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MYXOSPOREANS AND COCCIDIANS PARASITIC ON ENGRAULID FISHES FROM THE COASTS OF ARGENTINA AND URUGUAY

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Summary :

Two new species of the genus *Sphaeromyxa* (Myxosporea) from the gall bladder and one new species of the genus *Eimeria* (Coccidia) parasitizing the testes, are reported from engraulid fishes from the Argentinian shelf (including the Argentinean-Uruguayan Common Fishing Zone): *Sphaeromyxa bonaerensis* n. sp., found in *Engraulis anchoita* Hubbs & Marini, 1935 (argentine anchovy) (prevalence 0.2 %) and in *Anchoa marini* Hildebrand, 1943 (anchovy) (prevalence 24.2 %) caught in the Bonaerense region of the Argentinian Sea; *Sphaeromyxa argentinensis* n. sp. found in *E. anchoita* from all the sampled localities between 34° S and 46° S (prevalence 26.8 %) and from *A. marini* at Mar del Plata coastal zone (prevalence 1.01 %). *Eimeria patagonensis* n. sp. was found in *E. anchoita* living in the Patagonian region of the Argentine Sea (prevalence 0.45 %). Details of some ultrastructural features of *S. argentinensis* are provided.

KEY WORDS : *Sphaeromyxa, Eimeria, Engraulis anchoita, Anchoa marini,* parasites, Argentina, Uruguay.

Résumé : MYXOSPORIDIES ET COCCIDIENS PARASITES DES POISSONS DE LA FAMILLE ENGRAULIDAE PROVENANT DES CÔTES D'ARGENTINE ET D'URUGUAY

Deux nouvelles espèces du genre Sphaeromyxa *(Myxosporea) parasites de la vésicule biliaire et une nouvelle espèce appartenant au genre* Eimeria *(Coccidia) parasite des testicules, sont signalées chez des poissons de la famille Engraulidae, provenant des côtes d'Argentine (comprenant la Zone de Pêche Commune Argentine-Uruguay).* Sphaeromyxa bonaerensis *n.sp. a été trouvée chez* Engraulis anchoita *Hubbs & Marini, 1935 (anchois d'Argentine), (prévalence: 0,2%) et chez* Anchoa marini *Hildebrand, 1943 (anchois) (prévalence : 24,2 %) récoltés sur les côtes de la province de Buenos Aires.* Sphaeromyxa argentinensis *n.sp. a été recoltée chez* E. anchoita *provenant des prélèvements obtenus entre 34° S et 46° S (prévalence : 26,8 %), et chez* A. marini *capturé dans les eaux côtières de Mar del Plata (prévalence : 1,01 %).* Eimeria patagoniensis *a été trouvée chez* E. anchoita *provenant des eaux de la région Patagonique d'Argentine (prévalence : 0,45 %). Les nouvelles espèces sont décrites et figurées. Quelques caractéristiques ultrastructurales de* S. argentinensis *sont indiquées.*

MOTS CLÉS : Sphaeromyxa, Eimeria, Engraulis anchoita, Anchoa marini, *parasites, Argentine, Uruguay.*

INTRODUCTION

Most studies on myxosporidians from the Argentinean Sea were reviewed by Gaevskaya *et al.* (1985), who reported the presence of 31 species belonging to 14 genera, parasitizing fishes from the Falkland Islands (Malvinas)-Patagonian Region. Following Gaevskaya *et al.* (1985) only two new species were described (Kalavati *et al.*, 1995, 1996) and few authors studied other aspects of these myxosporidians, including morphology (Sardella, 1988a), pathogeny (Sardella, 1988b) and host-parasite relationships (Sardella & Roldán, 1989; MacKenzie & Longshaw, 1995; Sardella & Timi, 1996). Less known in the area is the coccidian parasitic fauna, represented at present by *Goussia clupearum* (as *Eimeria clupearum*) (Evdokimova, 1973), *Eimeria jadvigae* (Grabda, 1983) and *Goussia* sp. (MacKenzie & Longshaw, 1995).

In the course of a parasitological survey of the anchovies *Engraulis anchoita* Hubbs & Marini, 1935 (argentine anchovy) and *Anchoa marini* Hildebrand, 1943 (anchovy) caught in the Argentinean Shelf, representatives of two new species of the genus *Sphaeromyxa* Thélohan, 1895 and one new species of the genus *Eimeria* Schneider, 1875 were found; these parasites are described in the present paper.

MATERIALS AND METHODS

A total of 1,891 specimens of *Engraulis anchoita* Hubbs & Marini, 1935 and 99 specimens of *Anchoa marini* Hildebrand, 1943, were examined.

