON PHLEBOTOMINAE (DIPTERA: PSYCHODIDAE) IN A DISTRICT CALLED "BAIXADA DE JACAREPAGUÁ", IN THE COUNTY OF RIO DE JANEIRO, RJ, BRASIL

Carreira- Alves JR, Cunha SP, Souza MB*, Meira AM, Ponte CS, Andrade MV
FNS, RJ, DSRJ, Laboratório de Entomologia, Av. Pedro II, nº 278, 20941-070, Rio de Janeiro *DCB, Ensp, Fiocruz (e-mail: scunha@mandic.com.br)

"Baixada de Jacarepaguá" is a place located in the west zone of a county of Rio de Janeiro, between Pedra Branca and Tijuca hills, with the Atlantic Ocean in the south. It covers the administrative regional offices of Jacarepaguá and Barra da Tijuca districts. Cases of cutaneous leishmaniasis have been happening in this region since the 80's. Aiming at Keeping the fauna of sandflies updated as well as providing a more effective entomological observation, mainly concerning the evaluation of the watering of insecticidal products, two monthly four-hour captures were done in 06 (six) distinct areas, from October 1996 through October 1997. A total of 2,707 sandflies were captured and identified, such as : *Lutzomyia intermedia, L. migonei, L. firmatoi, L. pelloni, L. cortelezzii, L. fischeri* and *Brumptomyia nitzulescui*. The most abundant species were *L. intermedia*, with a frequency of 87,2 % , and *L. migonei*, with a frequency of 6 % out of the total. All of them were captured in the peridomestic environment. *L. intermedia* is considered one of the main vector of LTA in the Southeast region (Rangel et al., 1984; 1986; Rangel, 1995). Owing to its anthropophily, *L. migonei* is also suspected of transmitting this disease in the meridional region of the south- American Continent. (Forattini, 1973). It is important to state that even with 3 notices of visceral leishmaniasis cases, which happened in 1994 and 1995, *L. longipalpis* was not collected during the period we wored there.

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VE-59
RESISTANCE TO STARVATION OF *TRIATOMA MELANOSOMA* MARTÍNEZ, OLMEDO & CARCAVALLO, 1987 (HEMIPTERA, REDUVIIDAE)

Galvão C, Rocha DS, Carcavallo RU, Jurberg J, Cunha V
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

The resistance to starvation on triatomine bugs is know since long ago, according to Neiva & Lent, 1936, citing the observation of Darwin who maintained a specimen four months without any meal. The epidemiologic importance of that condition was pointed out by Pellegrino (1952) remaining that under adverse conditions those insects can move looking for a new favourable environment. Dias (1965) considered that resistance to starvation is an important survival strategy for domiciliated species that can wait in inaccessible places during the ective period of residual insecticides. The objective of this paper is to determine periods of starvation resistance for all the stages of *Triatoma melanosoma* Martínez, Olmedo & Carcavallo, 1987, species originally described as subspecies of *Triatoma infestans* (Klug, 1834) and later included in a specific complex that include *T. infestans, T. delpontei* Romaña & Abalos, 1947, *T. platensis* Neiva, 1913 and some forms "dark-mophs" recently found in the East of Bolivia by Noireau. Fifty eggs and fifty nymphs of each stage were selected from the LNIRTT Insectary, and grouped according to their stage. After hatching or moulting, insects were individualized in Borrel's flasks, maintained in climatized chamber at 28ºC +/- 1ºC and 80% +/- 5% of RH, and observed until they died. The mean values of survival for starvation were: 49.7 days for nymph I, 58.9 for nymph II, 91.5 for nymph III, 106.6 for nymph IV, 125.6 for nymph V, 51.5 days for adult males e 47.9 days for adult female. If we compared these results with other under similar conditions performed with other species, we find that *T. melanosoma* is one of the most resistent *Triatoma* species, surviving more than *T. brasiliensis* studied by Costa & Perondini (1976) and Costa & Marchon-Silva (1996), than *T. infestans* observed by Perlowagora-Szumlewicz (1969), that *T. sordida* and *T. vitticeps* observed by Juarez & Silva (1982) e Silva et al. (1985). The mean resistance to starvation of *T. melanosoma* was only shorter than those observed in North and Central American species: *T. nitida, T. dimidiata, T. lecticularia* (Zeledón et al., 1970; Jurberg & Costa, 1989; Galvão et al., 1996).

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VE-60
SAND FLIES (DIPTERA: PSYCHODIDAE) IN AREA OF LEISHMANIASIS: DISTRIBUTION OF THE SPECIES IN DEFORESTED AREA IN THE AMAZONIA OF MARANHÃO

Rebêlo JMM, Oliveira ST, Barros VLL, Silva FS, Costa JML, Ferreira LA
Núcleo de Patologia Tropical e Medicina Social, Departamento de Patologia, Universidade Federal do Maranhão, São Luis, MA, Brasil

The distribution pattern of the sand flies in amazon area of Buriticupu-MA, regarding the deforestation levels and the time of occupation by the rural communities, is discussed. The sand flies were captured by means of CDC light traps, Shannon's trap and as well as aspiration tube, from 6 P.M. to 6 A.M., once a month, over two years, in the forests and at the peri and intradomestic environment. The works were carried in two areas, one of recent colonization, the population of Lagoas with less than 12 years of human occupation, and one area of early colonization, the population of P1V5 with over than 20 years of occupation. A total of 10.164 specimens were captured, being 9.392 in Lagoas distributed in 38 species and 772 in P1V5 distributed in 27 species. In Lagoas, the species were significantly more frequent in the forest environment, while in P1V5 there was no significative difference between the two habitats. Such differences derive from the variation of period of settlement both areas and the antropic modifications in the natural ecotopes of the vectors. The variations was more expressive in the early colonization. With regard to the species, *L. whitmani* was dominant in both Lagoa (59%) and P1V5 (38%), occurring in the wild environmten as well as in domestic surroundings.

VE-61
SAND FLIES IN STATE OF TOCANTINS, BRAZIL (DIPTERA, PSYCHODIDAE)

Andrade Filho JD, Valente MB, Alves JCM, Falcão AL, Brazil RP
Laboratório de Leishmanioses, Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil

Leishmaniasis are a group of chronic diseases caused by different species of the protozoan *Leishmania*. The transmission occurs through bite of females of some species of sandflies. The state of Tocantins was established in 1988, and is constituted for an area of 278,420,7 Km^2, where the vegetation predominant is cerrado and the climate

is tropical semi -humid. In order to know the sandflies of fauna this region entomological survey were realized in december 1997, in 19 areas of four main cities in the state: Porto Nacional (7 areas), Monte do Carmo (5 areas), Paraíso do Tocantins (5 areas) and Monte Santo (2 areas). The ecotops chosen for the catches were domestic animals shelters, orchard and forest. We used CDC light trap in first three cities and human bait in last one (Monte Santo). Up to now we identified 549 specimens, belonging to 19 species: *Lutzomyia longipalpis, L. whitmani, L. evandroi, L. carmelinoi, L. sordellii, L. antunesi* and *L. peresi, L. aragaoi, L. sallesi, L. micropyga, L. flaviscutellata, L. bacula, L. christenseni, L. saulensis, L. termitophila, L. hermanlenti, L. longipennis, L. claustrei* e *Lutzomyia* sp. Monte Carmo showed a greater diversity of species (17), while Paraíso do Tocantins had the largest number of the sandflies caught (245). Of the species caught in this project, *L. longipalpis, L. whitmani, L. antunesi, L. flaviscutellata* are known vectors of leishmaniasis in several regions of Brazil. The catches using human bait carried in Monte Santo showed lower number of sandflies (14) caught, being *L. antunesi* the predominant species with 56%. Of the 19 species identified, only *L. micropyga* had been previously notified in this state. New survey will be realized in Tocantins in order to obtain a more significant number of sandflies and better characterize the fauna of this region.

