DOI 10.2478/s11687-014-0233-8

VERSITA

HELMINTHOLOGIA, 51, 3: 225 – 229, 2014

Severe granulomatous gastric lesions following migration of *Spiroxys contortus* larvae (Nematoda: Spirurida) in European pond turtles, *Emys orbicularis*

V. MICLĂUŞ[1], A. F. GAL[2*], C. CĂTOI[2], A. D. MIHALCA[3]

[1]Department of Histology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, Cluj-Napoca, 400372, Romania, E-mail: *adrianfloringal@yahoo.com*;
[2]Department of Pathologic Anatomy, Necropsy and Veterinary Forensic Medicine, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, Cluj-Napoca, 400372, Romania;
[3]Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, Cluj-Napoca, 400372, Romania

Summary

The European pond turtle gradually disappeared from most of its range due to various factors. Hence, conservation measures are of main concern in all European countries where it is still present. A decent methodology for assessing the effect of parasites on the health of wildlife is microscopic lesion description. In 2002 – 2007, eight *Emys orbicularis* were brought for necropsy. The presence of gastric nematodes, identified as adults and larval *Spiroxys contortus* was noticed in all the turtles. A discrete cellular infiltrate with mononuclear cells and eosinophils was noticed in the gastric mucosa. The most prominent lesions were severe granulomas with or without degenerated larval structures. Some of the granulomas presented a central area of coagulation necrosis surrounded by giant cells, epithelioid cells and macrophages. In mature granulomas, the cluster of macrophage cell line and necrosis were surrounded by a fibrous capsule. Vascular cuffs, hyperemia, edema and venous ectasia were also present.

Keywords: *Emys orbicularis*; granuloma; migration; *Spiroxys contortus;* stomach wall

Introduction

Wildlife parasitology is rarely approached from the medical point of view. Reports of clinical parasitism in wild animals are scarce. Hence, the understanding of the pathogenic effect in wildlife is often limited. A decent methodology for assessing the negative effect of parasites on the health of free-ranging animals can be lesion description, mainly at its microscopic level. With the exception of fish, the number of published accounts of microscopic lesions induced by parasites in cold-blooded vertebrates is relatively low. The parasitism with nematodes in free-ranging chelonians is commonly reported in the literature (Baker, 1987). However, most of the studies are simple new host/geographical records, morphological descriptions or brief epidemiological reports.

The European pond turtle, *Emys orbicularis* is one of the four autochthonous species of freshwater turtles from Europe. Although during early Holocene, the range of this turtle was relatively wide, it gradually disappeared from most of its European range due to synergic climatic deterioration and human activities (Sommer *et al*., 2007). The decline of the species still continues and conservation measures of *E. orbicularis* are of main concern in all European countries where it is still present (Fritz *et al*., 2007). Eleven species of nematodes (*Spiroxys contortus*, *Serpinema microcephalus*, *Falcaustra ararath*, *F. araxiana*, *F. armenica*, F. *lambdiensis*, *Anguisticaecum holopterum*, *Spauligodon eremiasi*, *Ascarops strongylina*, *Physocephalus sexalatus* and *Streptocara crassicauda*) are known to parasitize *E. orbicularis* (Sharpilo, 1976; Baker, 1987; Mihalca; 2007; Hidalgo-Vila *et al*., 2009). Except some previous observations on lesions produced by larval (Miclăuş *et al*., 2006; Miclăuş *et al.*, 2013) or adult (Miclăuş *et al*., 2008) *S. contortus* there are no other papers describing nematode-associated pathology in this turtle species. However, nematode-associated lesions were reported in introduced *Trachemys scripta elegans* from southern Spain, produced by a parasite probably acquired from native European pond turtles (Hidalgo-Vila *et al*., 2011).