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The samples of *E. anchoita* were collected during the research cruises of R.V. Cap. Oca Balda and R.V. Dr. E. Holmberg (Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)) OB 11/93 (October, 1993), OB 13/93 (November-December, 1993), H 04/94 (May, 1994), OB 08/94 (October, 1994), H 07/95 (September, 1995) and OB 14/95 (October, 1995), which covered the South West Atlantic shelf from 34°S to 46°S. The samples of *A. marini* were obtained in May, 1996 and June, 1998, from commercial catches at Mar del Plata Port (38° 08' S-57° 32' W).

Fish were fixed in buffered 10% formalin and transported to the laboratory, where they were dissected out and examined under a stereoscopic microscope.

Prevalence (percentage of parasitized fish) and intensity (number of parasites per host, calculated only for myxosporidian plasmodia) of infections were calculated according to Margolis *et al.* (1982) terminology.

The myxosporidians were studied in wet smears of the gall bladders. Air dried smears were refixed in methyl alcohol and stained with Giemsa's stain to highlight details of spore morphology. In a subsample of 30 specimens of *E. anchoita* and 20 specimens of *A. marini* the gall bladders were dissected out and the vegetative forms were removed with the aid of dissection needles. The vegetative forms and spores were measured.

For transmission electron microscopy (TEM) and scanning electron microscopy (SEM) a sample of fresh plasmodia was recovered from 30 fresh specimens of *E. anchoita* obtained from commercial catches at Mar del Plata Port (October, 1995). These anchovies were not included in the quantitative data. Plasmodia were fixed for 24 h at 4 °C with 3% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4 and post-fixed for 1h at 4 °C with 1% osmium tetroxide in 0.1M cacodylate buffer at pH 7.4. After fixation the material was dehydrated in progressive ethanol series and then for TEM observations the plasmodia were embedded in Spur at 70 °C, ultrathin sections were stained with lead citrate and uranyl acetate and observed in a Jeol T100 CX II TEM. For SEM observations, the specimens were subjected to CO2 critical point drying, coated with gold-palladium and scanned in a Jeol T100 SEM.

Descriptions of myxosporidians were made following Lom & Arthur (1989).

The coccidians were checked by squash method in only those testes showing transparent subepithelial spots under stereoscopic microscope and studied in wet smears.

Drawings were made with the aid of a camera lucida.

Collection number quoted refers to specimens deposited at the La Plata Natural Sciences Museum (LPNSM), La Plata, Argentina.

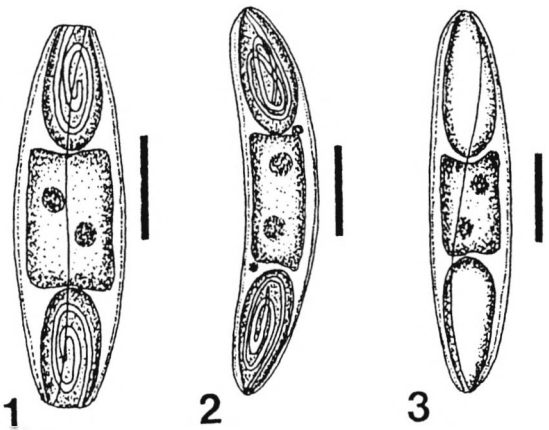
RESULTS

SPHAEROMYXA BONAERENSIS N. SP. (Figs. 1, 4)

Type host: *Anchoa marini* Hildebrand, 1943.
Other host: *Engraulis anchoita* Hubbs & Marini, 1935.
Type material: Giemsa stained spores, LPNSM Collection number: 002/1; one fixed plasmodium, LPNSM Collection number: 002/2.
Location in hosts: gall bladder.
Locality: Mar del Plata Port (38° 08'S - 57° 32'W); 37° 22'S - 56° 21'W; 37° 30'S - 56° 50'W; 39° 13'S - 60° 20'W; 38° 51'S - 58° 43'W.
Date: October, 1993; October, 1994; September, 1995.
Prevalence of infection: in *A. marini* 24 out of 99 (24.2%), in *E. anchoita* four out of 1,891 (0.2%).
Intensity (number of plasmodia per host): one.
Etymology: the specific name refers to the geographic zone where the species was found.

