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# BIOLOGY OF NOTOCOTYLUS NEYRAI GONZALEZ CASTRO, 1945 (TREMATODA)

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**Abstract.** Snails of the species *Lymnaea truncatula* and rodents of the species *Arvicola sapidus* and *Apodemus sylvaticus* were found infested respectively by larval and adult stages of *Notocotylus neyrai* in two biotopes on the province Salamanca (Spain). The findings gave the possibility of reproducing the life cycle of this trematode experimentally in the laboratory and studying some aspects of the ecology of the cycle in the nature.

During the studies of the systematics and taxonomy of the genus *Notocotylus* Diesing, 1893, the members of which are parasites of rodents in Europe (Simon-Vicente et al. 1985), also the biology of one of these species, *N. neyrai* Gonzalez Castro, 1945 was studied in detail. The results obtained in the Iberian Peninsula are reported in further text.

## MATERIALS AND METHODS

Molluscs of the species *Lymnaea truncatula* (Müller, 1774) collected in the nature were transferred to the laboratory, where a possible emission of cercariae was tested. Groups of five snails were put into glass containers of 100 cm$^3$ containing a small pellet of loam, leaves and stems of plants and water, all things coming from the same place of collection.

The rediae were obtained by dissection and studied in vivo. The cercariae isolated after emergence were studied in vivo by normal optic and phase contrast microscopy. They were placed in fresh water with or without neutral red and also fixed in diluted formalin and coloured with borax and acetic carmine. The metacercariae for the study and experimental infestations were obtained after encystment of cercariae on glass walls or leaves, where they have attached. The experimental infestations of the definitive hosts were carried out by feeding them either with isolated metacercariae or together with leaves. Adult trematodes from natural and experimental hosts were studied in vivo or after fixation with formol and staining with borax carmine.

## RESULTS

### GENERAL SCHEMA OF THE CYCLE

According to the experimental studies, the life cycle of *Notocotylus neyrai* follows the following pattern (Fig. 1):

— the eggs are released by the definitive host to the external medium with excrements;

— they are then ingested by an amphibian or aquatic mollusc serving as intermediate host within which the miracidium gives rise to two generations of sporocysts (according to the knowledge about the biology of notocotylids (see Yamaguti 1975); the sporocysts could never be found in our studies);

— the daughter sporocysts will probably give rise to two generations of rediae, in which the cercariae develop (rediae producing rediae have never been found in our studies);

— the cercariae of monostome type escape from the mollusc and live freely in water for very short time. Then they encyst on solid objects (vegetation in the nature, walls of glass container in the laboratory) giving rise to the stage of metacercaria;

— the definitive host, a mammal of the rodent group, becomes infested by ingestion of encysted metacercariae together with the vegetation. The adults develop in its caecum.

## DESCRIPTION OF LARVAL STAGES

The sporocysts and mother rediae have never been found in our studies in the nature. Therefore, the life cycle has been experimentally followed only starting with the daughter rediae producing exclusively cercariae.

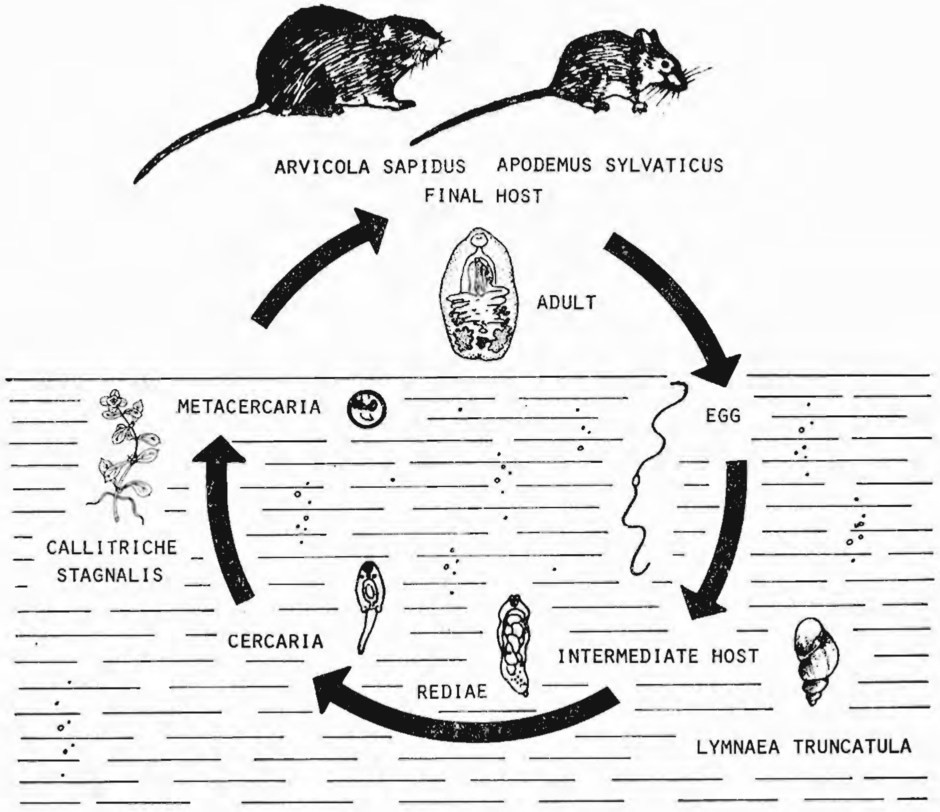

Fig. 1. Life cycle of *Notocotylus neyrai* Gonzalez Castro, 1945: schema showing the main pathways it follows in Salamanca (Spain).