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VE-62
SCANNING ELECTRON MICROSCOPE STUDY OF THE DEVELOPMENTAL STAGES OF *LUTZOMYIA LONGIPALPIS,* A NATURAL VECTOR OF *L. CHAGASI*

Secundino NFC, Pimenta PFP
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, Belo Horizonte, MG, Brasil Fax(031) 295-3115 (e.mail: pimenta@netra.cpqrr.fiocruz.br and nagila@netra.cpqrr.fiocruz.br)

The *Lutzomya longipalpis* has medical and veterinary interests because is the insect vector of *Leishmania chagasi*, the causative organism of American visceral leishmaniasis. We used the scanning electron microscopy in order to observe and clarify aspects of each distinct developmental stage of the sandfly. Our results show that the eggs have an asymmetrical oval shape with concave and convex surfaces. The external surfaces of the eggshell are covered with an exochorion arranged in several protuberant single lines intercalated with smooth surfaces. The exochorion lines have holes on its base, appearing to connect with the interior side of the eggs. Cracked eggshells exposed a smooth interior surface without any specific structures. The larvae stages were distinguished by the presence of one or two pairs of caudal filaments. The pair with caudal filaments characterized the first insthar larvae. The larvae's head is well individualized and sclerotised with preeminent mouthparts, like the mandible and the teeth. Its body is long and divided into twelve segments which are easily visualized from the ventral side. The first three segments are the thoracic partss and the other nine are the abdominal parts. The abdominal segments are recognized by the presence of stumps or projecting structures on the dorsal surface called prolegs. The last two segments are characterized by the presence of caudal filaments and an anal segment on the end of the insthar body. The lateroventral surface is covered with brush-like hairs with the exception of the caudal segment that has one or two pairs of setae-like hairs. The pupae stage were recognized by the cocoon shape. In the late pupae stage, it is easy to observe the abdominal segments with numerous spiculates, the wings and the legs. These observations represent an initial study which will be the fundamental base for further taxonomic consideration, in order to compare possible anatomical differences between *Lu. longipalpis* from distinct geographical regions.

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VE-63
SENSIBILITY OF PRECIPITIN TEST IN SEVERAL TRIATOMINES SPECIES ACCORDING TO DIFFERENT FASTING PERIODS

Lorosa ES, Andrade RE, Pereira CA, *Gonçalves TCM
Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz (e-mail: lorosa@gene.dbbm.fiocruz.com.br) *Núcleo de Morfologia, Anatomia Interna e Ultraestrutura de Artropodos Vetores, Departamento de Entomologia, Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

In order to verify the sensibility of precipitin technique according to different fasting periods, 5º stage nymphs of several triatomines species were studied. These nymphs were fed in birds (*Columba livia*) for two hours and, after a fasting period of 20 days, in rodents (*Mus musculus*) for the same time. This was the last blood meal. The blood volume ingested by these nymphs was verified by weighting before and after each blood meal. During all the experiment, the nymphs were maintained in BOD at 28 C ± 2 C and 80% ± 4UR.

Using bird and rodent anti-sera, after a period of 45, 70, 90, 110, 120 and 130 days, the technique showed the following sensibility to anti-bird-rodent and antisera-rodent respectively. *Triatoma infestans* 90 and 120 days; *T.brasiliensis* 70 and 110 days; *T.vitticeps* 70 and 90 days; *Panstrongylus megistus* 90 and 110 days and *Rodnius prolixus* 40 and 70 days.

	Number of Days	
	Anti-bird-rodent	anti-rodent
Triatoma infestans	90	120
T.brasiliensis	70	110
T.rubrofasciata	110	120
T.costalimai	110	130
T.melanossoma	120	—
T.vitticeps	70	90
Panstrongylus megistus	90	110
Rodnius prolixus	40	70

Com auxílio da FNS, Fiocruz e European Commission Latin American Triatominae Research Network, Eclat.

VE-64
SOME ASPECTS OF THE BIOLOGY OF *TRIATOMA JURBERGI* CARCAVALLO, GALVÃO & LENT, 1998 (HEMIPTERA: REDUVIIDAE: TRIATOMINAE), UNDER LABORATORY CONDITIONS

Silva MBA, Jurberg J, Cunha V, Carcavallo RU, Galvão C
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil

Triatoma jurbergi was recently described from insects collected and sent by the Fundação Nacional de Saude and reared in the insectary of the National and International Reference Laboratory on Triatominae Taxonomy. The original colony is originated in the State of Mato grosso, Municipality of Barra do Garças. Authors of the present paper had the objective of determine certain parameters of the btological cycles, such as the duration of each developmental stage from hatching to adult stage and how often they have a blood intake. Insects were kept in temperatures between 18ºC and 33ºC and humidity between 35% and 96%. Sixty three eggs were taken off and separated in groups to start the experiment. After hatching, specimens were kept in Borrel flasks with its own identification, and fed on pigeon (*Columba livia*) once a week. Results. a) Duration of each instar nymph: 1st instar: 14 to 30 days; 2nd instar: 10 to 26 days; 3rd instar: 26 to 85 days; 4th instar: 25 to 110; 5th instar: 48 to 97. b) Number of blood intakes during each stage: 1st instar 2 to 4 times; 2nd instar: 2 to 4 times; 3rd instar: 4 to 12 times; 4th instar: 4 to 17 times; 5th instar: 6 to 14 times.This research is being followed.

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VE-65
SOME INSUFICIENTLY KNOWN CUTICULAR STRUCTURES OF THE FIRST INSTAR NYMPH OF *TRIATOMA GUAZU* LENT & WYGODZINSKY, 1979

Silva MBA, Jurberg J, Galvão C , Barbosa HS*, Carcavallo RU
Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos
*Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil

Authors show some structures found in first instar nymphs of *Triatoma guazu* Lent & Wygodzinsky, 1979. Specimens, previously metallized were studied with scanning electronic microscopy (SEM), with magnifications between X 340 and X 3,000. Areas mainly studied were the prosternum, the rostrum, the antenniferous tubercle and the antennae. In this paper, authors publish the following structures:

Prosternum: the central area is granulose with lines surrounding a central part plain and simple and presenting some rudimentary horizontal lines, as a future stridulatory sulcus. On the anterior edge there are two strong and long setae with aspect of sensilla.

Antenniferous tubercle: is very rugose and it has a foliar structure very granulose in the inside edge of the base, and two hairs very strong, of possible sensorial functions.

First antennal segment: it has sensillae on the distal half and a structure, near the edge, with aspect of volcano.

Articulation of second and third antennal segments: on the apex of the second segment a granulose fosse is evident with an only central sensilla inserted at the entrance of a central depression; two large lateral sensillae are inserted in respective round annular depressions. The surface of the third segment base remains a beehive.

Articulation between the third and fourth antennal segments: Strong setae or sensillae are seen on the edge of the third antennite, surrounding a pleated membrane of granulose surface on the base of the proximal globular sector of the fourth segment.

Authors think that these structures need to be studied in other stages of the same species and in other Triatominae genera and species.

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VE-66
STUDIES ON SAND FLY FAUNA (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) IN PRESERVED AREAS OF ATLANTIC FOREST, IN RIO DE JANEIRO STATE

Souza NA, Vilela ML Andrade-Coelho CA, Rangel EF
Departamento de Entomologia, Instituto Oswaldo Cruz, Avenida Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil

The aim of this study is to contribute to the knowledge on the ecology of sand flies in Rio de Janeiro State in areas with preserved Atlantic Forest sites. The Biological Reserve "Poço das Antas" and "Bom Retiro" Farm, both considered as biological reserves controled by the Brazilian Government, are located in the municipalities of Silva Jardim and Casimiro de Abreu, respectively, far about 120km from Rio de Janeiro City. The collections were carried out in the forest and around farm (peridomiciliary areas), using Shannon trap in peridomestic places and in the forest, and CDC light traps in domestic animal shelters and close to the holes of silvatic mammals. The results were obtained from July/1996 till May/1998, with average temperature ranged from 18º to 32ºC, 90% as relative humidity and 404 mm^3 annual rainfall.

Considering all types of captures, these species were identified: I. Biological Reserve "Poço das Antas"- *Lutzomyia edwardsi, L. shannoni, L. barrettoi , L. whitmani, L.ayrozai, L. hirsuta , L. monticola ;* II. "Bom Retiro" Farm - *Brumptomyia guimaraesi , L. longipalpis,L. migonei, L. fischeri, L.shannoni , L. barrettoi , L. whitmani, L. intermedia, L. hirsuta, L. monticola, L. misionensis* (first occurrence in Rio de Janeiro State). In the peridomiciliary surroundings, *L.longipalpis, L.migonei, L. whitmani, L. intermedia* and *L. hirsuta* were collected biting man. *L. whitmani* and *L.intermedia* have been considered as important vectors for cutaneous leishmaniasis in Southeast Brazil. However, *L. hirsuta* was found infected by *Leishmania (Viannia)* sp. too, in Além Paraiba, Minas Gerais State, and is highly anthropophilic in silvatic habitats.

