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A new type of endogenous asexual proliferation in cyclophyllidean metacestodes

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Abstract

We describe 4 metacestodes classified according to their morphology as belonging to the genus *Mesocestoides* (Cest(Cyclophyllidea) in the abdominal cavity of an individual wood mouse, *Apodemus sylvaticus* (Rodentia, Muridae) trapped Spanish Mediterranean ecosystem. The morphological study of the metacestodes shows evidence of three types of asexual liferation: longitudinal fission (presence of supernumerary suckers in the scolex); budding; and a third form of asexual c sion not previously described in cestodes, with a mother tetrathyridium containing some daughter metacestodes (endopol ny). For the first time we have demonstrated different mechanisms of asexual division in a tetrathyridium-type metacest apparently of the genus *Mesocestoides*. Furthermore, this is the first report of endogenous asexual proliferation in any meta tode exhibiting primitive or gymnosonic development.

Key words

Mesocestoides, Cyclophyllidea, Cestoda, asexual division, proliferative metacestodes, *Apodemus sylvaticus*, Rodentia.

Introduction

Asexual proliferation among cestodes is very restricted, being almost exclusively confined to three cyclophyllidean families: Mesocestoididae, Hymenolepididae and Taeniidae. Several reports of anomalous proliferating cestodes from human and other hosts have been tentatively called *Sparganum proliferum*, and identified as pseudophyllidean plerocercoids, but the taxonomic status of these aberrant acephalic forms is uncertain (Kuntz *et al.* 1970, Noya *et al.* 1992). Unlike the Digenea, asexual proliferation of developmental stages within intermediate hosts is not an invariant part of cestode life-cycles. The phenomenon is, in fact, only relatively common in the family Taeniidae. In all cases where asexual proliferation does occur, it is characteristic of the metacestode developmental stage.

The process occurs in both of the major modes of metacestode ontogeny, that is, primitive or gymnosomic development which progresses without the production of an initial internal cavity or primary lacuna, in the metacestode, and neoteric or cystosomic development, which is characterized by the formation of this primary lacuna (Freeman 1973, Ubelaker 1983, Whitfield and Evans 1983). Although known in both types of development, asexual proliferation is much more common in species using the neoteric mode.

Among Cyclophyllidea, there is only one species primitive development which exhibits asexual prolifera the tetrathyridium of *Mesocestoides corti* Hoeppli, 192 *Mesocestoides vogae* (Etges, 1991)]. The tetrathyridium metacestode of the acetabulo-plerocercoid type, and in species proliferation is by fission (Specht and Voge 1 Hess 1972, Novak 1972, Etges 1991). Such fission requ the presence of at least one sucker (Hart 1968). Hess (1 reported an unique mitotically active "apical massif" betw the suckers of this species, and suggested that this organ responsible for the asexual fission. This interpretation supported by Conn (1988), who demonstrated that non- liferative tetrathyridia of *Mesocestoides lineatus* lack an cal massif.

Among the species exhibiting neoteric development, t types of metacestodes are distinguishable (Whitfield Evans 1983, Loos-Frank 2000). The first type includes t forms with exogenous scolices elaborated asexually on a gle metacestode. Within this group, it is possible to disting two subtypes of multiplying metacestodes: cysticercus- and strobilocercus-like. The first subtypes would compris species and subspecies: *Taenia crassiceps* (Zeder, 18 *T. endothoracicus* (Kirschenblatt, 1948), *T. martis m* (Zeder, 1803), *T. multiceps* Leske, 1780, *T. mustelae* Gm 1790, *T. pisiformis* (Bloch, 1780), *T. polyacantha polyac ha* Leuckart, 1856, *T. serialis serialis* (Gervais, 1847) and *T. twitchelli* Schwartz, 1927. Species presenting strobilocercus-like metacestodes are *T. krepkogorski* (Schulz et Landa, 1934), *T. parva* Baer, 1926 and *T. selousi* Mettrick, 1962.