### Rediae

The young rediae are white, elliptical and about 340 μm in size. Their digestive apparatus constitutes of a pharynx measuring 50—60 μm in diameter and a sacciform intestine. The body of these radiae contains numerous germ balls of different sizes and of granular appearance (Fig. 2A). The rediae measuring 700—800 μm and observed in vivo usually have a spheroidal diverticle at the posterior extremity joined to the body by a narrow contraction (Fig. 2B). The mature rediae are brown-black in color and measure 900-1 450 μm. They have more or less pronounced contractions at different parts of the body (Plate I, Fig. 1). Some of these rediae have a small terminal button (Fig. 2C). The intestine reaches an equatorial or post-equatorial level. They lack locomotor appendages and the birth pore is situated slightly anterior to midbody.

### Cercariae

The immature, intraredial cercariae are little pigmented, shortailed and with only lateral eyespots, not yet totally condensed (Fig. 2C). The mature, just emerged cercariae (Fig. 2D) are 325—435 μm long and 175—210 μm in maximal width in fixed specimens. The subterminal sucker has a diameter of 50—60 μm. The central eyespot, a little more elongate and less compact than the lateral ones, is situated 86 μm from anterior extremity and measures 20—25 μm. The lateral eyespots measure 19—22 μm and are situated more posteriorly.

The cystogenic cells are scattered throughout the body so that it is difficult to observe the digestive apparatus and primary and secondary excretory canals. These cutaneous gland cells are round, measure 15—18 μm and contain small, elongate, 3—4 μm large bodies.

There are two locomotor appendages. They are tuberous and contractile, localized on the posterior lateral parts of body, on both sides of the tail (Plate II, Fig. 1). The frontal section of one of these appendages is illustrated in Fig. 2E.

The excretory ring is of "monostomal" type. It is full of refringent granules. The flame cells are situated between its external outline and the body wall. Only 12 flame cells at each side could be counted with certainty because of the obstruction by the cystogenic cells. Two protonephridia greater or more patent than the others could be seen on the group of flame cells situated between the sucker and eyespots and on that localized on each posterior lateral extremity.

The tail is 510—540 μm long in the fixed specimens, but reaches 620 μm in the fresh, not fixed ones. The width of the tail at the level of its base is 54—60 μm. The tail possesses a central canal all along its length. This canal has no bifurcations, but it has irregular margins. In vivo, numerous small refractile granules move along it. At a slightly postequatorial or at midlevel of the tail, a terminal bifurcation of the excretory system opening in two always asymmetrical pores can be observed. It is impossible to elucidate if these excretory pores are functional or not (different authors mentioned the fact that the caudal tubes collapse and disappear and the original pores become functionless (Yamaguti 1975). But the impossibility of distinguishing the existence of the excretory central canal, which establishes the relationship between the excretory vesicle and the above-mentioned final bifurcation, is perhaps significative.

Attempts to study the cercarial chaetotaxy by impregnation with $AgNO_3$ never gave good results, because of the speediness of the beginning of the encystment process. Anyhow, the numerous preparations done allowed to deduce some aspects.

The chaetotaxy of the cercaria is very complicated, with a concentration of papillae on the oral sucker. The tail bears 8 papillae distributed laterally in 4 paires, 3 of them equidistant and anterior to the excretory pores and the last fourth pair more or less at midway between these pores and the end of the tail (Fig. 2D). This number and distribution of caudal papillae seem to be unique among the hitherto known cercarial chaetotaxies (Richard 1971, Bayssade-Dufour 1979).

