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Identification and distribution of *Echinostoma lindoense, E. audyi* and *E. revolutum* (Trematoda; Echinostomatidae)

Kian Joe Lie[1] and Ivan Kanev[2]

[1] Department of Epidemiology and International Health, University of California, San Francisco, California 94143, USA
[2] Central Laboratory of Helminthology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Abstract. The closely related *Echinostoma lindoense, E. audyi* and *E. revolutum* can be differentiated by morphological characteristics of their adults and cercariae. We have found *E. lindoense* and *E. audyi* in Southeast and Southwest Asia and Central Europe and the former species also in South America. However, using the morphological characteristics described by Beaver (1937) for *E. revolutum* which is assumed to be cosmopolitan, we did not find this species in these regions.

The adult stage of *Echinostoma lindoense* Sandground and Bonne, 1940 and *E. audyi* Lie and Umathevy, 1965 resemble very much the type species of the genus, *Echinostoma revolutum* (Froelich, 1802). Since the number, arrangement, and relative size of the cephalic spines are the same in the 3 species, separation of the species is often uncertain if based only on morphologic characters of the adult. The validity of *E. lindoense* or *E. audyi* as separate species depends on the recognition of stable morphologic features that can serve as reliable criteria of specific distinction. We have found that certain cercarial characteristics are constant and can be used to separate *E. lindoense* or *E. audyi* from *E. revolutum*. The most detailed description of the structure and life cycle of *E. revolutum* is to be found in Beaver's monograph (1937) which we use as reference for the species. Although the adult of *E. lindoense* differed from that of *E. revolutum* only in variable characters, such as the shape of the testes and the size of the cirrus sac, Sandground and Bonne (1940) created the species because there were biologic differences and a difference in the cercarial tail structure.

In 1964, one of us (KJL) established in experimental animals the complete life cycle of a Malaysian *Echinostoma* sp. with 37 cephalic spines, resembling in its marita stage *E. revolutum* and *E. lindoense,* but the cercarial tail of the Malaysian species was similar to that of *E. lindoense.* After long

Offprint requests to: K.J. Lie

Table 1. Differential characteristics of three species of *Echinostoma*

	E. revolutum[a]	*E. lindoense*	*E. audyi*
Adult: posterior end excretory bladder	straight	straight	coiled
Cercaria: tail fin folds	small, dorsal	2 dorsal, 2 ventral, 2 lateroventral	2 dorsal, 2 ventral, 2 laterovental
location of paraesophageal gland outlets	dorsolateral lips oral sucker?	around oral sucker, ventral and dorsal body surface between pharynx and acetabulum	around oral sucker, ventral body surface in pharynx region

[a] From Beaver (1937)

studies of numerous cercariae of this and other echinostome species, Lie and his coworker have identified certain cercarial features that can be used as reliable species characteristics (Lie 1964, 1966a, 1966b; Lie and Umathevy 1965). Using characteristics of adults and cercariae (Table 1), we have been able not only to separate *E. lindoense* from *E. revolutum*, but also to identify among the Malaysian echinostomes another closely related species *E. audyi* (Lie and Umathevy 1965). The adult *E. audyi* can be separated from *E. lindoense* and *E. revolutum* by the presence of a conspicuously coiled excretory bladder near the posterior end (Lie and Umathevy 1965; Kanev 1980). We have studied over the years a large number of adult worms of *E. audyi* and have found this characteristic stable, with little variation in degree of coiling and not altered by fixation or staining procedures. The posterior end of the excretory bladder in *E. lindoense*, determined by examining hundreds of adult specimens, is straight, sometimes slightly curved, but with no tendency to coil. The excretory bladder in *E. revolutum* (see drawings in Beaver, 1937) is similar to that in *E. lindoense*.

Of the cercarial features, the tail fin folds are most constant, with no noticeable variation in size and location. This constant characteristic has been observed not only in *E. lindoense* and *E. audyi*, but also in other echinostome species studied by us. Tail fin folds of *E. lindoense* and *E. audyi* are similar, with two ventral and two dorsal prominent fin folds and one pair of small ventrolateral ones (Lie 1964, 1968; Lie and Umathevy 1965; Lie et al. 1973). The tail fin fold in *E. revolutum*, as reported by Beaver (1937), consists of one small dorsal fold located near the tip of the tail.