VE-67
STUDY OF FEEDING SOURCES BY PRECIPITIN REACTION AND INFECTIVITY DEGREE IN *TRIATOMA RUBROFASCIATA* (DE GEER, 1773) COLLECTED IN SÃO LUIS ISLAND – MARANHÃO

Lorosa ES, Andrade RE, *Serra MGA, ** Rebelo JMM, ***Vinhaes MC
Laboratório Nacional e Internacional de Referência em Taxonomia de Triatonídeos, Departamento de Entomologia, Instituto Oswaldo Cruz *Gerência de Chagas FNS, São Luís, MA **Núcleo de Patologia Tropical e Medicina Social, Departamento de Patologia, Universidade Federal do Maranhão, São Luis, MA, Brasil ***Gerência Nacional do Programa de Controle da Doença de Chagas, FNS, Brasília, DF

From the 346 specimes of *Triatoma rubrofasciata* captured during the first semester of 1997 in São Luis Island, Maranhão, in domiciliary and peridomiciliary evironments 16,9% were infected by *Triapanosoma* sp and 283 had a single feeding source with 36,68% corresponding to rodent, 17,48% to dog, 10,31% to horse, 7,16% to opossum, 5,16% to human and 4,3% to bird. 34 specimens had more than one feeding source, dog-rodent 1,15%, bird-opossum 0,86%, human-bird 0,56%, bird-dog 0,29%, bird-rodent 0,29%, horse-rodent 0,29%, and 32% did not react with the antisera used in the imunologic reactions: human, bird, dog, cat, horse, goat/cow, pig, rodent, opossum and armadillo antiseras. It was observaded that none of the samples reacted with cat, goat/cow, pig and armadillo antiseras and the highest incidence of positive reactions occured with rodent antisera, followed by dog antisera in the simple reacitons. The same result was obtained in the double crossed reactions with rodent-dog and dog-opossum antiseras, demonstrading the feeding preference of this species for rodent. In this study, the identification of the feeding source of all specimens of *T.rubrofasciata* was determined by the precipitin test.

Com auxílio da FNS, Fiocruz e European Commission Latin American Triatominae Research Network, Eclat.

VE-68
STUDY OF THE PERITROPHIC MATRIX OF THE *LUTZOMYIA LONGIPALPIS,* A NATURAL VECTOR OF *LEISHMANIA CHAGASI*

Trycia Martins; Nágila F. C. Secundino and Paulo F. P. Pimenta*
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, Belo Horizonte, MG, Brasil Fax(031) 295-3115 (e.mail: pimenta@netra.cpqrr.fiocruz.br and nagila@netra.cpqrr.fiocruz.br)

One of the first barriers that *Leishmania sp* needs to overcome inside the sandfly vector after blood feeding, is the peritrophic matrix (PM). The PM starts to form immediately after blood intake by the female insects when the

meal goes directly into the midgut filling the whole cavity. The PM has been the object of studies during decades on several insects, but there is not much knowledge about the sandfly PM. In general, it is accepted that all the PMs, are constituted by chitin and non-characterized glycoproteins. We conducted this initial study, using histological and confocal microscopies to characterize the sandfly PM. Initially, the female flies were fed either with blood directly from anesthetized hamsters or with latex particles through a membrane feeding device. The sandfly's midguts were dissected 12h and 24h after the feeding and fixed in a glutaraldehyde soution. Some guts were embedded in historesin and processed for light microscopy. Other guts were processed for anti-chitin and WGA-lectin bindings for observation under the confocal microscope. The historesin sections were stained with toluidin blue, hematoxilin-eosin and Giemsa. It was observed a thick steady PM separating the bloodmeal from the epithelium 24 h after the feeding. In the midgut of sandflies fed with latex beads, it was possible to observe PM formation in about 12 h after the artificial feeding. It was possible to dissect the latex-formed PM which represents the entire sandfly PM components without blood contaminants. The confocal studies showed the fluorescent specific bindings of anti-chitin antibody and WGA-lectin around both meals. These observations characterized the chitin presence in the sandfly PM even when they were fed artificially with latex beads. This model will be used for biochemical and molecular biological studies in order to characterize other sandfly PM components

This investigation received financial support from Fiocruz, CNPq, Pronex.

VE-69
SURVEY OF THE DOMESTIC VECTORS OF CHAGAS DISEASE, IN THE STATE OF GOIÁS, DURING THE PERIOD 1994/95

Silva JL, Camargo MF, Oliveira AWS, Isac E*, Santos AH*, Silva E, Abrão N
Fundação Nacional de Saúde *Universidade Federal de Goiás, Goiânia, GO, Brasil

The variety of species which have synantropical and anthropophilical habits demand frequent monitoring because their habits vary. This study airms at evaluating the performance of the antivectorial campaigns of 1994 and 1995 and at accompanying the possible changes in behaviour of triatomines in people's homes in the State of Goiás.

The vectorial survey was carried out in homes and outbuildings using the PCDCH method, of the National Health Foundation.

A total of 39,747 triatominoes were caught in 191 municipalities with the following infestation frequency: *Triatoma sordida* - 87,29%, *R. neglectus* - 7,20%, *P.megistus* - 4,54%, *T. pseudomaculata* - 1.51%, *T. costalimai* - 0,06% e *T. infestans* - 0,10%. The *T.cruzi* positivity of these species was 1,79% for *T. pseudomaculata*, 1,11% for *P. megistus*, 0,76% for *R. neglectus* and 0,16% for *T. sordida*.

The index of domestic infestation of *T. sordida* was greater than all the synantropical species together. However, its *T. cruzi* possitivity was lower, which could be related to the number of triatominoes examined.

VE-70
SURVEY OF THE PHLEBOTOMINE FAUNA IN THE *SERTÃO* AREA OF PERNAMBUCO

Balbino VQ, Kido EA, Luna LKS*, Santos ESC, Sá BH**, Andrade PP
Departamento de Genética Universidade Federal de Pernambuco, Caixa Postal 7818, Recife, 50732-970, PE *Bolsista Biologia, PET, UFRPE **Fundação Nacional de Saúde, Recife, PE , Brasil

Visceral and tegumentar leishmaniasis are widely spread endemic diseases, being present in about 80% of the counties of Pernambuco. Data from the National Health Foundation (NHF) do not support any assumption on which species may be involved in the maintenance of tegumentary leishmaniasis in this state. This question is more critical in the *Sertão* area, where the fragmentary data originate only from NHF's entomological inquiries. In this work, we analyse the *Lutzomyia* species in the *Sertão* area (26 counties), based on data collected both by the NHF (1994-96) and the UFPE (1997). Manual and light (CDC) trappings were done indoors and in the peridomicile of rural residences. 10,183 sandflies were identified, belonging to 9 species. *L. longipalpis* was the most abundant, followed by *L. oswaldoi* and *L. lenti*. After *L. longipalpis*, the species *L. intermedia*, *L. migonei* and *L. evandroi* are important, as they may have a role in the transmission of *Leishmania braziliensis*. Of the total, 21.08% was captured indoors, and showed a male/female (m/f) rate of 0.70:1, while in the peridomicile this rate was 5.55:1. The consistent presence of sandflies indoors, associated with a high relative frequency of females in this environment, may be related with possible processes (in progress) of domiciliation. Therefore, studies approaching biological and behaviour aspects are necessary and already in progress, analysing data collected in subsequent periods and/or years.