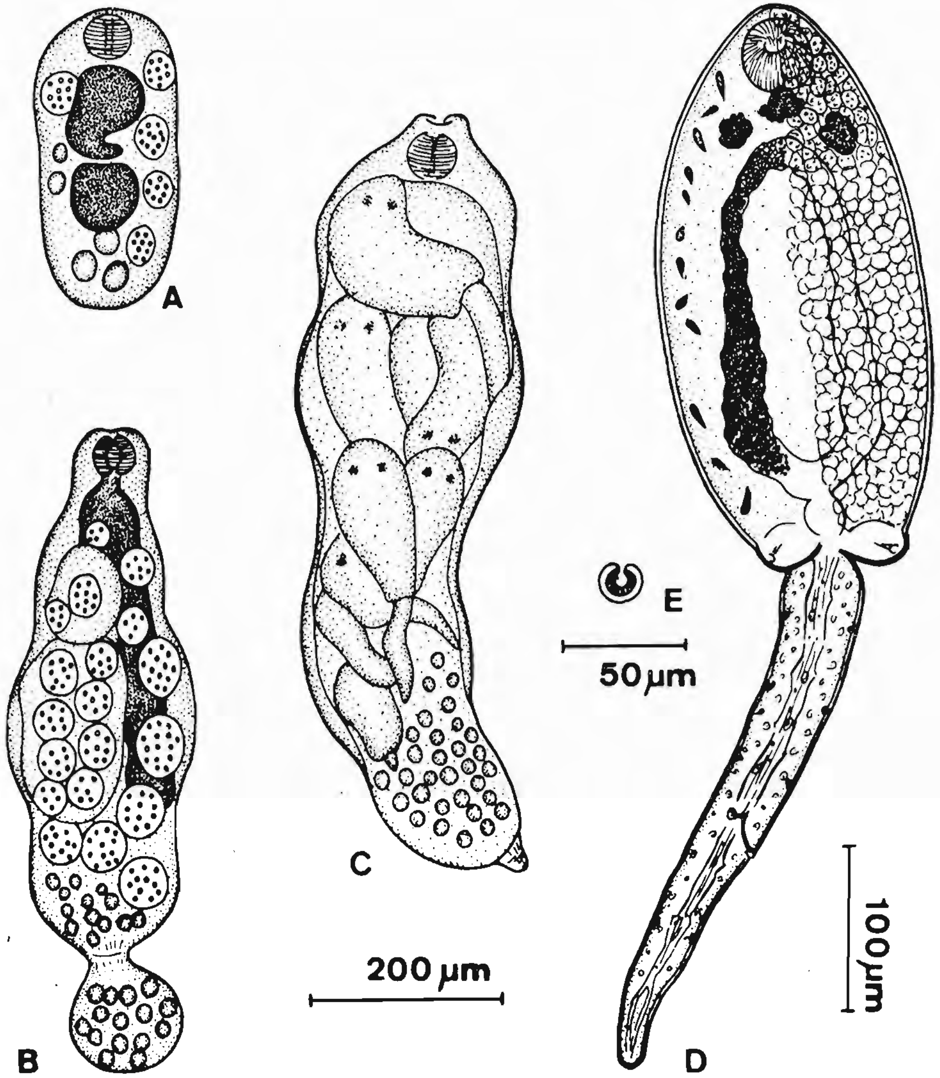

Fig. 2. Larval stages of *Notocotylus neyrai* Gonzalez Castro, 1945. A — young redia, B — immature redia showing posterior spheroidal diverticle, C — mature redia showing terminal bottom, D — mature, just emerged cercaria, E — frontal section of locomotor appendage. A, B, C: scale 200 µm; D: 100 µm; E: 50 µm.

### Metacercariae

The cyst, just formed, has a dimeter of 175—180 µm (Plate I, Fig. 3). Its form is like a flat convex or semispheroidal lens. The brilliant and transparent wall is 6—8 µm wide, increasing a little posteriad. Outside water, the wall loses quickly its transparency and buckles by drying.

## ECOLOGY OF THE CYCLE

### Characterization of the studied biotopes

Both selection places studied, 80 km distant from each other, have similar characteristics. They are land areas with slight inclination and moistened by water drainages, which run into bordering ponds forming water streams of little velocity and scarce depth. The place situated near the village of Los Villares de la Reina is a land canal 4 m wide and 300 m long, with lateral side-slopes of 2 m mean height. In the place near Guadramiro, the water running follows the soil gradient through a course less deep than the above-described one.

The vegetation is typical of communities of little deep watercourses and ponds of fresh water, which may dry in summer (class Potametea R. Tx. et Preising, 1942). The most frequent plants at both places are *Nasturtium officinale*, *Callitriche stagnalis*, *Lemna minor* and *Stellaria media*.

On borders, at water line or at higher levels, the entrances of the refuges of *Arvicola sapidus* can be found. It is a rodent species with clear aquatic preferences, which deposit its excrements on borders, principally on clay-sand soil, near water line (Plate II, Fig. 2). *Lymnaea truncatula* is on the loam at borders or in the water, on dead waters and small pools covered by a water layer of few centimeters.

### Parasitation percentages found in molluscs in the nature

Seasonal observations done during several consecutive years on the biotope near Los Villares de la Reina gave significative data.

From December till February no specimen of *L. truncatula* can be found on the ground. At the end of this last month, if the temperature is moderately temperate, the molluscs begin to appear at places covered by water. They measure 7—10 mm and may be infested by young rediae at percentages of 4—5 %. In the following months their number increases, being very high in May—June and sometimes the parasitation reaches 50 % or more. There are a lot of mature rediae. The emergence of cercariae, which is slow and discontinuous in March, is more rapid and constant at the end of spring.