DESCRIPTION

Spores: the spores are fusiform, slightly arcuate in valvular view; straight in sutural view and with truncated ends. The shell valves are longitudinally striated. The sutural ridge is oblique. The two oval and equal polar capsules are situated on each end of the spore. The polar filament makes five longitudinal folds in the capsule. The capsular nuclei are sometimes visible beneath the polar capsules. The space between the polar capsules is filled with a finely granular sporoplasm. Two sporoplasm nuclei are often visible.
Measurements in micrometers, based on 20 spores from *A. marini* (Type host); mean is followed by the range in parentheses: spore length: 17.33 (16.50-18.75); spore width: 4.45 (3.75-4.80); spore thickness: 4.16



Figs. 1-3. – Fig. 1. *Sphaeromyxa. bonaerensis* n. sp., spore, sutural view. Fig. 2. *Sphaeromyxa argentinensis* n. sp., spore, valvular view. Fig. 3. *S. argentinensis* n. sp., spore, sutural view. Bars: 5 µ.

(3,45-4,50); polar capsule length: 4.08 (3.00-4.50); polar capsule width: 2.81 (1.95-3.30). Measurements of 10 spores from *E. anchoita* were coincident with those from the Type host.

Vegetative form: the plasmodium is found floating freely in the gall bladder fluid, coiled upon itself. Attempts to unfold the plasmodia were unsuccessful because they were obtained from fixed hosts. Maximum diameter, based upon two folded plasmodia: 1.10 mm and 1.53 mm respectively.

REMARKS

Laird (1953) divided the genus *Sphaeromyxa* into two groups, the *balbianii* group with straight or slightly curved fusiform or ovoid spores, having ovoid polar capsules and the *incurvata* group with arcuate spores, having pyriform polar capsules. The species described above belongs to the *balbianii* group.

In the Argentine Sea, so far only one species of *Sphaeromyxa* has been recorded: *S. schulmani* Kovaleva & Gaevskaya, 1982 parasitizing *Salilota australis*. This species also belongs to the *balbianii* group; it can be distinguished from the new species by having smaller spores (18.6-20.0 µ × 4.0-5.98 µ), larger polar capsules (4.65-5.98 µ × 2.66-3.32 µ), smooth spore valves and one sporoplasm nucleus (Kovaleva & Gaevskaya, 1982).

Love & Moser (1983) cited the presence of *S. reinhardti* Jameson, 1929 in another engraulid species, *Engraulis mordax* from the Pacific coast of United States. This species belongs to the *balbianii* group, but its spores, according with the description of Jameson (1929) are larger than those of the new species (21.25-23.30 µ × 3.75-5.00 µ).

The new species resembles *S. balbianii* Thélohan, 1892, a parasite of several fish species from the Mediterranean Sea, the Adriatic Sea, the Atlantic coasts of Europe, Canada and the United States and West Africa (Senegal) (Laird, 1953; Khan *et al.*, 1986; Lubat *et al.*, 1989; Kpatcha *et al.*, 1996; Gracia *et al.*, 1997). *S. balbianii* differs from the new species by having wider spores (5.00-9.50 µ) and larger polar capsules (6.0-7.0 µ × 3.0-4.7 µ) (Laird, 1953; Khan *et al.*, 1986; Lubat *et al.*, 1989). Nevertheless, Kpatcha *et al.* (1996) reported a smaller length in the spores of *S. balbianii* from Senegal than those from European and North American waters (13.5-14.6 µ); these measurements are even smaller than those of the species described in the present paper.

The other members of the *balbianii* group are readily distinguished from the new species by having spores of different size (Noble, 1939, 1941; Laird, 1953; Schulman, 1966).

On the basis of the differences listed above, a new species, *Sphaeromyxa bonaerensis*, is proposed.

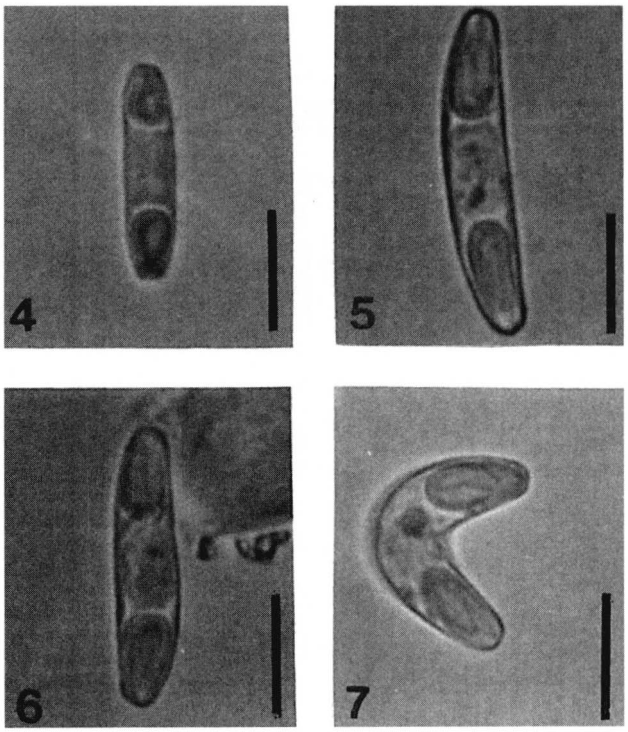
The values of prevalence in both host species suggest that *A. marini* is the main host for this myxosporean in the studied area. This assumption is supported by the presence of *S. bonaerensis* n. sp. only in specimens of *E. anchoita* proceeding from the area where both host species overlap their distributions (Northern zone) (Fuster de Plaza & Boschi, 1961).