A second category of proliferative metacestodes consists of those with endogenous scolices. In this category are included the polycercus of the dilepidid *Paricterotaenia paradoxa* (Rudolphi, 1802), the cystic hydatid of *Echinococcus granulosus* (Batsch, 1786), and the alveolar hydatid of *E. multilocularis* Leuckart, 1863.

The third category of proliferative metacestodes is found within some hymenolepidid life-cycles as in *Staphylocystis pistillum* (Dujardin, 1845), *S. scalaris* (Dujardin, 1845) or *Pseudodiorchis prolifer* (Villot, 1890). In these examples, metacestodes with irregularly branched bodies are found, and each bud can develop into a differentiated cysticercoid-like form. Such budding metacestodes have been called staphylocystic metacestodes or multigermino-cysticercoids.

Up to this point we have provided an update to the different types of proliferative metacestodes known to date among cyclophyllideans. This in turn serves as a starting point for describing a new type of proliferative metacestodes detected by us. The metacestodes were found in the abdominal cavity of a specimen of the wood mouse, *Apodemus sylvaticus* (Linnaeus, 1758) (Rodentia, Muridae). This capture occurred during research initiated more than seven years ago with the aim of clarifying the epidemiological behavior of helminthiases of terrestrial mammals after an environmental catastrophe – in this case, after a wild fire (Galán-Puchades and Fuentes 1996, Galán-Puchades *et al.* 1999).

Materials and methods

The metacestodes were found in the Serra Calderona Mountains, located between the provinces of Castellón and Valencia in Spain (0º15′21? and 0º43′31″W, 39º51′9″N) (Fig. 1). This mountainous area suffered a wild fire in the summer of 1992, which affected a total of 9500 ha of littoral holmoak and corkoak plantations, as well as cultivation zones let down.

The specimen of *A. sylvaticus* was an adult male captured in September 1994 in an area of postfire regeneration. The wood mouse was dissected and all organs and viscera were helminthologically studied in the laboratory under a binocular microscope.

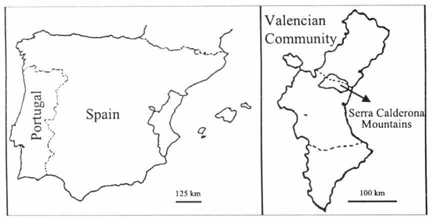

Fig. 1. Geographical location of the Serra Calderona Mountains (Valencian Community, Spain)

The metacestodes found were fixed in hot 70% ethanol, followed by staining in alcoholic hydrochloric carmine for 24 hours. The metacestodes were differentiated with acidified alcohol, dehydrated in an alcohol series, cleared with xylene and mounted *in toto* in Canada balsam.

All measurements are in micrometers unless otherwise stated.

Results

The metacestodes were found free in the abdominal cavity of the wood mouse. The isolated parasites consisted of four me-

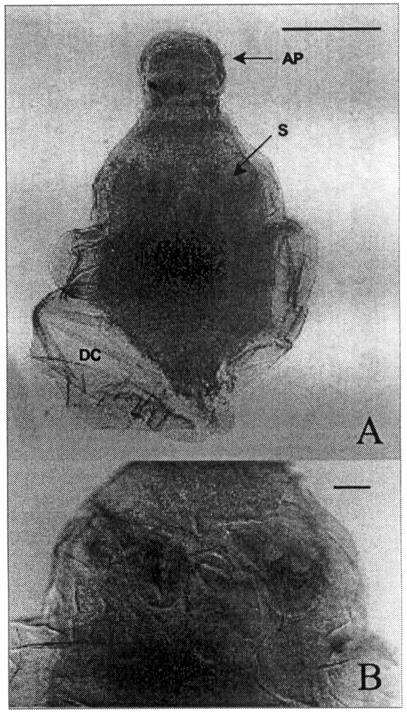

Fig. 2. Metacestode found in the abdominal cavity of *Apodemus sylvaticus*. **A** – general morphology, **B** – detail of an apparent stage of suckers duplication. AP – apical protuberance, S – suckers, DC – dehiscent cover; scale bars: A = 500 µm, B = 100 µm