The population of snails appeared at the end of winter and consisting of survivors of the precedent year according to their size, increases in spring with other new generations. During summer the small watercourses diminish and there remain zones with scarce humidity on the borders. High mortality takes place, affecting specially molluscs of greater size. In July—August, *L. truncatula* disappears from the habitat.

From the middle of September till November, the presence of snails can be observed, but usually in a lower number than in spring. The parasitation continues during these months showing the mean percentages of 30 % and superior limit values similar to those observed at the end of spring but always greater than 3 mm in populations of heterogeneous size.

The studies on the parasition of molluscs by larval stages of trematodes in the nature did not concern only *L. truncatula* in the above-mentioned locality, but comprised all species of snails at 40 different places of the province of Salamanca during 3 years including seasonal and mor or less continuous studies. The results of these studies (Simón-Vicente 1979) revealed a marked specificity of the larval stages of *N. neyrai* to *L. truncatula*. The existence of several species of Planorbidae (species of the genera *Planorbis* and *Planorbarius*) and Physidae (species of the genus *Physa*) and even other species of the same genus *Lymnaea* (*L. auricularia*, *L. lagotis*, *L. stagnalis*) should also be considered in the same biotopes. The above-mentioned species, genera and families of molluscs are usual intermediate hosts of *Notocotylus* species (Yamaguti 1975).

## Emergence and behaviour of cercariae

The molluscs collected in May, maintained at 18—20 °C under artificial light in the laboratory, begin to shed cercariae at 8 a. m. in fresh-water coming from the natural habitat. The emergence continues till 12 a.m. of the same day. The cercariae usually emerge from the snail isolately, one by one, with irregular intervals ranging between 1 and 10—15 minutes. Sometimes two cercariae come out almost successively. They show a marked phototropism, accumulating in zones where light falls into. The cercariae swim shaking vigorously the tail, remembering the fly of a butterfly. They stop sometimes on the surface of the glass walls or on the leaves and stems of plants, trying them with their locomotor appendages and contracting and extending the tail.

After swimming for 15—30 min, the cercariae attach to the glass with their suckers, shake strongly the tail to the sides and after 5 min or less, they lose it. Then they begin to turn round, while the cystogenous cells discharge their contents forming the cyst (Plate I, Fig. 2).

The metacercariae encysted on the shell of snails have never been found, either in the natural medium or in the laboratory. In the laboratory, the majority of them encyst on the glass wall nearer to the light source. Scarce cercariae encyst on the bottom or on the leaves and stems collected in the natural biotope and put into a glass container.

In the natural habitat, a great number of metacercariae are found on floating leaves attached to their inferior face which is always contacting water, in spite of the changes of water level. Metacercarial cysts are almost never found on petioles or stems of plants. The leaves of *Callitriche stagnalis* usually carry a greater number of the attached cysts. Only scarce metacercariae attach to other species of plants and apparently none to *Lemna minor*.

## Natural and experimental infestation of definitive hosts

Respecting nature, 3 specimens of *Apodemus sylvaticus* (L.) (Muridae) and 2 of *Arvicola sapidus* (Miller) (Arvicolidae) were captured in the biotope near Los Villares de la Reina between February and May. Two of *A. sylvaticus* harbored 8 gravid adults of *N. neyrai*, whereas 250 and 920 adults of the same species at different development stages could be found in the two *A. sapidus* specimens, respectively. This suggests accumulative infestations. At the biotope near Guadramiro, 4 *A. sylvaticus* captured were negative, whereas 1 *A. sapidus* harboured 124 adults of the trematode species in question.

In the laboratory, a series of feeding experiments was carried out with different bird and mammal species as possible definitive hosts in order to evaluate the specificity sensu stricto of the adults of *N. neyrai*. The results are shown in detail in Table 1. They suggest a marked specificity of the digenean species for mammals of the rodent group. The dimensions and characteristics of the adult specimens experimentally obtained from mouse and rat are given in Table 2. No preference for any rodent species could be distinguished.

**Table 1.** Summary of experimental infections of *Notocotylus neyrai* in bird and mammal hosts

| Experimental hosts | | | Metacercariae | | | Autopsy | | |
|---|---|---|---|---|---|---|---|---|
| Species of exposed hosts | Age of exposed hosts | No. exposed hosts | No. feeded metacercariae per host | Age of feeded metacercariae | Provenance of metacercariae | Days after exposition | Results | Flukes obtained |
| Chicken | 8 days | 3 | 200<br>+200 | 21 days<br>22 days | glass walls<br>glass walls | 8 | — | — |
| Chicken | 8 days | 2 | 400 | 21 days | glass walls | 16 | — | — |
| Cock | 1 month | 1 | 150 | 8 days | glass walls | 15 | — | — |
| Pigeon | unknown | 1 | 100 | 1 week | leaves of plants | 25 | — | — |
| Duck | young | 2 | 200 | 1 week | leaves of plants | 20 | — | — |
| Mouse (lab. white × wild-trapped) | unknown | 1 | 80 | 8 hours | glass walls | 17 | — | — |
| Mouse (wild-trapped) | unknown | 1 | 80 | 1 week | glass walls | 30 | + | 15 adults (12 gravid +3 immature) |
| Rat (lab. white) | unknown | 1 | 80 | 1 week | glass walls | 18 | + | 8 adults (3 gravid +5 immature) |
| Guinea-pig | unknown | 1 | 200 | unknown | leaves of plants | 14 | — | — |
| Guinea-pig | unknown | 1 | 200 | unknown | leaves of plants | 14 | + | 5 adults (all immature) |

