

## OPALINID PROTOZOANS OF ANURANS FROM LOS TUXTLAS, VERACRUZ, MEXICO.

Opalinid protozoan are parasites or endocomensals of cold blooded vertebrates, especially of amphibian anurans (Corliss, 1990. *Handbook of Protoctista*: 239-245; Wessenberg, 1978. *Parasitic Protozoa*: 551-581). Scattered records exist for snakes, salamanders, caecilians and fishes (Foissner *et al.*, 1979. *Zool. Anz.*, 202: 71-85; Sandon, 1976. *Trans. Amer. Micros. Soc.*, 97: 357-366). About 400 species are known worldwide belonging to six genera: *Protoopalina*, *Zelleriella*, *Cepedea*, *Opalina*, *Hegneriella* and *Bezenbergeria*. This last two genera not accepted because no type specimens are available and because of the dubious quality of their original description (Corliss, 1990; B. L. J. Delvinquier, pers. com; Wessenberg, 1978).

Mexico holds an amazing rich herpetofauna, especially in the humid rain forest and tropical dry forests that provide suitable habitats for many species of amphibians and reptiles. Unfortunately, little is known regarding the details of the symbiosis between opalinids and Mexican herpetofauna. To date, 24 amphibian species have been examined from fourteen states in Mexico, and 21 species of opalinids have been described (Beltrán, 1925 *Trans. Amer. Micros. Soc.* 44: 222-223; 1941a. *Rev. Soc. Mex. Hist. Nat.* 2: 127-137; 1941b. *Rev. Soc. Mex. Hist. Nat.* 2: 267-273; Metcalf, 1923. *Bull U.S. Nat. Mus.* 120: 1-484).

The bulk of the opalinids was described from the states of Oaxaca, Guerrero and Distrito Federal. Only three species are known described from the Gulf coast of Mexico, in the states of Veracruz, Tabasco, and Campeche.

Los Tuxtlas is a region that comprises a large zone of lowland rain forest and grassland in southern Veracruz, Mexico. From this area, 45 amphibians and 107 reptiles have been recorded (Pérez-Higareda, *et al.*, 1987. Instituto de Biología, UNAM). However, only three records of opalinids exist for the region. (Beltrán, 1941a; Metcalf, 1923). I report on additional collections of opalinids from Los Tuxtlas, intended as a contribution to the study of protozoan symbiosis with vertebrates in Mexico.

I collected frogs and toads from the vicinity of the Estación de Biología "Los Tuxtlas" and at a nearby site known as "Las Escolleras", near Alvarado in southern Veracruz. Anurans were collected by hand at night using a spotlight, searching under rocks or wood and in small ponds or rivers and lagoons. Some species like hylids were collected by hand under the leaves and small tree branches. All the specimens were killed with chloroform. From each, the large intestine and caecum were dissected out and placed in a Petri glass containinig 0.6% physiological solution.

**Table 1.** Species of Mexican anurans examined and species parasited with opalinids in Los Tuxtlas, Veracruz, México.

Host	Result A - B
<i>Rhinophrynus dorsalis</i> <sup>1</sup>	7 - 5
<i>Bufo cavifrons</i> <sup>2</sup>	5 - 5
<i>Bufo marinus</i> <sup>2</sup>	18 - 18
<i>Eleutherodactylus rugulosus</i> <sup>3</sup>	1 - 0
<i>Eleutherodactylus rhodopis</i> <sup>3</sup>	1 - 0
<i>Leptodactylus melanonotus</i> <sup>3</sup>	1 - 0
<i>Agalychnis callidryas</i> <sup>4</sup>	6 - 0
<i>Hyla ebraccata</i> <sup>4</sup>	5 - 4
<i>Hyla picta</i> <sup>4</sup>	13 - 10
<i>Smilisca baudini</i> <sup>4</sup>	15 - 15
<i>Smilisca cyanosticta</i> <sup>4</sup>	3 - 0
<i>Gastrophryne usta</i> <sup>5</sup>	4 - 2
<i>Rana berlandieri</i> <sup>6</sup>	4 - 2
<i>Rana vaillanti</i> <sup>6</sup>	16 - 16