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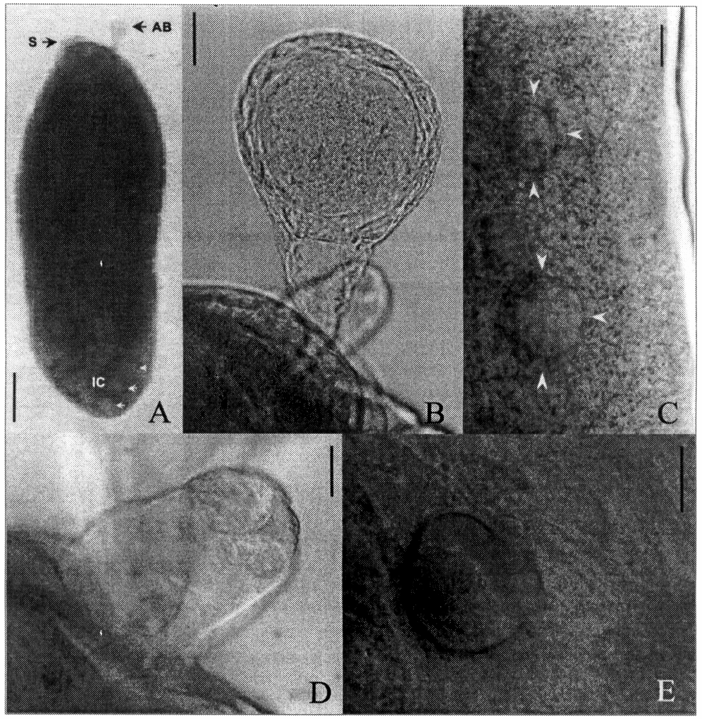

Fig. 3. Budding metacestode. **A** – general morphology, **B** – detail of a pedunculated apical bud, **C** – arrows delimitating internal cavities, **D** – a view of a sessile bud, **E** – one of the internal cavities showing tissue mass developing inside. AB – apical buds, IC – ?, S – suckers; scale bars: A = 500 μm; B, C and E = 50 μm, D = 100 μm

tacestodes and, based on their morphology, the specimens were initially classified as tetrathyridia of the genus *Mesocestoides*. Detailed microscopic study revealed the following morphological and structural features.

One of the metacestodes measured 1.9 mm in length (1890 μm) by 859 in width (Fig. 2) with an apparently dehiscent cover, an apical protuberance and suckers (Fig. 2A) measuring 165 × 192. On examining the metacestode in detail there are supernumerary suckers apparently in the process of duplication, measuring 125 × 167 (Fig. 2B).

A second metacestode measured 3.9 mm in length by 1.5 mm in maximum width (Fig. 3). The metacestode had four suckers, apical buds and internal cavities (FIg. 3A). The suckers measured 176 × 211. The pedunculated apical bud measured 345 × 207 (Fig. 3B). The smallest bud was sessile, measuring 86 × 120. This bud in turn contained vesicles (Fig. 3D). The internal cavities appeared to follow the trajectory of the excretory canals of this "mother" metacestode (Fig. 3C). These vesicles measured 48–97 × 58–105. It was possible to distinguish a tissue mass developing inside some vesicles (Fig. 3E).

The third metacestode, measuring 4.3 mm in length and almost 3 mm at its maximum width, appeared to be a mother metacestode in a more advanced stage of development (Fig. 4). It contained many internal vesicles, each enclosing a developing daughter metacestode (Fig. 4A). The numerous internal vesicles make visualization of the suckers difficult. One of these vesicles showed an external vesicle containing