Species	Indoor (m/f)		Peridomicile (m/f)		Sub-total		Total	Rel. Freq.
L. longipalpis	786	1202	6,729	1,207	7,515	2,409	9,924	97.45 %
L. oswaldoi	50	41	19	3	69	44	113	1.11 %
L. lenti	36	11	42	24	78	35	113	1.11 %
L. intermedia	5	7	0	0	5	7	12	0.12 %
L. evandroi	7	0	6	0	13	0	13	0.13 %
others	1	1	5	1	6	2	8	0.08 %
Total	885	1,262	6,801	1,235	7,686	2,497	10,183	100 %

VE-71
TEGUMENTARY LEISHMANIASIS IN COFFEE PLANTATIONS OF MINAS GERAIS: PREVALENCE OF INFECTION IN HUMAN RESIDENTS AND PRESENCE OF PHLEBOTOMINE SAND FLY VECTORS OF *LEISHMANIA*

Alexander B, Oliveira EB*, Haigh E*, Santos MC*
Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, MG, Brasil *Faculdade de Farmacia, Universidade de Alfenas, 37130-000 Alfenas, MG, Brasil

Although transmission of *Leishmania braziliensis* is an important public health problem in the coffee-growing regions of Colombia, little information is available on the situation in southern Minas Gerais, where 50% of Brazilian coffee is produced. A total of 271 cases of tegumentary leishmaniais were recorded from the Alfenas region between 1992-1996, of which 45 (16.6%) were from the muncipality of Machado. A pilot study was therefore carried out in Machado from November 1997 to January 1998, based on application of the Montenegro skin test to residents of coffee farms, a questionnaire survey to determine knowledge among these people of leishmaniasis of the disease and its transmission, and a sampling survey of phlebotomine sand flies in three types of coffee plantations: *"convencional"*, *"adensado"* and *"orgânico"*. In the first two coffee is grown as a monoculture with bushes (varieties "catuaí" or "novo mundo") planted in full sunlight at densities of 1,000 or 10,000 plants/hectare respectively. Insecticides are regularly used in these plantations against coffee pests. In organic plantations coffee is shaded by large trees in an ecologically diverse habitat with no insecticides used. A total of 193 people on 22 farms were skin tested, with 14 (7.0%) giving a positive response indicating prior contact with *Le. braziliensis*. In addition three active cases were encountered. None of the subjects interviewed were aware of phlebotomine sand flies or knew that these insects transmitted *Leishmania*. Sand fly collections were made using CDC light traps and Shannon traps from 18.00-20.00, with only one night devoted to each plantation type. Five insect species were collected, with *L. whitmani* present in the three types of plantation; *L. misionensis* in organic and dense ones; *L. migonei* and *L. fischeri* only in organic plantations and *L. intermedia* only in the conventional plantation. The total number of insects collected (47) was too small to permit meaningful comparisons among the three coffee-growing systems, but our results illustrate that *L. whitmani* and *L. intermedia* are able to adapt to unshaded monocultures of coffee where insecticides are regularly used. Both species are suspected vectors of *Le. braziliensis* in Minas Gerais and are presumably responsable for autochthonous transmission of this parasite to coffee farm residents. We are currently attempting to study this epidemiological situation in more detail, in order to determine the influence of different coffee-growing practices on *Leishmania* transmission in southern Minas Gerais.

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VE-72
THE CLEANING APPARATUS OF THE *TRIATOMA INFESTANS* LEG

Campanucci VA, Insausti TC, Lazzari CR
Departamento de Cs. Biológicas, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina. (e-mail: lazzari@bg.fcen.uba.ar)

On the inner side of forelegs of all stages of the blood-sucking bug *Triatoma infestans*, there is a structure composed by a group of bristles ordered in a comb-like fashion and a cavity of smooth cuticle. This structure is entirely absent in the middle and hindlegs. The comb is composed by a group of lined short hairs (20-25 spines). In *T. infestans*, the length of the hairs decreases from the edges (ca. 60 mm) to the middle of the comb (ca. 45 mm), in 5th instar larvae. This structure locates in the lateral (inner) side of the leg, delimiting the proximal edge of a cuticular depression, which underside presents a flat surfaces, were no cuticular process can be observed. Thick and long hairs that are densely distributed over the distal tibia round the whole structure. In histological preparations, a pluricellular gland could be found inside the tibia. This structure presents a dilated conduct that seems to serve as a reservoir for the secretion products of the associated glandular cells. The duct runs through the cuticular, opening very close to the base of a thick hair, near and lined to the comb-like structure. The functional role of this apparatus should be sought in relation to the cleaning behaviour of these bugs. Under certain circumstances, *T. infestans* and

also other triatomines use to clean their antennae using forelegs. The insect cleans the antennae by lifting both fore legs and drawing first one antenna and then the other through the small comb on the inner side of the fore tibia. In *T. infestans* this behaviour is performed with higher frequency in the presence of host-relate stimuli (i.e., warmth, odours, etc.), under low stimulation levels or situations in which the animal is unable either to locate the source or to reach it, e.g., non-directional stimulus, or open-loop conditions.

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VE-73
THE DAILY PATTERN OF LOCOMOTION ACTIVITY OF *TRIATOMA BRASILIENSIS*

Guarneri A, Reisenman C*, Lorenzo M*, Diotaiuti L, Lazzari C*
Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, MG, Brasil *Laboratorio de Fisiología de Insectos, Univ. de Buenos Aires, Buenos Aires, Argentina

Several activities and physiological processes in triatomines vary according to the time of the day. Locomotion activity, oviposition, egg hatching, the preference for certain temperatures, flight activity and ecdysis occur at specific temporal windows, mostly either at dusk or at dawn. Some of this processes showed to be under the control of endogenous circadian clocks, i.e., are relatively independent from external conditions. The adaptive value of this temporal organisation is related, among others, to the activity pattern of vertebrate hosts, which play a dual role as food sources and predators. In this work, we analysed the activity pattern of *Triatoma brasiliensis*, a species living in association with night active rodents. Fed adult bugs were individually placed in actometers and exposed to a cycle 12hs light (25 lux) and 12hs darkness. Their movements recorded by a computerised data acquisition system designed *ad hoc*. *T. brasiliensis* exhibited a particular pattern of activity, which main features were: 1) a main burst of activity that occurs during first two hours of the scotophase; 2) a secondary burst after dawn and 3) a low, but sustained activity during the whole photophase. The two first points resemble the daily pattern exhibited by other species of triatomines, but differing in the intensity and timing of the nocturnal activity, given that 87% of it concentrates in just 2hs. In addition, in contrast to other related species that display their activity almost exclusively during the scotophase, *T. brasiliensis* displays a significant amount of its activity during the day (34% of the total activity of a day). We suggest that this particular pattern can be related to particular requirements of this species concerning food search and adaptation to their habitat.

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VE-74
THE EFFECT OF RELATIVE HUMIDITY ON THE ECLOSION SUCCESS OF *TRIATOMA PSEUDOMACULATA* AND *TRIATOMA SORDIDA* EGGS

Guarneri A, Diotaiuti L, Lazzari C*, Lorenzo M*
Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, MG, Brasil *Laboratorio de Fisiología de Insectos, Universidad de Buenos Aires, Buenos Aires, Argentina

Relative humidity (RH) seems to affect the biology of most triatomine species, although in a variable fashion in different species. Whereas it does not affect the eclosion success of eggs of *Triatoma infestans*, low RH is deleterious for *Rhodnius prolixus* eggs. In the present work, we studied the effect of relative humidity on the eclosion success of eggs of two secondary vector species, *T. pseudomaculata* and *T. sordida*. Furthermore, we analysed the effect of this environmental parameter on the mortality and moulting success of *T. pseudomaculata*. In a first experiment, we placed batches of eggs of each species at different RHs (ca. 0%, 33%, 56%, 75% and 100%). The number of hatched eggs was counted for each RH and, in the case of the emerged *T. pseudomaculata* larvae, their mortality and moulting success were determined for the five groups. Our results show that the eclosion of eggs of *T. pseudomaculata* is lowest at 0% and almost as low at 100% RH. However, for *T. sordida* eggs, 100% was the worst RH, followed by 0%. Besides, the mortality of *T. pseudomaculata* larvae was strongly affected by low RHs. Conversely, the moulting success of first instar larvae was worst at 100% RH. The present results are discussed in relation to the natural habitats of these species, as well as their physiology.