## Modalities of the life cycle in the nature

According to the studies done in the natural biotopes and in the laboratory, the life cycle of *N. neyrai* develops in nature as follows:

— first intermediate host: *Lymnaea truncatula;*

— encystment substratum of metacercariae: preferably leaves, rarely petioles or stems, of fresh-water plants, principally *Callitriche stagnalis;*

— definitive hosts: *Arvicola sapidus, Apodemus sylvaticus.*

The strict specificity (= oioxeny) of this digenean species for *L. truncatula* as intermediate host is worth mentioning. It was verified in Salamanca province, and shall probably occur in the whole Iberian Peninsula. Malacological research studies

**Table 2.** Morphoanatomic comparison of experimentally obtained adults of *Notocotylus neyrai*

| Experimental host<br>Ranges and means | Mouse | | White rat | |
|---|---|---|---|---|
| | E. V. | $\bar{X}$ | E. V. | $\bar{X}$ |
| Length | 1350—3400 | 2120 | 2200—2500 | 2310 |
| Maximum width | 610—1400 | 920 | 924—1104 | 1010 |
| Oral sucker | 157—267 | 212 | 202—224 | 215 |
| Testis | 350—420<br>380—490 | 385<br>435 | 386—470 | 432 |
| Ovary | 192—310 | 268 | 303—450 | 397 |
| Cirrus sac | 488—650 | 565 | 598—644 | 621 |
| Cirrus (evag. part) | 157—374 | 257 | 368 | — |
| Metraterm (total length) | 112—156 | 131 | 138—156 | 147 |
| Metraterm/cirrus sac ratio | 1/4.5 | — | 1/4.3 | — |
| No. ventral papillae/row | 12—14 | — | 12 | — |
| No. vitelline follicles/side | 6—9 | — | 8—9 | — |
| Eggs | 18—20/10—12 | — | 19—21/12.8—14.2 | — |

All measurements in µm. E.V. = extreme values (= ranges); $\bar{X}$ = means. Measurements done by F. Simón-Vicente.

done in the South (Granada) and centre (Soria) of Spain have shown the existence of specimens of *L. truncatula* in biotopes of similar characteristics, shedding triocellate cercariae with the same morphoanatomic features as those of *N. neyrai*, and presumably belonging to the same species. The snail *L. truncatula* becomes a new intermediate host for *Notocotylus* species. Anyhow, other species of the genus *Lymnaea* have already been mentioned acting as hosts of the larval stages (Yamaguti 1975).

The marked ecological specificity of the adults for *A. sapidus* is undoubtedly related to the aquatic ethology of this arvicolid rodent all over the Iberian Peninsula and to its eminently herbivorous diet. The evident preference of encystment of metacercariae on leaves of *C. stagnalis* is clearly related to these facts. The very different ethology and diet of *A. sylvaticus* explain the lower parasitation frequency and adult fluke number in this murid, in spite of the coexistence of both rodent species in the same biotopes.

## DISCUSSION

Similarly as the adult forms, the larval stages also pose great problem in identification and differentiation. In addition to great morphoanatomic similarities, there are the problems caused by the little clear correlation between the larval forms and intermediate hosts (Stunkard 1966).

Among the Notocotylinae species parasitizing mammals listed by Yamaguti (1971, 1975), those whose life cycles are well known are:

— *Notocotylus mamii* Hsü, 1954: known from China, it uses a prosobranchial mollusc of the genus *Melania* as intermediate host and was obtained from naturally infested aquatic birds and experimentally only from rabbits (Hsü 1957);

— *Notocotylus noyeri* Joyeux, 1922: known from Europe, it has planorbids of the genera *Bathyomphalus* and *Anisus* as intermediate hosts and *A. terrestris* and other arvicolids as definitive hosts; attempts of experimental infestation of birds (*Fulica atra, Gallus bankiva, Anas plathyrhynchos*) were unsuccessful (Odening and Bockhardt 1965, Odening 1966);

— *Quinqueserialis quinqueserialis* (Barker et Laughlin, 1911) Hardwood, 1939 (Syn.: *Notocotylus urbanensis* sensu Harrah, 1922 pro parte): known from North America and Europe, it uses species of *Lymnaea* and *Physa* as intermediate hosts in the USA and rodents as definitive hosts in the nature; experimental adults have been also obtained in chicken and ducks (Acholonu and Olsen 1967).