A. Number of specimens examined.

B. Number of specimens with opalinids.

1. Rhinophrynidae. 2. Bufonidae. 3. Leptodactylidae. 4. Hylidae. 5. Microhylidae. 6. Ranidae.

Opalinids were mainly in the caecum, attached to the intestine walls or mixed with feces. Therefore I separated them from the intestine wall using a Pasteur pipet. Opalinids were then fixed directly by heat onto the slide using Zenker, Schaudinn or 4% formaline. The rest of the specimens were concentrated by centrifugation at 500 rpm, and fixed with the same solutions mentioned above. These slides were then washed and air dried in order to stain them and get permanent slides. Slides were stained according to common techniques including Delafield hematoxylin, Heidenhain iron hematoxylin, Giemsa and some silver impregnate stains. Each slide was microscopically examined to identify species. The most important features in determination of opalinid species are, according to Sandon (1976), shape and body size, nucleus size, arrangement of ciliation, and falx shape. In this work, the main features used were shape and size of the body, (Earl 1970. *Protistologica*, 7: 491-503).

Fourteen anuran species were examined comprising six families (Table 1) and five opalinid species were determined, another one may be a new species and two more that have not yet been determined owing to their special features. Slides are deposited at the International Protozoan Type Slide Collection, United States National Museum (USNM).

*Protoopalina xyster*. From *Gastrophryne usta*, from Laguna Zacatal, "Los Tuxtlas", Veracruz. This record is in accord with the host recorded in the original description (Metcalf, 1923). USNM 47739.

*Zelleriella microcarya*. Abundant populations were encountered in the large intestine of *Rana vaillanti* from Laguna Escondida, "Los Tuxtlas". USNM 47740-41.

*Zelleriella antunesi*. From to the cane toad *Bufo marinus*, Estación de Biología Tropical, Los Tuxtlas. USNM 47745.

*Zelleriella bufoxena*. A few specimens of this species were found associated with *Rana berlandieri*, from Laguna Zacatal, los Tuxtlas. USNM 47742-43.

*Opalina obtrigonoidea*. Many individuals of this species were found living in the large intestine of *Smilisca baudini*, from the Estación de Biología and ten specimens of *Hyla picta* from Laguna Zacatal. USNM 47746-47.

*Zelleriella* sp. This is the first record of opalinids from the burrowing toad *Rhinophrynus dorsalis* collected at Las Escolleras, Alvarado, Veracruz. USNM 47744.

Two other species of anurans had opalinids in to the large intestines, but the opalinid species are not yet determined.

The records of opalinids in Mexican anurans comprise 21 species described; only six of the fourteen anuran species examined in this work had been examined previously: *G. usta* from Tehuantepec, Mexico describing *Protoopalina xyster* (Metcalf, 1923); (*Hyla baudini*) in association to *Cepedea baudini* and *Opalina guatemalae* both from Cordova, Mexico (*sic*); *Zelleriella antilliensis* was recorded in the toad *Bufo marinus* examined by Beltrán (1941a); and finally, *Rhinophrynus dorsalis* was examined and no opalinids recorded (Metcalf, 1923).

In this work the first opalinid associated to *R. dorsalis* is recorded and five species of opalinids belonging to three genera are redescribed. Six new host records of anurans were also recorded. Hence it is evident that the knowledge of Mexican protozoan symbionts remains incomplete, and that many other species of reptiles, amphibians and fishes may also be potential hosts for protozoan opalinids.

#### ACKNOWLEDGEMENTS

I thank Dr. Ben L. J. Delvinquier, Dr. Rafael Lamothe and Dr. Eucario López Ochoterena for their help in the development of this project. Thanks also to Dr. Fernando Cervantes and Dr. Fernando Chiang for comments on the manuscript. Financial assistance was provided from DGAPA-UNAM, Instituto de Biología.

GEORGINA SANTOS-BARRERA. Museo de Zoología, Facultad de Ciencias. UNAM, Apartado postal. 70-399, 04510, México, D.F.