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VE-75
THE EFFECT OF RELATIVE HUMIDITY ON *TRIATOMA BRASILIENSIS*: HYGROPREFERENCE AND ECLOSION SUCCESS

Guarneri A, Diotaiuti L, Lazzari C*, Lorenzo M*
Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, MG, Brasil *Laboratorio de Fisiología de Insectos, Universidad de Buenos Aires, Buenos Aires, Argentina

The geographical distribution of most triatominae bugs usually shows a close association with climatological regions. However, the preference of most species of the group on paramenters such as temperature and relative humidity was not studied yet. This aspect appears as relevant, given the particular microenvironmental properties of triatomine habitats, that can differ from regional climate. In this work, we studied the RH preference in *Triatoma brasiliensis* by means of a gradient design. Besides, we analised the eclosion success of eggs of the same species confronted to different RHs (ca. 0%, 33%, 56%, 75% and 100%). We also determined the mortality of the resulting first instar larvae, and their moult success at each RH. In the first experiment, 50 fourth-instar larvae were fed, and one day after, placed into the gradient determined by means of presenting five different saturated salt solutions below a plastic mesh arena. During the following 5 days the position of insects was determined every 3 hours by means of recording the output of an IR sensitive videocamera. The same procedure was performed during an equivalent 5 day period at which moulting occurred. The insects preferred to stay at ca. 0% RH during the first five days after feeding. Moreover, the same preference was observed before and during ecdysis. Newly moulted bugs also preferred this RH level, but almost 10% of these insects were found at higher RHs. The eclosion of eggs showed to be affected by extreme RH values, but the data on the mortality of larvae as well as those on their moulting success reflect that these parameters are not affected by RH.

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VE-76
THE ESTABLISHMENT OF A CLOSED COLONY OF *LUTZOMYIA LONGIPALPIS*, A NATURAL VECTOR OF *LEISHMANIA CHAGASI*, IN A SHORT PERIOD OF TIME

Secundino NFC, Pimenta PFP
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, Belo Horizonte, MG, Brasil, Fax(031) 295-3115 (e.mail: pimenta@netra.cpqrr.fiocruz.br and nagila@netra.cpqrr.fiocruz.br)

A closed colony of the phlebotomine *Lutzomyia longipalpis* from theLapinha cave (Minas Gerais State, Brazil) was established in a short time in our laboratory. The intention was to obtain enough flies in order to develop a series of experiments to study the interaction of the sandfly with *Leishmania chagasi*, a natural vector and with other *Leishmania sp* for comparitive studies. The colony started with 850 females from the wild life caught during night-time with CDC light traps. After the females came from the field, they were blood fed from anesthetized hamsters by series of four days. The engorged females were separated in lots of 40 individuals and placed in pots of plaster for oviposition. The pots were pre-saturated with distilled water to force the female oviposition and maintained at 26 ^{o} C in a BOD incubator. Three days later, they put an average of 600 eggs in the pots and the flies died. The corpses were carefully removed from the pots with forceps. An average of 85% of the eggs released larvae which were daily fed on a special diet. The food was made up of an extensive fermentation process. The process started from blending dry rabbit feces with commercial rabbit food (1:1). The mixture was then humidified and stored for fermentation in the dark in a closed plastic container for a period of at least 60 days with weekly observations. After the growing of several types of fungus and its complete depletion, the food was put to dry in the sunlight. The dried food was blended again in fine granules and was ready to use. The larvae fed with this food had their life cycle completed in a maximum period of 35 days until the release of the flies. The flies were then fed with aqueous solution of sugar for three days until they be bloodfed with hamsters. The loss of individuals in the colony happened from no hatching of the eggs, death of some 2th to 4th insthar larvae, and death of engorged flies before oviposition. Today, seven months later, the succeed colony is in the 6th generation with a production of 3,000 adult flies. The flies are being used for morphological studies and for experimental infections with *Leishmania* parasites.

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VE-77
THE EXTRACELLULAR MEMBRANES OF THE INTESTINE OF THE INTESTINE OF *RHODNIUS PROLIXUS*

Albuquerque JMC, Graciano GL, Nogueira-Higino NFS, De Souza W
Laboratório de Biologia Celular e Tecidual, CBB, UENF, Campos RJ, Brasil

The ultrastructural analysis of midgut epithelium of *Rhodnius prolixus* demonstrated that it possess two differents cell types: the columnar cells and indifferentiated cells. The columnar cells of intestine of *R. prolixus* presents a complex system of membranes, known as the extracellular membrane layer, which establish contact with the surface of the epithelial cells. The extracellular membranes are more developed around ten days after meal. In the present study we used a cytochemical approach to characterize further this membrane system.

The binding of cationized ferritin demonstrated the presence of anionic groups on their surface. We also used colloidal iron hydroxyde particles at pH 1.8 to label anionic sites localized in the membranes. A significant binding

of the particles was observed. This binding was significantly decreased, and even abolished, by previous incubation of the intestine fragments with neuraminidase from *Clostridium perfrigens*, thus indicating the presence of sialic acid residues in the membranes.

Cytochemical detection of Mg^{2+}-ATPase and alkaline phosphatase revealed labeling of the extracellular membranes supporting the idea that they indeed represent a true membrane. However, no activity for 5'Nucleotidase, a classical plasma membrane marker, was detected.

We used digitonine to probe, using electron microscopy, the presence of sterols in the extracellular membranes. The results obtained showed the presence of sterols in the membranes.

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VE-78
THE MICROBIOTA OF THE DIGESTIVE TRACT OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE)

Oliveira SMP, Morais BA*, Gonçalves CA, Loureiro MM*, LeandroMJF, Giordano-Dias CM, Vilela ML, Brazil RP***, d'Almeida JM**, Asensi MD*, MelloRP
Departamento de Entomologia *Departamento de Bacteriologia **Departamento de Biologia, IOC, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil ***Lab. de Leishmaniose, Centro Pesq. Reneé Rachou, Fiocruz, Belo Horizonte, MG, Brasil

Several species of *Lutzomyia* are vectors of leishmaniasis and *Lutzomyia longipalpis* (Lutz & Neiva, 1912) is the main vector of visceral leishmaniasis in the New World. Phlebotomine sandflies, like some other hematophagous insects, feed also on sugar meal as a source of carbohydrates. The chances of microorganisms contamination are higher when insects are maintained in laboratory colonies. We have identified the bacterial flora of the digestive tract of 300 females of *L. longipalpis* separated into 2 groups of 150 each. Group 1: flies fed only on hamster blood. Group 2: flies fed on hamster blood and a saturated solution of sucrose. Groups of 30 flies were dissected and the pool of their guts was processed by bacteriological methods.

Gram negative bastonets were isolated and identified in both groups. In group 1 we have identified the following species, most of them belonging to the no sugar fermenters group: *Acinetobacter baumannii, Stenotrophomonas maltophilia, Burkolderia cepacia, B. gladioli, Pseudomonas aeroginosa, P. fluorescens, Enterobacter cloacae* and *Serratia marcescens*. In group 2, the following species were found, the great majority belonging to the sugar fermenters group: *Enterobacter cloacae, E. aerogenus, E. gergoviae, E. sakazakii, Serratia marcescens, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Yokenella regensbourgei, Acinetobacter lwoffii*, and *A. baumannii*.

Due to the importance of the microbiota in the nutrition and survival of sandflies, the identification of the bacterial fauna in the gut of *L. longipalpis* may account for a possible biological control.

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VE-79
THE PERFORMANCE OF YEAST-BAITED TRAPS WITH *TRIATOMA SORDIDA, TRIATOMA BRASILIENSIS, TRIATOMA PSEUDOMACULATA* AND *PANSTRONGYLUS MEGISTUS* IN LABORATORY ASSAYS

Pires H, Diotaiuti L, Lazzari C*, Lorenzo M*
Laboratório de Biologia de Triatomíneos e Epidemiologia da Doença de Chagas, CPqRR, Fiocruz, Belo Horizonte, MG, Brasil *Laboratorio de Fisiología de Insectos, Universidad de Buenos Aires, Buenos Aires, Argentina

The effectiveness of a trap for triatomines baited with yeast cultures has been previously demonstrated for *Triatoma infestans* in laboratory assays. We report here results from laboratory assays testing yeast-traps for *Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata* and *Panstrongylus megistus*. All assays were conducted in an open experimental arena 100 x100 cm, with two traps placed at opposite ends, one containing a yeast culture, and the other with the saccharose solution used as culture medium, as a control. The results obtained clearly demonstrate that yeast-baited traps are effective for capturing both *T. sordida* and *P. megistus* at the laboratory. For *T. sordida*, yeast-baited traps captured significantly more bugs than their controls (*t*-test, P=0.0302, k=6). For *P. megistus,* yeast-baited traps also captured significantly more bugs, when compared to their controls (*t*-test, P=0.0357, k=6). In the experiments with *T. brasiliensis,* traps were able to capture the insects, but the attractive effect of yeast volatiles was extremely weak if detectable (*t*-test, N.S.). For *T. pseudomaculata*, the results show that even though the insects appear to perceive the odours emanated by the cultures (*t*-test, P=0.013, k=4), their attractive effect appears to be weak. These results indicate that yeast-traps are capable of capturing significant numbers of *T. sordida* and *P. megistus* in one night, at the laboratory. For the remaining species, other chemical attractants should be studied for their use as baits for traps. The potential use of yeast-traps for the detection and capture of these triatomine bugs is discussed.