Distribution of paraeosophageal gland outlets (Lie 1966b) is also a useful feature in identifying species of *Echinostoma*. Outlets connected to irregular, often dilated gland ducts are vividly stained with various vital stains, e.g. dark red in diluted neutral red. The number of gland ducts and outlets stained is variable, but the location of the outlets is characteristic for each species. Paraesophageal gland outlets in *E. audyi* cercariae are located symmetrically around the oral sucker, in 6 or fewer pairs; in the pharynx region

of the ventral body surface these outlets are arranged in 2 mesial rows, usually 1 to 3 in each row. Outlets in *E. lindoense* cercariae are many, located symmetrically around the oral sucker in up to 12, but usually fewer, pairs; they are found also on the dorsal and ventral body surface between pharynx and acetabulum. The outlets on the body surface are arranged in 8 rows, 2 mesio-ventral, 2 latero-ventral, 2 mesio-dorsal, and 2 latero-dorsal. The number of outlets observed varies from 1 to 5 in each row, except in the latero-dorsal rows, where the maximum is 4 (Lie 1966b). Beaver (1937) studied *E. revolutum* cercariae stained with neutral red. If *E. revolutum* cercariae had paraesophageal gland ducts and outlets similar in location to that in *E. lindoense* or *E. audyi*, Beaver probably would have noticed them. Instead, he found 3 pairs of irregular, coiled ducts with their outlets on the dorsolateral lips of the oral sucker. These ducts are presumably similar to the 2 pairs of ducts with outlets also on the dorsolateral lips of the oral sucker, which were described in cercariae of *Echinostoma rodriguesi* (Hsu et al. 1968). On the basis of the staining properties, Hsu et al. (1968) assumed that the ducts in *E. rodriguesi* cercariae were probably those of paraesophageal gland cells. The ducts described by Beaver (1937) in *E. revolutum* cercariae also may be paraesophageal gland ducts.

Patterns of integumentary papillae in *E. lindoense* and *E. audyi* cercariae are similar (Lie 1966a; Kanev 1980, Vasilev and Kanev 1981); patterns of these papillae in *E. revolutum* cercariae have not been studied. Flame cell patterns are extremely difficult to determine in echinostome cercariae and, therefore, cannot be used for species identification. Number of penetration gland cell outlets located on the dorsal lip of the oral sucker may be constant. There are 3 pairs in *E. lindoense*, 2 in *E. audyi* (Lie 1966b) and 3 in *E. revolutum* cercariae (Beaver 1937). Shape and size of cystogenous cell contents and number of esophagus cells seem also to be constant and deserve further investigation.

Echinostoma lindoense and *E. audyi* appear to be widespread. They are common parasites in Indonesia, Malaysia, and Thailand (Lie et al. 1973). The former species is common also in Brazil (Lie 1968), but *E. audyi* has not been found in that country.

One of us (Kanev) took part in an extensive study of the structure, ecology, and taxonomy of echonostomes with 37 collar spines from Europe and Southwest Asia (Vassilev et al. 1978, 1982; Vassilev and Kanev 1979, 1981; Kanev and Vassilev 1977, 1978, 1980, 1981, Kanev and Odening 1982; Kanev et al. 1982; and Kanev 1980, 1982). The results show that echinostomes similar in its structure and biology to *E. lindoense* and *E. audyi* are common in Bulgaria, Poland, Czechoslovakia, German Democratic Republic, Austria, England and Georgian Soviet Socialist Republic, U.S.S.R. Kanev and coworkers have confirmed that characteristics of adults and cercariae (Table 1) used to identify *E. lindoense* and *E. audyi* are stable and useful. We have compared *E. lindoense* from Malaysia and Brazil and *E. audyi* from Malaysia with similar species of *Echinostoma* found in Bulgaria. We observed no significant morphologic differences and consider them identical.

An unexpected problem has developed as a result of our surveys carried out in Southeast and Southwest Asia, South America, and Europe. Using Beaver's criteria for cercariae (Table 1), we did not find the cosmopolitan *E. revolutum* in these countries, although we looked for it. The species of *Echinostoma* found to be cosmopolitan was *E. lindoense*. Inability to find echinostomes similar to those described by Beaver (1937) as *E. revolutum* in Central Europe, the type locality for the species, is striking. It might be that the parasite described by Beaver (1937) was not *E. revolutum*, but rather a closely related species occurring only in America. Such an error could have been made since no adequate descriptions of *E. revolutum* cercariae existed before Beaver undertook his studies.

In an attempt to solve this problem one of us (I.K.) in collaboration with helminthologists in Central Europe and U.S.A. has determined: (1) the structure, biology, and systematics of the North American species of *Echinostoma* described by Beaver (1937) as *E. revolutum*; (2) the cercarial characteristics and the complete life history of species of *Echinostoma* with 37 collar spines from the same locality where *E. revolutum* was first found by Froelich in 1802 in Central Europe; (3) the adult and cercarial characteristics of *E. revolutum* being maintained in some laboratories in Europe and USA; and (4) the structure of the adult of the species of *Echinostoma* with 37 collar spines, kept in museums in Vienna and Berlin as type and paratype specimens. The results of these and other studies will be reported separately (I.K.).