SPHAEROMYXA ARGENTINENSIS N. SP. (Figs. 2-3, 5-13)

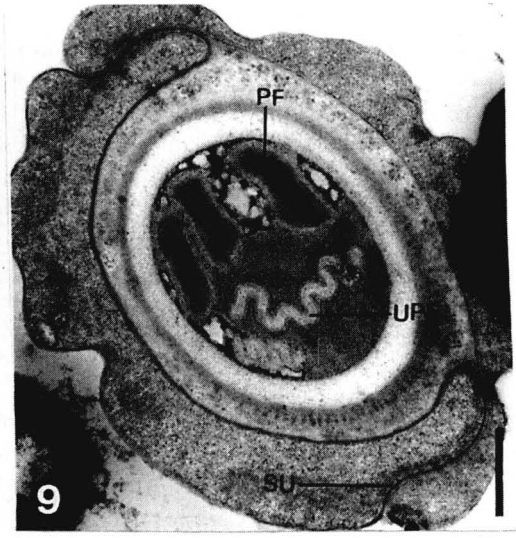
Type host: *Engraulis anchoita* Hubbs & Marini, 1935.
Other host: *Anchoa marini* Hildebrand, 1943.
Type material: Giemsa stained spores, LPNSM Collection number: 003/1; two fixed plasmodia, LPNSM Collection number: 003/2.
Location in hosts: gall bladder.
Locality: all sampled localities in the Argentine Sea.
Date: October-December, 1993; May, October, 1944; September-October, 1995.
Prevalence of infection: in *E. anchoita* 506 out of 1,891 (26.8 %), in *A. marini* one out of 99 (1.01 %).
Intensity (number of plasmodia per host) : one-four.
Etymology: the specific name is derived from the presence of this species along the coast of Argentina.

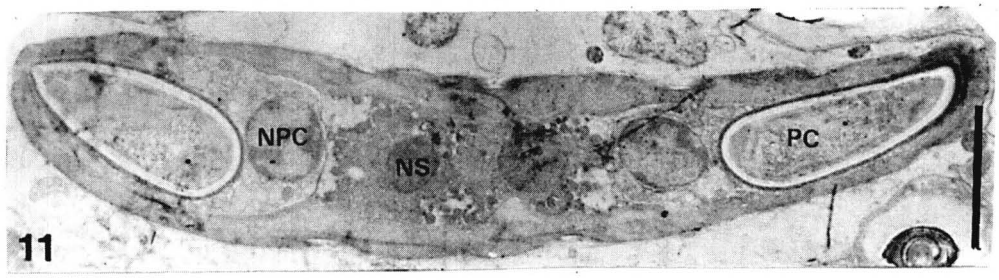
DESCRIPTION

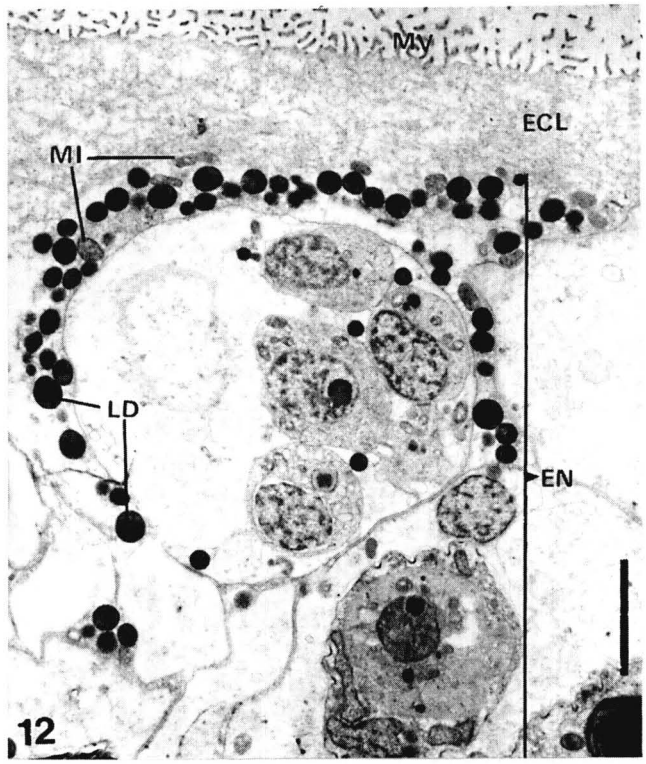
Spores (Figs. 2-3, 5-11): the spores are elongate and fusiform, arcuate in valvular view, sometimes