The spirurids (order Spirurida) are a group of nematodes that use arthropods (i.e. insects or crustaceans) as intermediate hosts for the initial larval stages and adults develop usually in the upper digestive system of vertebrates (Anderson, 2000). Species of genus *Spiroxys* live in the stomach mucosa of fresh water turtles and use aquatic crustaceans as intermediate hosts (Mader, 2006). *S. contortus* (Rudolphi, 1819) Schneider, 1866 was described in Europe

from *E. orbicularis* (the type-host). Its geographic range includes Western Palearctic and Nearctic. *S. contortus* is a worldwide distributed parasite of the digestive tract in a large variety of aquatic turtles: *Chelydra serpentina*, *Chrysemys picta*, *Deirochelys reticularia*, *Emydoidea blandingii*, *Graptemys geographica*, *Kinosternon subrubrum*, *Pseudemys scripta*, *Sternotherus odoratus*, *Terrapene carolina, Trionyx spiniferus*, *E. orbicularis* (Edwards *et al.,* 1974; Baker, 1987; McAllister *et al.,* 1993; Anderson, 2000; Platt, 2000; Gonzales, 2010).
Hedrick (1935) provided detailed information on development of larval *S. contortus* encapsulated in the stomach of the painted turtle, *C. picta*. Various cyclop species (family Cyclopidae) serve as intermediate hosts (Hedrick, 1935). The infective 3rd stage larvae were found encapsulated on the mesentery of various naturally infected paratenic aquatic hosts (dragonfly nymphs, snails, fish, amphibians, reptiles).
The current paper describes the histological aspects of granulomas induced by larval stages of the nematode *S. contortus* in the stomach wall of in naturally infected *E. orbicularis*.

Materials and methods

Between 2002 and 2007, eight European pond turtle, *E. orbicularis* were brought for necropsy to the Department of Pathology, Faculty of Veterinary Medicine Cluj-Napoca, Romania. The animals were in fresh stage and originated from Danube Delta (Tulcea Country, Romania) being found drowned in fishing nets. During complete necropsy, at gross examination of the stomach we have noticed the presence of nematodes in all the turtles. The nematodes (n = 226) were collected in 70 % ethanol and submitted to the Department of Parasitology for specific identification. The morphological characters used for morphological identification are presented in details by Hedrick (1935) and Mihalca (2007). In three of the eight turtles, at the stomach level we have noticed the presence of gross lesions (focal whitish spots and thickening of the gastric wall). From these specimens, we have collected small pieces of gastric tissues (cca. 0.5 cm) which were fixed in neutral 10 % buffered formalin. Tissues were subsequently embedded in paraffin wax, sectioned at 5 µm, and stained with Goldner's trichrome method.

Results

Based on morphological characters, all nematodes collected were identified as adults (n = 196) and larval stages (n = 30) of *S. contortus*.
Superficial epithelium did not show pathological changes following mechanic irritation induced by the nematodes (Fig. 1). A discrete cellular infiltrate with mononuclear cells and eosinophiles was noticed in the gastric mucosa. The cellular lymphohistiocytic and eosinophilic infiltration had a focal lymphofollicular character and was located in the superficial and deep *lamina propria*.
The submucosa contained small to large perilarvar granulomatous lesions. In the central part of some granulomas larval structures were still distinguishable, while in others the larvae were in various stages of degeneration and/or were replaced by a central area of coagulation necrosis. The granulomatous inflammation was characterized by a focal infiltration with macrophages, epithelioid and giant multinucleated cells around a central necrotic area (Fig. 2). In older, mature granulomas, the cluster of macrophage cell line and necrosis was surrounded by a fibrous capsule.

Fig. 1. Adult of *Spiroxys contortus* in the gastric lumen of *Emys orbicularis* (Goldner's trichrome)