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VE-80
THE PERMEABILIZATION OF *ANOPHELES ALBITARSIS* EGGS ALLOWS AN ULTRASTRUCTURAL ANALYSIS OF CHORION LAYERS

Monnerat AT, Soares MJ*, Lima JBP**, Rosa-Freitas MG, Manso PPA**, Valle D
Departamento de Bioquímica e Biologia Molecular *Departamento de Ultraestrutura e Biologia Celular, Fiocruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil **US Army Medical Research Unit, Instituto de Biologia do Exército, Rio de Janeiro, RJ, Brasil

The construction of transgenic mosquitoes refractory to the *Plasmodium* is an alternative to malaria vector control presently under development. However, in order to succeed, this strategy requires knowledge of mosquito developmental biology. The first step towards the study of the morphological and molecular events governing *Anopheles* embryology is the removal or, alternatively, the clarification and permeabilization of the melanized and sclerotized chorion.

The protocol classically used for the chorion removal of *Drosophila* eggs was applied, with partial efficacy, to eggs from one of the neotropical malaria vectors, *Anopheles albitarsis*. Each step was monitored by Scanning Electron Microscopy and the results point out to possible exochorion similarities and endochorion differences between both species.

As an alternative to chorion removal, mosquito egg permeabilization was attempted. At oviposition mosquito eggs are soft and bright, becoming hard and dark as embryogenesis proceeds. This is due to the process of sclerotization, dependent upon the synthesis, at the mosquito egg chorion, of N-acetyldopamine, which in turn cross-links proteins. Benserazide is an inhibitor of Dopa Decarboxilase, one of the enzymes involved in the sclerotization process. Its use at oviposition induced a delay in egg darkening. The influence of this inhibitor over embryo morphology (monitored by Laser Scanning Microscopy) and egg permeabilization efficiency (verified by Transmission Electron Microscopy-TEM) was also tested.

Permeabilization of mosquito early embryos chorion to Epon resin used in TEM was achieved, allowing the observation of chorion ultrastructure. *An. albitarsis* embryos are surrounded successfully by an endochorion, homogeneous in nature and ranging in width from 0.6 to 1.2mm, and a compound exochorion, comprised of an internal lamellar sheet and external and protruding tubercles. The lamellar sheet of the exochorion consists of two lamina filled in by spherical structures. External exochorionic tubercles present the same electrondensity as the spherical structures inside the lamellar sheet, suggesting a unique chemical nature and raising the possibility of tubercles assemblage by coalescing of the spherical structures during oogenesis. While dorsal tubercles are independently attached to the subjacent lamellar sheet, adjacent ventral tubercles are joined to each other as well as to the float and the frill by their uppermost portion. This arrangement at the ventral side, that rests below the water line during embryonic development, would serve to held a gas film by the superficial network, contributing to the buoyance of the egg.

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VE-81
THE PRESENCE OF LEISHMANIOSIS VECTORS IN AN AGRICULTURAL SETTLEMENT, IN THE WEST OF THE STATE OF GOIÁS

Camargo MF, Oliveira AWS, Abrão N, Santos AH*, Silva EA, Isac E*
Fundação Nacional de Saúde *Universidade Federal de Goiás, Goiânia, GO, Brasil

Leishmaniosis is a very important infectious disease, prevalent principally among young people. The most important factor in its transmission cycle is the presence of phlebotoms. This study aims at evaluating the occurrence, density and behaviour of these vectors in areas of agricultural settlements.

The area studied was the Cantoneiras settlement, on the Ubá farm, 70 kilometres from Nova Crixás, in the western part of the State of Goiás. National Health Foundation technicians caught specimens using people as bait and Shannon and CDC*(Center for Disease Control of Atlanta*) lighted traps, between 6pm and 6am.

A total of 61 examples of *Lutzomyia* were caught, of which 53 (86,88%) were of the *Lu. Longipalpis* species, 06 (9,83%) were *Lu. Lenti* and 02 (3,27%) were *Lu. Whitmani*.

The presence of these vectors would suggest that the cases of tegumental leishmaniosis registered among the settlers are autochthonous and associated with the clearing of the forest in order of build houses and roads and prepare areas for planting.

VE-82
USE OF AN EFFECTIVE PROTOCOL FOR OBTAINING HIGH-TTTER POLYCLONAL ANTISERA AGAINST *ANOPHELES* DIGESTIVE PROTEASES

Hamedi A, Alcântara GC, Lima JBP*, Valle D
Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *U.S. Army Medical Research Unit, Instituto de Biologia do Exército, RJ, Brasil

An alternative strategy to malaria control based on the establishment and release in the field of *Anopheles* mosquito vector lineages refractory to *Plasmodium* is presently under development. One of the requirements to viabilize this strategy is the characterization of strong *Anopheles* gut-specific and blood meal-induced promoters: the gut is the site where the interaction between malaria vector and agent first takes place, after a blood meal. Insect genes coding for digestive proteases are natural candidates to fulfill these prerequisites. In order to study the expression profile of digestive proteases in the colonized neotropical *Anopheles aquasalis* and *Anopheles albitarsis*, we raised antisera against *Anopheles gambiae* recombinant trypsins and chymotrypsins, previously purified through nickel affinity chromatography. Polyadenylic-polyuridylic acid was used as an aqueous adjuvant, enabling not only less harmfull intravenous injections but also the utilization of low amounts of antigen in each rabbit (60 mg per booster). A long immunizing scheme was followed, in order to assure high specificity of produced antibodies. One single animal was immunized against each of four antigens. Each animal was heart-bled three times with 40-50 days intervals between sucessive bleedings, always done twelve days after a booster. Titer and specificity (tested against the different produced *An. gambiae* recombinant proteases) of the various obtained sera were separately determined, through Dot and Western blots. If on one hand titers of up to 1:30.000 have been obtained by this procedure, on the other hand, differences in titer and specificity among the various bleedings of the same animal were found, making possible the use of a single animal to obtain sera with distinct characteristics.

These antisera are being presently used to detect the *An. gambiae* homologous digestive proteases in neotropical *Anopheles* and to follow its expression pattern.

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VE-83
THERMOPREFERENCE TO STAY AND LAY EGGS IN *RHODNIUS PROLIXUS*

Schilman PE, Lorenzo MG, Lazzari CR
Departamento de Ciencias Biológicas, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina. e-mail: lazzari@bg.fcen.uba.ar

Several attempts have been made to find a reliable climatic indicator of the distribution of triatomines, in particular to predict changes originated in regional or global climate modifications. In this sense, it is relevant to know what microenvironmental conditions bugs actually experience in the nature, given that they may differ from regional ones. As well, the conditions that insects actively prefer should be determined, since they could to be able to influence their body temperature behaviourally. In this work, we analysed the active preference of *Rhodnius prolixus* to remain and oviposit in a temperature gradient. Fifteen males or 15 females were placed in an experimental arena where a temperature gradient and a 12:12 hs light:dark cycle were established, during 20 days. The position of the bugs was recorded every hour in videotapes, with the aid an IR sensitive video camera and a computerised control system. Thereafter, the position of every bug was read for each hour, and the data fed to a computer. Males and females exhibited a slightly different thermopreference (in average, 25.00°C vs. 25.41°C, respectively). This preference varied according to the time of the day and starvation. On the one hand, bugs moved towards lower temperatures when increasing starvation. On the other hand, they remained at the highest value of the day, at the beginning of the night, when they begin to their daily activity. When compared with other triatomines, both displacements showed lower amplitude. Females laid most of their eggs in temperatures ranging from 22° to 30°C, with a maximum occurring at 25°-26°C. The distribution of eggs in the gradient cannot be fully explained by simply considering the time that the insects spent at these temperature values during the experiment and suggest the possible existence of an active thermopreference to oviposit in this species. In summary, *R. prolixus*, as other triatomines, seems to display behavioural thermorregulatory strategies, that allow them to fit the most adequate temperature for different physiological processes (i.e., metabolic rate, egg development, etc.).