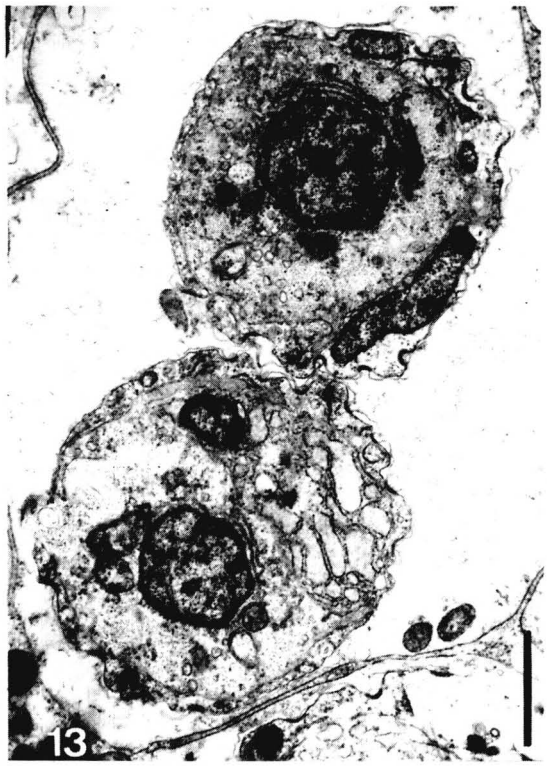


Figs. 4-7. – Fig. 4. *Sphaeromyxa bonaerensis* n. sp., spore, valvular view. Fig. 5. *Sphaeromyza argentinensis* n. sp., spore, valvular view. Fig. 6. *S. argentinensis* n. sp., "S"-shaped spore. Fig. 7. *S. argentinensis* n. sp., "U"-shaped spore. Bars: 10 µ.









Figs. 8-13. – Spores and plasmodium of *Sphaeromyxa argentinensis* n. sp. Fig. 8. SEM image of spore, VS: valvular striations (bar: 10 µm). Fig. 9. TEM image of cross section of developing spore, SU: suture, UPF: undulate section of polar filament, PF: polar filament (bar: 0.5 µm). Fig. 10. SEM image of spores. Fig. 11. TEM image of longitudinal section of developing spore, PC: polar capsule, SP: sporoplasm, NPC: nucleus of polar capsule, NS: nucleus of sporoplasm (bar: 10 µm). Fig. 12. TEM image of cross section of plasmodium, MV: microvillosities, ECL: ectoplasmic layer, EN: endoplasm, MI: mitochondria, LD: lipid droplets (bar: 4 µm). Fig. 13. TEM image of disporeous pansporoblast (bar: 2 µm).

"S"-shaped and exceptionally "U"-shaped; straight in sutural view and with bluntly rounded ends. SEM examination of spores evidenced longitudinally striated shell valves and "S"-shaped sutural ridge. The two equal pyriform polar capsules are situated on each end of the spore. TEM examination of spores showed the polar capsules opening sub-apically and the polar filament making five longitudinal folds in the capsule; the polar filament has an undulate proximal part in cross section and a compressed distal end. The capsular nuclei are sometimes visible beneath the polar capsules. The space between the polar capsules is filled with a finely granular sporoplasm. Two sporoplasm nuclei are often visible.

Measurements in micrometers, based on 20 spores from *E. anchoita* (Type host); mean is followed by the range in parentheses: spore length: 24.90 (23.25-27.50); spore width: 4.78 (4.50-5.25); spore thickness: 4.95 (4.50-5.25); polar capsule length: 7.25 (6.00-9.00); polar capsule width: 3.32 (2.55-4.50). Measurements of ten spores from *E. anchoita* were coincident with those from the Type host.