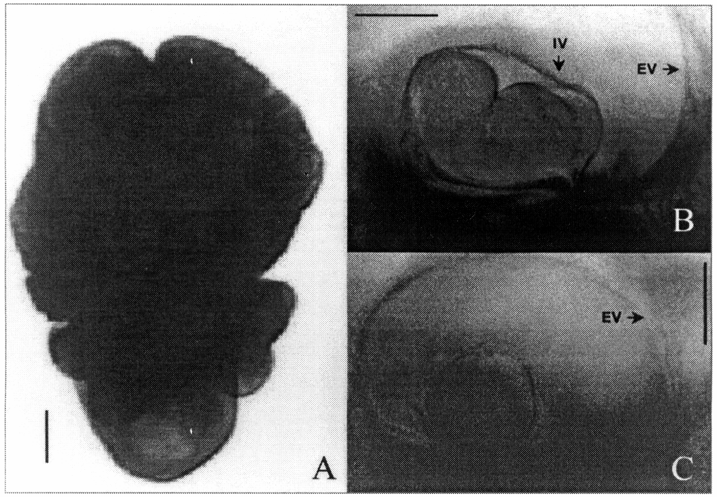

Fig. 4. Young mother metacestode. **A** – general morphology, **B** and **C** – two daughter metacestodes enclosed in the external and internal vesicles. EV – external vesicle, IV – internal vesicle; scale bars: A = 500 μm; B and C = 50 μm

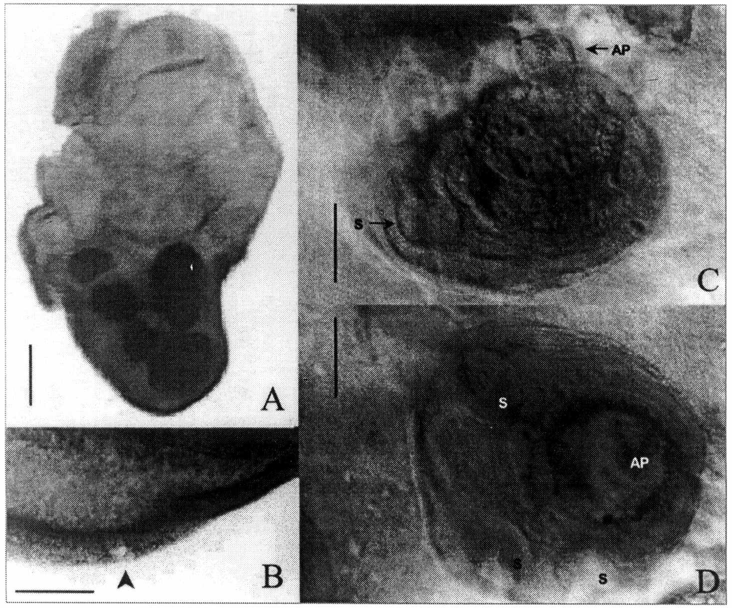

Fig. 5. Mature mother metacestode. **A** – general morphology, **B** – detail of the excretory pore (arrow), **C** and **D** – daughter metacestodes. AP – apical protuberance, S – suckers; scale bars: A = 1 mm; B-D = 50 μm

another internal pedunculated vesicle enclosing a developing daughter metacestode (Fig. 4B, C) 126 long (Fig. 4B).

The fourth metacestode found within the abdominal cavity of the wood mouse appeared to be a partially ruptured mother metacestode, where following development the daughter metacestodes are released (Fig. 5). This particular mother metacestode contained four daughter metacestodes, and constitutes the largest metacestode, measuring 6.5 by 3.8 (Fig. 5A). The fully developed daughter metacestodes (mean length × width = 169 × 190) showed the apical protuberance, suckers (Fig. 5C, D), and the excretory pore (Fig. 5B).

Discussion

The metacestodes seem to present three different processes of asexual proliferation: (1) development of supernumerary suckers, which would be indicative of possible posterior fission; (2) budding, with the development of outgrowths or buds; and (3) endogenous proliferation with the formation of daughter tetrathyridia within a mother metacestode.