From the morphoanatomic and biologic points of view, it is evident that the Notocotylinae species studied in Berlin (G.D.R.) by Odening and Bockhardt (1965) and Odening (1966) under the name *Notocotylus noyeri* is the species showing characteristics more similar to the digenean studied in the present paper. According to the morphoanatomy of the adult and to the conclusions reached in the above exposed systematic part of the paper, in our opinion the species studied by the German authors was in reality *N. neyrai*, that is, the same species we have studied in Salamanca (Spain). From the bioecologic point of view, clear similarities can also be observed. Clear similarities are the marked specificity of adults for rodent host, the morphologic characteristics of rediae lacking lateral apophysis and the evident similarity of cercariae concerning body size, dimensions of oral sucker, nature of eyespots, and form and situation of the excretory ring, typical of the "monostomi" group.

Anyhow, several differences between the observations of Odening (1966) and ours can be distinguished. First, this author did neither mention nor draw the posterolateral locomotor appendages of the cercaria. These organs are very conspicuous in the Spanish cercariae (Plate II, Fig. 1) similarly as in cercariae of other species of the genus *Notocotylus* ("grubige Organe" of Szidat 1930, and Yamaguti 1938; "eversible and retractile locomotor appendages" of Stunkard 1966). Odening (1966) omitted their reference, probably presuming their normal presence. Respecting the excretory apparatus, the number of flame cells seems to differ. Nevertheless, nothing can be assured to this point, because, unfortunately, we cannot consider our observations as definitive. Odening (1966) mentioned a total of 36 flame cells, a number which he considers typical of all species of the genus. However, this number does not agree with the observations of Stunkard (1966) on the species *N. atlanticus* Stunkard, 1966.

But, if the above listed morphoanatomic differences are insignificant and undoubtedly product of incomplete observations, there are two very remarkable bioecologic differences:

— the specificity at the level of intermediate molluscan host: according to Odening (1966), in Germany the trematode is oligoxenous, using different species of aquatic snails of the family Planorbidae, which never come out of water (*Bathyomphalus contortus, Anisus leucostomus* and *A. vortex*); in Spain the digenean is oioxenous, parasitizing only one species of amphibious snail of the family Lymnaeidae, which usually comes out of water and remains on loam (*L. truncatula*); it shall be added here that Odening (1966) considers that the findings of the cercariae "*Cercaria ephemera*" from *A. vortex* by Wesenberg-Lund (1934), of *Notocotylus* sp. and *N. ephemera* from *Gyraulus albus* by Žďárská (1963, 1964), and of *Cercaria fischeri* from *Planorbis carinatus* by Dönges (1962) probably refer to the same species studied and named *N. noyeri* by him (= *N. neyrai* after our conclusions);

— the behaviour of the cercaria in relation to the preferred substratum for encystment: according to Odening and Bockhardt (1965) and Odening (1966), in Berlin the encystment takes place simply on hard and flattened objects in water; Odening (1966) figures shells of molluscs and aquatic insects at the corresponding level within the life cycle schema. Anyhow, this fact does not agree with the eminently herbivorous diet of all natural (non experimental) definitive hosts, which the German author lists according to the paper of Petrov and Chertkova (1962) (Arvicolidae: *A. terrestris*, *A. sapidus*, *Microtus oeconomus*, *M. agrestis* and *Clethrionomys glareolus*); in Salamanca (Spain) the trematode shows a marked preference for encysting on vegetation, above all on aquatic plants with floating leaves such as *Callitriche stagnalis*.

In our opinion, both above-mentioned small bioecological differences are not enough for distinguishing the *Notocotylus* of Germany and Spain at species level. Thus, they shall be considered as biological strains or geographic races, even perhaps with taxonomic category of subspecies (Macko 1983), as a consequence of different adaptation phenomena according to the offerings and availabilities of the natural medium and the inhabiting molluscs and rodents. Only the elucidation of different cercarial chaetotaxies would allow to sustain two different species, in spite of the impossibility of morphoanatomic differentiation at all other levels, larval and adult stages (as has been demonstrated, it may occur in other genean families such as Brachylaimidae and Plagiorchiidae). Nevertheless, the speediness of the action starting of the cystogenous cells makes the realization of chaetotaxic preparations very difficult.

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## БИОЛОГИЯ ТРЕМАТОДЫ *NOTOCOTYLUS NEYRAI* GONZALEZ CASTRO, 1945 (TREMATODA)

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**Резюме.** Обнаружены моллюски вида *Lymnaea truncatula* и грызуны *Arvicola sapidus* и *Apodemus sylvaticus*, зараженные личинками и половозрелыми экземплярами *Notocotylus neyrai*, в двух биотопах провинции Саламанка (Испания). На основе этих находок в лаборатории экспериментально репродуцирован жизненный цикл этой трематоды и изучены некоторые аспекты экологии этого цикла в природе.