Vegetative form and sporogenic stages: the plasmodium is found floating freely in the gall bladder fluid, coiled upon itself. Unfolded plasmodium is discoidal to oval, measuring in fresh condition 1.9 to 4.4 by 2.0 to 4.7 mm, some of them exceeding the gall bladder itself in length and breadth.

TEM examination of plasmodium (Figs. 12-13) showed network of microvillosities covering its external surface. In cross section, an ectoplasmic layer covering a vacuolated endoplasm is observed. As the ectoplasm passes into the endoplasm, there is a concentration of mitochondria and lipid droplets. The endoplasm has a vacuolated appearance, with vegetative nuclei, generative cells, developing sporoblasts and ripe spores floating among the vacuoles. The pansporoblasts are all disporous.

REMARKS

The species described above is a member of the *incurvata* group (Laird, 1953); there are five species of *Sphaeromyxa* belonging to this group, which show resemblance in the spore size with the new species: *S. sabrazesi* Laveran & Mesnil, 1900, from the Mediterranean Sea and the Atlantic coasts of Europe (Laird, 1953, Lubat *et al*, 1989); *S. hellandi* Auerbach, 1909, from the Atlantic coasts of Norway and Barents Sea (Laird, 1953; Schulman, 1966); *S. maiyai* Morrison & Pratt, 1973 from the Oregon coasts (United States of America) (Morrison & Pratt, 1973); *S. dighae* Sarkar & Majumder, 1983 from the Bay of Bengala (India) (Sarkar & Majumder, 1983) and *S. bareni* Sarkar, 1984, from India (Sarkar, 1984).

However, according to Laird (1953), *S. sabrazesi* has slender spores (3.0-4.30 µ), longer polar capsules (8.0-10.0 µ) and truncated ends, although Lubat *et al.* (1989) reported larger sized spores (28-30 µ) and polar capsules (10.5 µ) in some specimens from the coasts of Montenegro (Adriatic Sea). *S. hellandi* differs from the new species in having widder spores (5.4 µ), larger polar capsules (10-10.8 µ) and truncate ends (Laird, 1953), although Schulman (1966) cited smaller spores (20.5-23 µ) for this species. *S. maiyai* shows widder spores (5.6 µ) and longer polar capsules (9.3 µ) (Morrison & Pratt, 1973). *S. dighae* has slender spores (3.33) and ellipsoidal polar capsules (2.32 µ) (Sarkar & Majumder, 1983). Finally *S. bareni* shows smooth and thicker spores (5.60-8.87 µ) and oval to ellipsoidal longer polar capsules (8.87-10.27 µ) (Sarkar, 1984).

Other described species, members to the *incurvata* group, differs from the new species in the dimentions of the spores and/or polar capsules (Laird, 1953; Sarkar, 1984; Schulman, 1966; Su & White, 1994).

The ultrastructural features of the plasmodia and the developmental stages of the spores agree with the findings of Lom (1969), exhibiting structural patterns apparently common to all species of *Sphaeromyxa*.

On the basis of the differences listed above, a new species *Sphaeromyxa argentinensis* n. sp. is proposed.

The values of prevalence in both host species suggest that *E. anchoita* is the main host for *S. argentinensis* n. sp. in the studied area.

EIMERIA PATAGONENSIS N. SP. (Figs. 14-21)

Type host: *Engraulis anchoita* Hubbs & Marini, 1935.

Type material: one parasitized testes preserved in formalin 4%, LPNSM Collection number: 004.

Location in host: testes.

Date: October, 1995.

Number of males parasitized: four of 892.