The first asexual multiplication process has been known for the tetrathyridium of *Mesocestoides corti* for the past 35 years (Specht and Voge 1965, Hess 1972, Novak 1972, Etges 1991). Budding is the most common type of asexual reproduction among cestodes (Kennedy 1983); however, this sort of proliferation has not been demonstrated in *M. corti*. Specht and Voge (1965) described budding as one of the types of asexual division in *M. corti* metacestodes, but both Hess (1972) and Novak (1972) disagreed with this interpretation.

To the best of our knowledge, endogenous proliferation has never before been described in metacestodes exhibiting primitive development, that is, without a primary lacuna. In metacestodes exhibiting neoteric development, endogenous proliferation such as that shown by hydatid cysts originates from the oncosphere, and the result is the formation of protoscolices. In the present case, the mother metacestode is of the tetrathyridium type, which in turn gives rise to new daughter metacestodes that morphologically appear to be of the same type. Therefore, this type of endopolygeny would be unique among the cestodes. In addition, we find metacestodes that in nature show simultaneous exogenous and endogenous proliferation capacities.

A search of the literature revealed some cases of parasitism by metacestodes that could resemble our own findings. The affected hosts were dogs, cats, a horse, a snake, non-human primates and a human case (Joyeux and Baer 1932, Kuntz *et al.* 1970, Beaver and Rolon 1981, Conn 1990, Conn and McAllister 1990, McFarlane *et al.* 1994). However, none of these exhibited endogenous proliferation, there were no cases – where scolices were detected, and in most of them the metacestodes were described as vacuolated hindbodies. Thus, until recently there was no basis to report the former as anything other than unidentified metacestodes (Conn 1990, Conn and McAllister 1990). However, Crosbie *et al.* (2000) have now presented molecular evidence that at least some similar acephalic metacestodes from dogs in North America are actually species of the genus *Mesocestoides*.

We have studied a total of 622 small mammals for helminths. The only metacestode stages detected in these hosts to date have been *Taenia parva*, *T. martis* and normal non-proliferative tetrathyridium metacestodes of the genus *Mesocestoides*. These tetrathyridia have been detected in 5 *Apodemus sylvaticus*, 4 *Mus spretus* Lataste, 1883, 3 *Rattus rattus* (Linnaeus, 1758) (Rodentia, Muridae) and 2 *Crocidura russula* (Hermann, 1780) (Insectivora, Soricidae).

In the enclave where the wood mouse containing the four metacestodes described here was captured, an additional 205 *A. sylvaticus* were studied, and three of them were infected with classical *Mesocestoides* metacestodes, with no evidence of asexual division. Since the helminthological spectrum of the zone has been firmly established as a result of six years of study, it is most likely that the metacestodes correspond with *Mesocestoides*, since the absence of hooks and absence of a primary lacuna differentiate them from *T. parva* or *T. martis*.

If we compare the first metacestode described (Fig. 2A) and one of those still contained within the mother metacestode (Fig. 5C), the former might be a second generation metacestode already freed from it. It presents the same apical protuberance as the daughter metacestodes still within the mother metacestode. The cover observed might correspond to remains of the internal vesicle in which it was contained, and the apical protuberance might be an expansion of the metacestode by which active movement could be the mechanism used by the daughter metacestodes to free themselves from the mother metacestode and/or the containing vesicles – this structure not being subsequently observed. This developmental process will remain speculative until further experimental data have been obtained. Elucidating the complete life- cycle of at least one species of the genus *Mesocestoides* will be critical. Other helpful work would involve making further progress on development of the metacestodes *in vitro* and/or *in vivo*. Verification of the identity of all similar asexual metacestodes is also an important goal for future research, using PCR or other techniques to determine whether the metacestodes reported here are closely related to known *Mesocestoides* spp., or other species not yet identified. This approach was used successfully in resolving some relationships among North American *Mesocestoides* by Crosbie *et al.* (2000). The present work will provide a theoretical framework for continuing such exploration of this cestode group. In any case, the unique mother metacestode reported here represents a form of asexual reproduction previously unknown among the cestodes.

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