## REFERENCES

ACHOLONU A. D., OLSEN O. W., Studies on the life history of two Notocotylids. Proc. Helm. Soc. Wash. 34: 43—50, 1967.

BAYSSADE-DUFOUR Ch., L'appareil sensoriel des cercaries et la systématique des Trématodes Digénétiques. Mém. Mus. Nat. Hist., Mat. sér. A, Zool. 113: 3—81, 1979.

DÖNGES J., Entwicklungsgeschichtliche und morphologische Untersuchungen an Notocotyliden. Z. Parasitenkd. 22: 43—67, 1962.

HSÜ P. K., On the life history of *Notocotylus mamii* Hsü, 1954. Acta Zool. Sinica 9: 121—128, 1957. (In Chinese.)

MACKO J. K., On the subspecies in parasites with respect to helminths. Folia parasit. (Praha) 30: 107—116, 1983.

ODENING K., Physidae und Planorbidae als Wirte in den Lebenszyklen einheimischer Notocotylidae (Trematoda: Paramphistomida). Z Parasitenkd. 27: 210—239, 1966.

—, BOCKHARDT I., Der Entwicklunszyklus des Trematoden *Notocotylus noyeri* Joyeux, 1922, im Raum Berlin. Deutsch. Akad. Wiss. Berlin 7: 51—52, 1965.

PETROV A. M., Trematodes of subfamily Notocotylinae Kossack, 1911, parasitic in rodents. Tr. VIGIS 9: 91—102, 1962. (In Russian.)

RICHARD J., La chétotaxie des cercaires. Valeur systématique phylétique. Mém. Mus. Nat. Hist. Nat., nouv. sér. A, Zool. 67: 1—179, 1971.

SIMON-VICENTE F., *Lymnaea truncatula*, hospedador intermediario de *Notocotylus neyrai* (= *N. noyeri?*). I. Cong. Nac. Parasit. (Granada, 29. 9.—2. 10. 1976), sec. G (1): 67, 1976.

—, Trematodes larvarios y sus moluscos hospedadores en Salamanca. Rev. Ibér. Parasit. 39: 241—250, 1979.

—, MAS-COMA S., LOPEZ-ROMAN R., TENORA F., GALLEGO J., Review of *Notocotylus* species (Trematoda: Notocotylidae) parasitizing rodents in Europe. Folia parasit. (Praha) 32: 21—33, 1985.

STUNKARD H. W., The morphology and life-history of *Notocotylus atlanticus* n. sp., a digenetic trematode of eider ducks, *Somateria mollissima* and the designation of *Notocotylus duboisi* nom. nov., for *Notocotylus imbricatus* (Looss, 1893) Szidat, 1935. Biol. Bull. 131: 501—515, 1966.

SZIDAT L., Die Parasiten des Hausgeflügels. 4. *Notocotylus* Diesing und *Catatropis* Odhner, zwei, die Blinddärme des Geflügels bewohnende monostome Trematodengattungen, ihre Entwicklung und Uebertragung. Arch. Geflügelkd. 4: 105—114, 1930.

WESENBERG—LUND C., Contribution to the development of the Trematoda Digenea. Part II. The biology of the fresh-water cercariae in Danish freshwaters. Mém. Acad. Roy. Sci. et Lettres Danemark, sect. Sci. 5: 1—223, 1934.

YAMAGUTI S., Zur Entwicklungsgeschichte von *Notocotylus attenuatus* (Rud., 1809) und *N. magniovatus* Yamaguti, 1934. Z. Parasitenkd. 10: 288—292, 1938.

—, Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. Keigaku Publishing Co., Tokyo, 1074 pp., 1971.

—, A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates. Keigaku Publishing Co., Tokyo, 590 pp., 1975.

ŽĎÁRSKÁ Z., Larval stages of trematodes from aquatic snails in the territory of Czechoslovakia. Čs. Parasitol. 10: 207—262, 1963. (In Czech.)

—, Contribution to the development and species independence of *Notocotylus ephemera* (Nitzsch, 1807) (syn. *N. thienemanni* Szidat L. et Szidat U., 1933). Čs. Parasitol. 11: 309—318, 1964. (In Czech.)