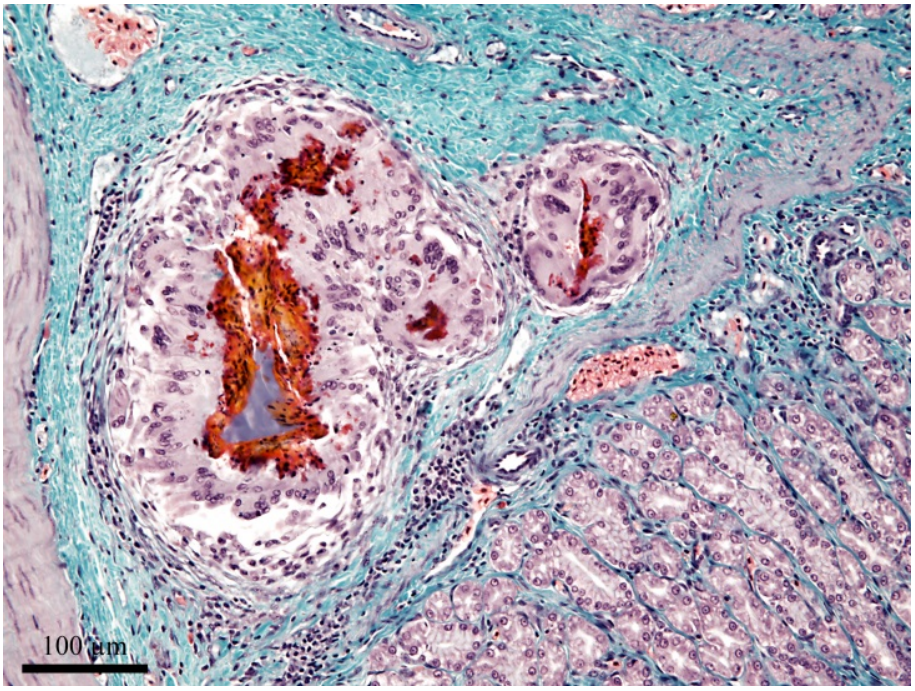

Fig. 2. Granulomas in the gastric submucosa of *Emys orbicularis* containing macrophages, epithelioid and giant multinucleated cells around a central necrotic area (Goldner's trichrome)

The inner part of the capsule had a loose fibrous tissue invaded by lymphocytes and few heterophils, and the outer part was fibrous. There was a typical morphology of the giant cells encountered in these granulomas, such as: (a) fine cytoplasmic prolongations that bordered necrotic areas of the granuloma, and (b) the disposition of the giant cells nuclei opposite to the necrotic bulk (i.e. to the periphery of the granuloma). Additionally, scattered mononuclear infiltrate (i.e. vascular cuffs) and vascular changes such as hyperemia, edema and venous ectasia were present in the submucosa.

Similar granulomatous lesions were found in the *muscularis externa* and serosa of the stomach wall. The central part of some granulomas from the *muscularis externa* contained degenerating or degenerated larvae. The granulomas had a similar morphology with that from the submucosa (Fig. 3). Vascular changes also occurred in the *muscularis externa* (i.e., sanguine congestion, perivascular and

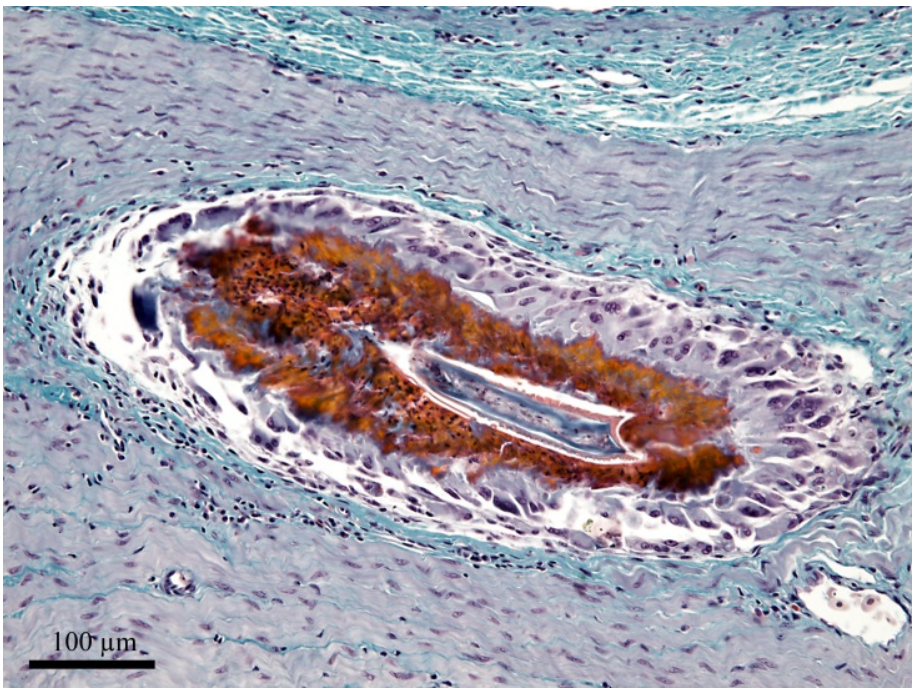

Fig. 3. Granulomatous lesion in the gastric *muscularis externa* of *Emys orbicularis* containing degenerating larvae of *Spiroxys contortus* (Goldner's trichrome)