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VE-84
THERMOPREFERENCE TO STAY AND TO LAY EGGS IN *TRIATOMA BRASILIENSIS*

Guarneri A, Pires H, Diotaiuti L, Lazzari C*, Lorenzo M*
Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, MG, Brasil *Laboratorio de Fisiología de Insectos, Universidad de Buenos Aires, Buenos Aires, Argentina

The geographical distribution of most triatominae bugs usually shows a close association with climatological regions. However, the microenvironmental properties of their habitats and behavioural thermoregulation make this relation quite complex to be interpreted. A main point is the knowledge of the active preference of a given species for certain environmental variables. In this work, we studied the temperature preference of *Triatoma brasiliensis* by means of a gradient design. We analysed the thermopreference of adult bugs under different physiological conditions to remain, as well as the preference of females to lay eggs. A group of insects was initially fed and placed

inside an arena, where a temperature gradient was established. We recorded for every hour the position of all bugs by means of a video-setup controlled by a computer. In a second assay, we placed a group of fed females at the same arena and after two periods of approximately 30 and 40 days, we counted the number of laid eggs at each temperature. Results evinced that recently fed adult *T. brasiliensis* prefer to stay at a mean temperature of 29.3±0.1°C. After a starvation period of 40 days, they shifted their preference towards 26.2±0.2°C. After feeding the same insects again, they moved to about 30.1±0.9°C. Moreover, the daily dispersion shown by starved bugs was almost twice that of recently fed ones, suggesting a more intense food search activity. Females laid most of their eggs between 28 and 32°C, with a relative maximum occurring at 30°C. Our experiments show that *T. brasiliensis* have a preference for relatively high temperatures, particularly regarding egg-laying, when compared to other triatomine species analysed. This should be interpreted in accordance to the particularecological and physiological requirements of this species.

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VE-85
ULTRASTRUCTURAL ASPECTS OF THE *PLASMODIUM GALLINACEUM* SPOROZOITES IN THE OOCYSTIS AND IN THE SALIVARY GLAND

Duque-de-Mello M, Pimenta PFP
Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto Lima 1715, 30000 Belo Horizonte, MG, Brasil
Fax(031) 295-3115 e.mail: pimenta@netra.cpqrr.fiocruz.br and mduque@netra.cpqrr.fiocruz.br

The malaria disease is one of the most important and serious subject in the world's medical research. The disease causes millions of death. Our work is to elucidate some of the principal questions about thedifferentiation, invasion and infection inside the insect. In order to do, we made routine electron microscopy of *Aedes fluviatilis* infected with *Plasmodium gallinaceum*, making possible to observe the sporozoite formation inside the oocysts, and the salivary gland sporozoites. Which is extremely important, because it is through the salivary gland that the sporozoites are injected into the vertebrate host. As we know, the ookinete invades the epithelium cells of the mosquito's midgut and develops a thick electron dense capsule, forming the oocyst. We could see that the immature oocyst has its wall thicker than the mature. Also, the cytoplasm of the growing oocyst is subdivided into pieces of masses, that will originate the sporozoites. These young parasites leave the oocysts and invade the salivary gland of the mosquito. Inside the salivary gland, the parasites do not change their shape. But, some changes happen in the sporozoite inside the salivary gland making them infective. Studies already showed that only the parasites that passes through the salivary gland are infective for the vertebrates. Our comparative study showed that the coat surface of the salivary gland sporozoites is a little bit thicker than the oocyst parasite. Also, we noted that in the salivary gland, these forms are seen in large number inside the secretory cells but in fewer number inside the salivary duct. This fact suggests that only some parasites are able to invade the salivary duct. Another question to answer, is how these parasites can cross the chitin line of the salivary duct, before being injected into the vertebrate host, during the mosquito feeding. Maybe theses forms have some kind of a chitinase enzyme that permits their entrance. Also, in the salivary gland we could observe some parasite degradation, which suggests that not all sporozoites are able to survive in contact with the mosquito salivary enzymes. Further studies are necessary to understand these and other aspects of the infection process.

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VE-86
USE OF ESBIOTHRIN AS CHEMICAL OUSTMENT OF TRIATOMINES

Vitta ACR, Pires HER, Porto FA*, Coelho VA*, Diotaiuti L
Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil *Hoechst Shering AgrEvo do Brasil, Caixa Postal 1935, 01059-970 São Paulo, SP, Brasil

Dorine is an insecticide pyrethroid whose active ingredient is the esbyothrin, formulated as a concentrate emulsion 10%, in association with the butoxide of piperonil as sinergist. Its insecticide action against cockroaches is known, being used in the doses of 2,5 mg ai/m^2 against *Periplaneta americana* and 17,5 mg ia/m^2 against *Blatella germanica*. As all the insecticide pyrethroid, is expected that the initial contact with the active molecule causes irritation and excitement in the insects. This is an interesting effect, that promotes the displacement of the insects from its hiding place, allowing its capture more easily. In this work, for the first time the action of this product is being tested against triatomines, especially being investigated the possibility of its use as oustment. Initially, the experiments were accomplished exposing for 24 hours 60 fifth instar nymphs of *Triatoma infestans*/dose to filter papers impregnated with the doses of 8, 10, 12, 14, 16 and 18 mg ai/m^2. The doses of 14, 16 and 18 mg ai/m^2 promoted an intoxication between 56,7% and 58,3% ($p>0,05$), preceded for an excite-repellent effect. Seven days

after the insecticide contact, about 70% of the intoxicated insects were considered normal, obtaining a very low final mortality.A second series of experiments was accomplished in an experimental arena of glass of 1m x 1m, contends in one of the extremities a hiding place constituted by filter paper impregnated with Dorine in the doses of 16, 18, 24 and 26 mg ai/m ^{2}; in the other extremity was placed another identical hiding place but without insecticide. The insects were loosened in the centre of the arena at the beginning of the night, and at the following morning they were counted in accordance of the triatomines position in the arena. Notable preference of the insects was observed by the places without insecticide, however the number of insects that presented intoxication symptoms in the doses of 16 and 18 mg ai/m^2 was significantly larger, demonstrating that before their dispersion to another ranches, the insects were presented in the hiding place with insecticide. In the higher doses, on the contrary, the insects avoided the contact and were less intoxicated.It is concluded by the perspective of use of Dorine CE as oustment, having been the doses of 24 or 26 mg ai/m^2 the most suitable, once, if applied in the fields, the survival insects allows the examine for *Trypanosoma cruzi* infection at the laboratory. The field tests are scheduled to begin immediately.

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VE-87
YEAST CULTURE VOLATILES AS ATTRACTANTS FOR *RHODNIUS PROLIXUS*

Lorenzo M, Manrique G, Pires H*, de Brito Sánchez M, Diotaiuti L*, Lazzari C
Laboratorio de Fisiología de Insectos, Universidad de Buenos Aires, Buenos Aires, Argentina *Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, MG, Brasil

The orientation of triatomine bugs towards the host is aided by diverse cues, being the heat and odours released by hosts those fundamentally used. Only a few chemical substances were demonstrated to attract these insects in the host searching context, and CO_2 appears as playing an important role, as occurs with most blood-sucking arthropods. The exploitation of this attractive effect for baiting traps for triatomines has been proposed showing that yeast cultures, a source of CO_2, are highly attractive for *Triatoma infestans*. Moreover, a trap prototype that was able to capture important numbers of larvae from that species when it was baited with yeast cultures had been presented. In this study, responses to air currents carrying volatiles from yeast cultures were measured by means of electroantennograms in the haematophagous bug *Rhodnius prolixus*. Besides, we also performed laboratory experiments in which yeast-baited traps were tested for this species. The volatiles liberated by yeast cultures were capable to generate a clear electrophysiological response. The amplitude of the electroantennogram measured in response to yeast volatiles was 0.140 ± 0.0079 mV (mean ± s.e.). This amplitude was much higher than that obtained during the stimulation by clean air (0.0009 ± 0.0009 mV). Yeast-baited traps showed able for capturing *R. prolixus* larvae in single-night assays. Moreover, the addition of yeast cultures to the traps dramatically increased the capture ability of these devices (k= 4, *t*= 7.554, df= 3, P= 0.0024). A modification introduced to previously reported traps showed to be effective for this species, as the bugs were immobilised once they fell into the device. Our results demonstrate that yeast culture volatiles are attractive for *R. prolixus*. The results obtained by means of electroantennogram techniques represent the first electrophysiological evidence of the perception of yeast volatiles by the antennae of a triatomine bug. We discuss the relevance of our results in view of currently used triatomine sampling techniques and control strategies.