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References

Beaver PC (1937) Experimental Studies on *Echinostoma revolutum* (Froelich), a fluke from birds and mammals. Illinois Biol Monog 15:1–96

Froelich J (1802) Beiträge zur Naturgeschichte der Eingeweidewürmer. Naturforscher (Halle) 29:5–96

Hsu KC, Lie KJ, Basch PF (1968) The life history of *Echinostoma rodriguesi,* sp.n. (Tremotoda: Echinostomatidae). J Parasitol 54:299–319

Kanev I (1980) Study on some echinostome species (Trematoda) found in Bulgaria. IV. On the development and ecology of *Echinostoma audyi* Lie and Umathevy, 1965 (in Bulgarian). Helminthologia 9:39–50

Kanev I (1982) Comparative investigations on the morphology and biology of *Echinostoma nudicaudatum* Nasir, 1960, *Cercaria deficipinnatum* Khan, 1960 and *Echinoparyphium aconiatum* Dietz, 1909 (in Bulgarian). Helminthologia 14: in press

Kanev I, Vassilev I (1977) On some echinostome species found in Bulgaria. Proc. 3rd Nat Conf Parasitol Albena Bulgaria, 12–14 Oct pp 150–152

Kanev I, Vassilev I (1978) On some echinostome species found in Bulgaria. Proc. 4th Int Cong Parasitol Warszawa Poland, 19–26 Aug p 20

Kanev I, Vassilev I (1980) On the validity of *Echinostoma robustum* Yamaguti, 1935 (Trematoda). Morphologic and biologic studies. Proc 3rd Europ Multicoll Parasitol 7–13 Sept Cambridge England, p 128

Kanev I, Vassilev I (1981) Examination of *Echinostoma* species found in Europe. 9th Int Conf of W.A.A.V.P. Budapest Hungary, 13–17 July, p 186

Kanev I, Odening K (1982) Further studies on *Cercaria spinifera* La Valette, 1855 (Trematoda: Echinostomatidae) in Central Europe. Helminthologia 14: in press

Kanev I, Chumburadze K, Varalamovich G (to be published) On some echinostome species found in Georgia. Helminthologia, in press

Lie KJ (1964) Studies on Echinostomatidae (Trematoda) in Malaya. VII. The life history of *Echinostoma lindoense* Sandground and Bonne, 1940. Trop Geogr Med 16:72–81

Lie KJ (1966a) Studies on Echinostomatidae (Trematoda) in Malaya. XIII. Integumentary papillae on six species of echinostome cercariae. J Parasitol 52:1041–1048

Lie KJ (1966b) Studies on Echinostomatidae (Trematoda) in Malaya. XIV. Body gland cells in cercariae of *Echinostoma audyi* Lie and Umathevy and *E. lindoense* Sandground and Bonne. J Parasitol 52:1049–1051

Lie KJ (1968) Further studies on the life history of *Echinostoma lindoense* Sandground and Bonne, 1940 (Trematoda: Echinostomatidae) with a report on its occurrence in Brazil. Proc Helminthol Soc Wash 35:74–77

Lie KJ, Umathevy T (1965) Studies on Echinostomatidae (Trematoda) in Malaya VIII. The life history of *Echinostoma audyi* n. sp. J Parasitol 51:781–788

Lie KJ, Nasemary S, Impand P (1973) Five echinostome species from Thailand. Southeast Asian J Trop Med Pub Hlth 4:96–101

Sandground JH, Bonne C (1940) *Echinostoma lindoensis* n. sp., a new parasite of man in the Celebes with an account of its life history and epidemiology. Am J Trop Med 20:511–536

Vassilev I, Kanev I (1979) Study of *Echinostoma* species (Trematoda) in Bulgaria. III. Determination of two species of echinostome cercariae with 37 collar spines. (in Bulgarian) Helminthologia 8:6–23

Vassilev I, Kanev I (1981) Study of *Echinostoma* species (Trematoda) in Bulgaria. V. On the morphology and ecology of *Echinostoma lindoense* Sandground and Bonne, 1940. (in Bulgarian) Helminthologia 11:3–18

Vassilev I, Komandarev S, Michov L, Kanev I (1978) Comparative electrophoretic investigations of certain species of the genus *Echinostoma* with 37 collar spines. (in Bulgarian) Helminthologia 6:31–38

Vassilev I, Świetlikowski M, Kanev I, Busta J (1982) Finding of *Echinostoma lindoense* Sandground and Bonne, 1940 in Poland and Czechoslovakia. (in Bulgarian) Helminthologia 13:12–22

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