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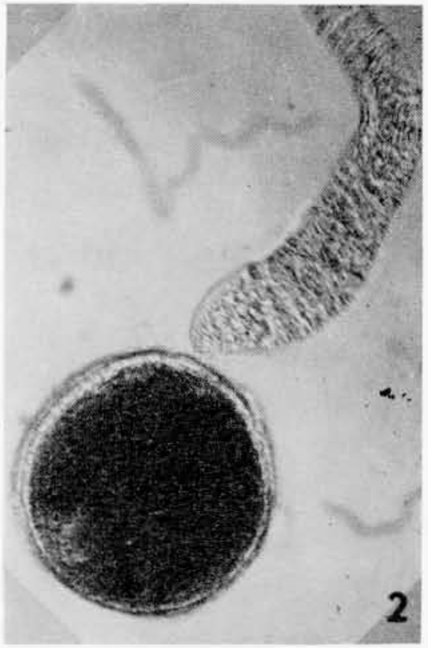

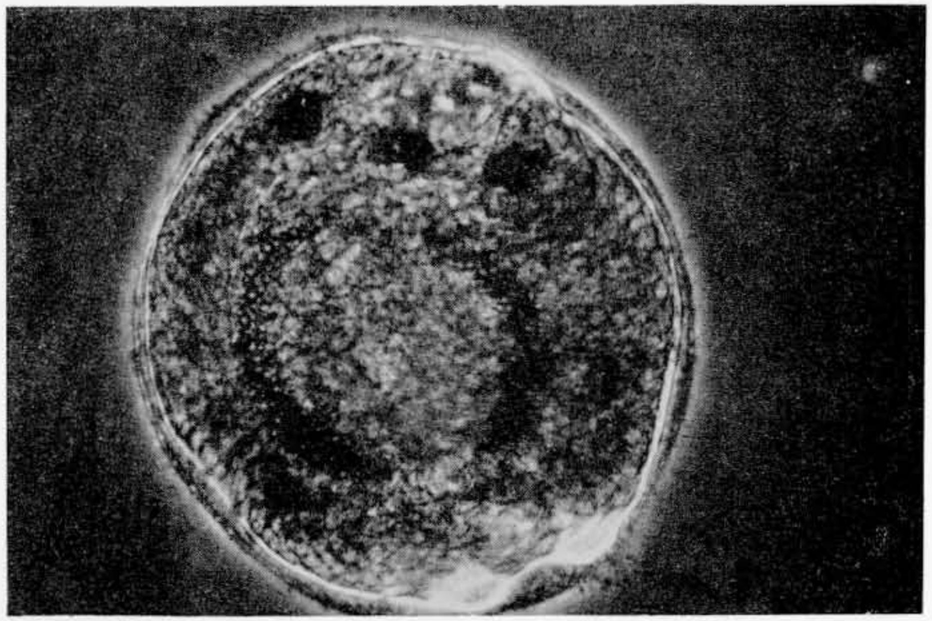

**Fig. 1.** *Notocotylus neyrai* Gonzalez Castro, 1945: rediae in vivo (×60).
**Fig. 2.** *Notocotylus neyrai:* cercaria just before losing the tail in first moments of encysting (×200).
**Fig. 3.** *Notocotylus neyrai:* just encysted metacercaria (×450).

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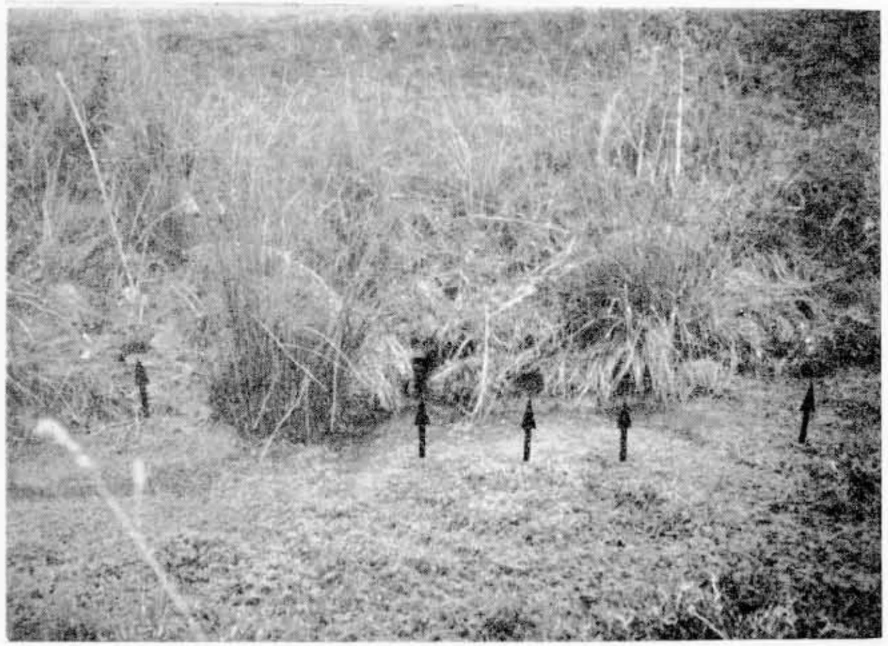

**Fig. 1.** *Notocotylus neyrai* Gonzalez Castro, 1945: posterior part of cercarial body showing the two locomotor appendages on terminal corners and the base of the tail (×550).
**Fig. 2.** *Notocotylus neyrai:* aspect of the biotope studied near the village Los Villares de la Reina (prov. Salamanca, Spain), where the life cycle of the Digenean takes place (April 1977): note the entrances of the refuges of *Arvicola sapidus* (arrows).