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VE-88
LEISHMANIA (L.) CHAGASI CAUSES VISCERAL DISEASE AND WIDESPREAD ATYPICAL CUTANEOUS LEISHMANIASIS

Belli Alejandro/*, Garcia David **, Palacios Xiomara*, Rodriguez Betzabe*, Videa Elsa*, Valle Sonia**, Tinoco Ena***, Marin Francisca ****, Harris Eva
School of Public Health, University of California, Berkeley, 140 Warren Hall, Berkeley, CA 94720, USA *Departamento de Parasitologia, Centro Nacional de Diagnostico y Referencia, Ministerio de Salud, Apartado 2900, Managua, Nicaragua **Direccion de Epidemiologia, Ministerio de Salud, Leon, Nicaragua ***Hospital Dermatologico Nacional, Managua, Nicaragua ****Programa Nacional de Control de Leishmaniasis, Ministerio de Salud, Managua, Nicaragua

Leishmania chagasi is the causative agent of visceral leishmaniasis in the Americas, a disease that is fatal if untreated. Recently, the same parasite species has been associated with atypical cutaneous lesions in several Central American countries; however, little comprehensive information about this disease is available.

We have analyzed clinical, epidemiological, and parasitological characteristics of 44 cases of American visceral leishmaniasis (VL) and of 178 cases of atypical cutaneous leishmaniasis (ACL) from Nicaragua. The VL cases were

characterized by classical symptoms and ranged in age from 9 months to 32 years (x =3D 3.8 years); 84% were four years of age or younger. ACL cases presented with cutaneous nodules (3-30 mm) on the face and extremities. The lesions were generally few in number, with 83% of the patients presenting with three or less nodules. The lesions were present for 1 month to 15 years, with an average duration of 2.7 years. The mean age of the ACL patients was 15.9 years; 76% were between the ages of 5 and 20. A complete house-to-house survey of 100 houses in one affected community revealed a prevalence of ACL of 12%. Sandflies were collected near houses of VL and ACL patients; both *Lutzomyia longipalpis* and *L. evansi* were identified.

Dermal scrapings from ACL patients were analyzed by a *Leishmania* genus-specific PCR, and 83% of cases were positive. In constrast, only 23% the specimens revealed amastigotes by microscopy. The parasites were classified as *L. chagasi* using two PCR assays specific to the *L. donovani* complex. Schizodeme analysis of PCR-amplified minicircles using either of two restriction enzymes indicated that parasites from LV and ACL cases are genetically similar, if not identical.

The ability of *L. chagasi* to cause both visceral and cutaneous disease could be determined by a number of factors, including the status of the host immune system, the genetic identity and characteristics of the parasite, and the sandfly responsible for its transmission. Our results indicate that ACL is more prevalent than previously thought. This has significant public health implications since ACL seems to be caused by the same parasite as the potentially fatal visceral disease.

VE-89
SAND FLY SALIVA AND MAXADILAN: DISSOCIATION BETWEEN VASODILATION AND *LEISHMANIA* INFECTION-ENHANCING EFFECTS

Castro F, Paranhos-Silva M, Sherlock I, Paixão MS*, Sales LA, Pontes-de-Carvalho LC, dos-Santos WLC
Laboratório de Imunologia Molecular e Celular, CPqGM, Fiocruz *LACEN - Secretaria da Saúde do Estado da Bahia. Salvador, BA, Brasil

Sand fly saliva has been shown to enhance *Leishmania* infection in mice (*Science 239:1306,1988; Infect Immu 59:1592, 1991*). This effect is attributed to maxadilan, a polypeptide that also produces vasodilation and, presumably, inhibits the killing of *Leishmania* by macrophage *in vitro*. In this study, we tested the ability of maxadilan to enhance the infection of macrophages by *L. major* and *L. braziliensis in vitro* and of CBA and BALB/c mice by *L. major* and *L. braziliensis*, respectively, *in vivo*.

Groups of 6 and 18 CBA mice, and 6 BALB/c mice, were used in three separate experiments. Three different batches of recombinant or synthetic maxadilan were used. As expected, each batch produced diarrhoea in mice and/or cutaneous hyperaemia in rabbit. Salivary glands were isolated from *L. longipalpis*, and lysates prepared with these glands produced skin hyperaemia in rabbits. The CBA and BALB/c mice were infected with 10^5 fourth *in vitro* passage, stationary phase, *Leishmania* promastigotes in 1) phosphate buffered saline containing 1% bovine serum albumin (PBS-0.1%BSA) alone, or 2) PBS-0.1%BSA containing half acinus of salivary gland of *L. longipalpis* (only CBA mice), or 3) PBS-0.1%BSA containing maxadilan.

No differences in the size of the lesion, parasite burden (as measured by limiting dilution), or dissemination of *Leishmania* to spleen, liver and lung, were observed between groups of CBA mice infected with *L. major* in presence or absence of maxadilan or salivary gland lysate, during 9 or 14 weeks of observation. There was a slight increase in the lesion of BALB/c mice infected with *L. braziliensis* and maxadilan compared with the animals infected with *L.braziliensis* alone. In both groups of animals the lesion subsided after 14 weeks of infection. There was no increase in macrophage infection with *L. major* or *L. braziliensis* in presence of different concentrations of maxadilan. The data presented herein show that the induction of vasodilation by sandfly saliva is not by itself sufficient to enhance *Leishmania* infection. They also suggest that the enhancing effect of maxadilan or salivary gland lysate on *Leishmania* infection is not consistently observed, and its relevance to the infectivity of *Leishmania*, requires further studies

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VE-90
PRELIMINARY ANALYSIS EXTERNAL MORPHOLOGY OF *RHODNIUS BRETHESI* MATTA, 1919 BY SCANNING MICROSCOPY (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

Ferro ZPA, Junqueira ACV*, Moreira CJC*, Spata MCD*, Silva LM**, Gonçalves TCM
Departamento de Entomologia *Depto. Medicina Tropical **Depto. Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Rhodnius brethesi is a sylvatic species which has been described in "piassaba palms" (*Leopoldina piassaba* Wallace) in Brazil (Amazonas and Pará) and Venezuela. It takes blood in animals seeking shelters and has also reported attacking humans during harvesting palm fiber (Lent & Wygodzinsky, 1979). Previous taxonomic studies

regarding this species were based on optical microscopy (Matta, 1919; Lent & Jurberg, 1969). In the present study the scanning electron microscopy was used to demonstrate ultraestructural aspects of external morphology with taxonomic purpose. Specimens of *R. brethesi* were collected in Barcelos district, located at the right bank of "Rio Negro", on the northern part of the State of Amazon, Brazil (Coura et al., 1994) and subsequently maintened in the bug colony at the Department of Medicine Tropical in Oswaldo Cruz Fundation. The insects were placed individually into Borrel flasks and submitted to starvation until death. Micrographs were performed in a Digital Scanning Microscope (DSM-940, Zeiss). The head, thorax and legs of the first and fifth instar nymphs and adult specimens were analysed. Concerning to morphological caracters in the head, it could be observed a granulose cuticle, a trichobotria on the second antennal segment, labrum with star-like structures. The thorax shows a stridulatory sulcus with horizontal parallel bars. Another structures are being analysed in the same instars as well as other instar nymphs.