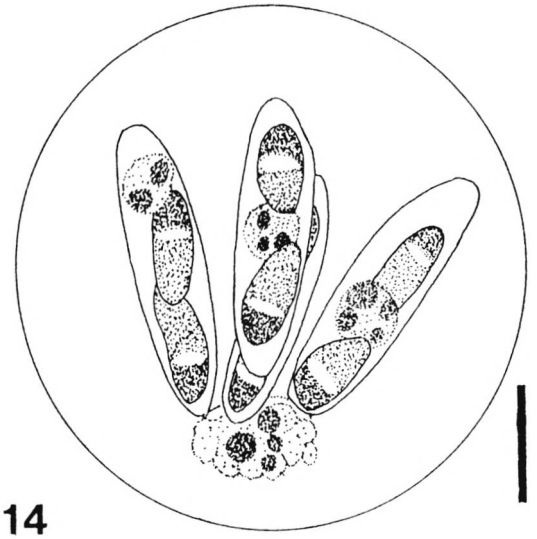
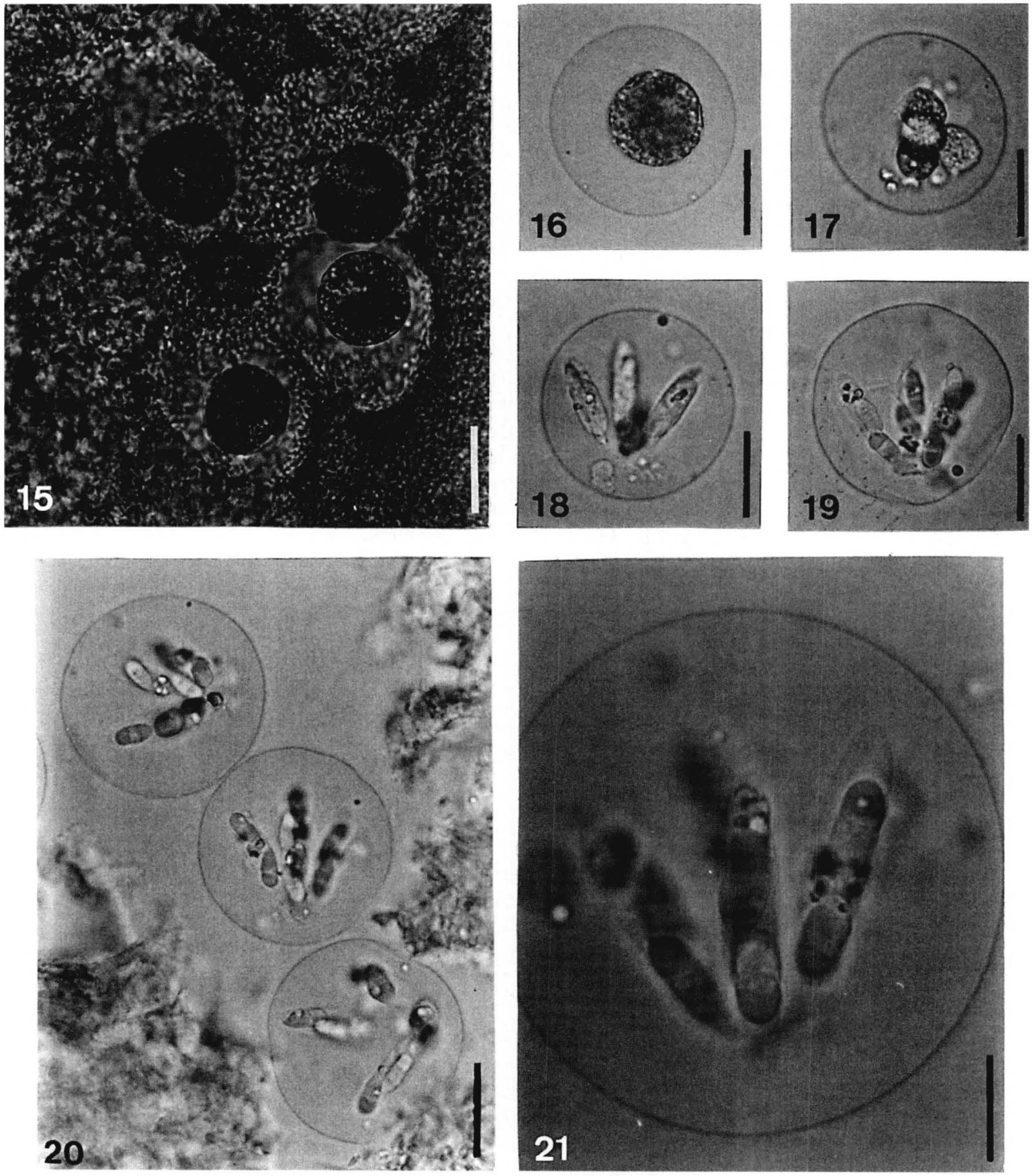


Fig. 14. – *Eimeria patagonensis* n. sp, mature oocyst (bar: 10 µm).



Figs. 15-21. – *Eimeria patagonensis* n. sp. Fig. 15. young oocyst in testes (tissue impression) (bar: 25 µm). Fig. 16. young oocyst (bar: 25 µm). Fig. 17. oocysts containing sporoblasts (bar: 25 µm). Fig. 18. oocysts containing developing sporocysts (bar: 25 µm). Figs. 19-20. mature oocysts (bar: 25 µm). Fig. 21. detail of mature oocyst (bar: 10 µm).

Prevalence of infection: including all the samples: four out of 892 males (0.45%); in the parasitized sample (42° 45'S - 60° 40'W): four out 26 (15.4%).

Etymology: the specific name refers to the geographic zone where the species was found.

DESCRIPTION

Undivided oocysts (Figs. 15-16), oocysts containing sporoblasts (Fig. 17) and oocysts containing developing sporocysts (Fig. 18) were observed. Mature oocysts (Figs. 14, 19-21) thin-walled and sphaerical and with no micropyle or polar granule. Oocyst residuum composed by three or four coarse refringent granules, immersed in a thin mass. Sporocyst elongated and fusiform, does not fill the entire oocyst, forming a triradiate star or a pyramid, confluent extremes in contact with the oocyst residuum. Stieda body absent. Sporozoites ovoid, not in contact with the sporocyst wall, laying head to tail with the sporocyst residuum at one end, or situated one after other with the sporocyst residuum in the center. Sporocyst residuum in form of two to four coarse refractile granules embedded in a sphaerical transparent mass.

Measurements in micrometers, based on 20 mature oocysts from only one fish; mean is followed by the range in parentheses: oocyst diameter: 43.3 (43.0-44.1); oocyst residuum: 12.7 (11.9-13.0) × 15.5 (15.1-16.5); sporocyst length: 31.9 (30.9-33.0); sporocyst width: 8.0 (7.2-8.2); sporozoite length: 10.3 (8.2-12.4); sporozoite width: 5.3 (4.1-6.2); sporozoite residuum diameter: 5.1 (4.1-6.2).

In undivided and immature oocysts from other host specimens, the measurements based on 20 specimens were: oocyst diameter: 57.1 (43.3-59.8); immature sporocyst length: 28.2 (25.8-30.9); immature sporocyst width: 7.5 (6.5-8.2).

REMARKS

Records of eimeriid species parasitizing the testes of fish form Argentina are inexistent. In the North Hemisphere, species belonging to the genus *Eimeria* have been reported from the testes of clupeiform fishes: *Eimeria sardinae* (Thélohan, 1895) from the Atlantic Ocean (Dyková & Lom, 1983; Lom & Dyková, 1992; Diouf & Toguebaye, 1994) and the Adriatic Sea (Daoudi *et al.*, 1989); *E. nishin* Fujita, 1934 from the Pacific Ocean (Arthur & Arai, 1980); *E. brevoortiana* Hardcastle, 1944 from the Atlantic (Hardcastle, 1944; Lom & Dyková, 1992; Upton *et al.*, 1984) and *E. ethmalosae* Diouf & Toguebaye, 1994 from the Senegal coasts (Diouf & Toguebaye, 1994).

The new species can be distinguished from all the species listed above by having a big oocyst residuum and by the arrangement of the sporocysts in the oocyst (triradiate star or pyramid).

The dimentions of the new species overlap the measurements range of *E. sardinae* given by Lom & Dyková (1992) (oocyst diameter: 40-60 µ, sporocysts: 25-35 × 7-8 µ), but this species has vermiform sporozoites and a small oocyst residuum. Daoudi *et al.* (1989) reported smaller sized oocysts (31-40 µ) in specimens from *S. aurita* from the Adriatic Sea, although the sporocysts have similar dimensions (24-32 × 7-9 µ) than those of the new species.

Diouf & Toguebaye (1994) found a great variability in the size of the oocysts of *E. sardinae* from *Sardinella aurita* and *S. maderensis* in Senegal coasts and reported the presence of Stieda body in giant-sized oocysts from *S. aurita*. Nevertheless, *E. sardinae* does not have Stieda body (Morrison & Hawkins, 1984); this feature, as well as the great variability of measurements found by Diouf & Toguebaye (1994), could indicate that there are more than one species parasitizing both hosts.

No differences between *E. nishin* and *E. sardinae* were found by Arthur & Arai (1980), who suggested that they might be considered as a synonymy.

The oocysts of *E. ethmalosae* are variable in size (27-53 µ) (Diouf & Toguebaye, 1994), but this coccidian differs from the species described above by having ellipsoidal sporocysts, Stieda body and vermiform sporozoites.

Eimeria brevoortiana has smaller oocysts and sporocysts (17.5-30 µ and 16.4 × 6.3 µ, respectively), and the sporozoites are cigar-shaped (Hardcastle, 1944; Upton *et al.*, 1984).

Based upon these differences and taking into account the host species and the geographic procedence of this coccidian, a new species, *Eimeria patagonensis*, is proposed.

The observed differences in size between mature and immature oocysts from different hosts could be due to the developmental stage and/or to a great intraspecific variability also observed in other species of *Eimeria* from the testes of fishes, such as *E. sardinae* and *E. ethmalosae* (Diouf & Toguebaye, 1994).

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