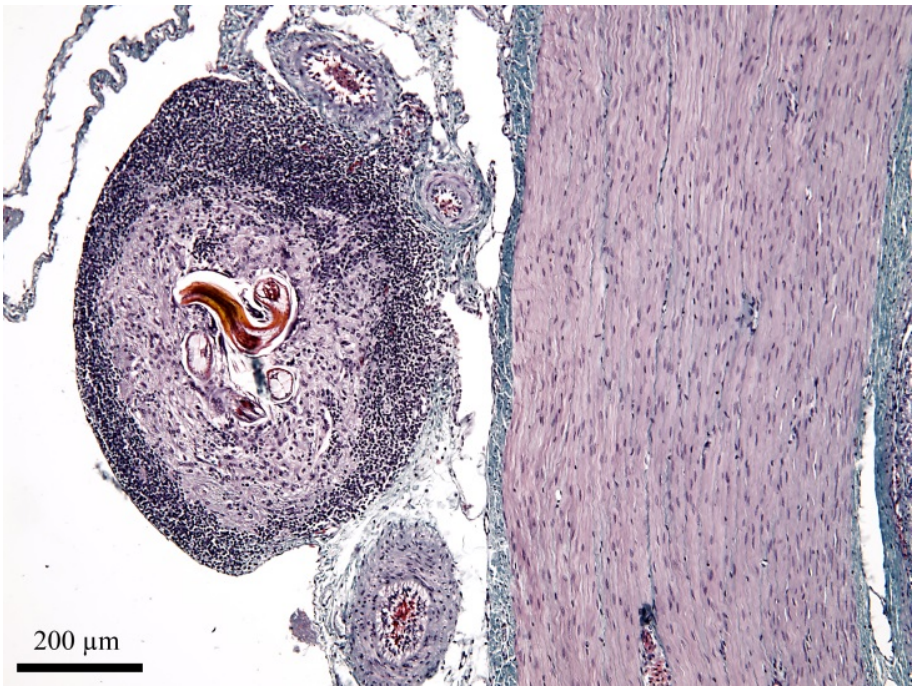

Fig. 4. Granulomas in the gastric serosa of *Emys orbicularis* containing non-degenerated parasites of *Spiroxys contortus* bordered by macrophages, multinucleated giant cells and heterophils (Goldner's trichrome)

interfibrillar edema and discreet perivascular and interfibrillar mononuclear infiltrate).

Similar granulomatous lesions containing non-degenerated parasites were noticed in the gastric serosa (Fig. 4). A marked hypertrophy of the gastric serosa and omental ligament occurred following intense fibroplasia. Clusters of lymphocytes and scattered heterophils were present in the gastric serosa.

Discussion

Although *S. contortus* is the most commonly reported nematode in *E. orbicularis*, the pathological descriptions of the lesions are almost absent. The single study (McAllister *et al.,* 1993) reported that *S. contortus* induced similar granulomatous lesions in the stomach of *Apalone spinifera pallida*. Nematodes were surrounded by non-caseating granulomas in the gastric submucosa and muscularis externa. Newly formed granulomas consisted of macrophages, heterophils, occasional multinucleated giant cells, and cuffs of lymphoid cells. Larvae in these early granulomas were not surrounded by a fibrous capsule. There were central areas of necrotic debris and eosinophilic coagulum. In older, mature granulomas *S. contortus* was surrounded by a fibrous capsule. Clusters of macrophages and heterophils were noted adjacent to the nematode cuti cle. The bulk of each granuloma was composed of concentric swirls of fibrous connective tissue and mononuclear cells, and was surrounded by dense cuffs of lymphocytes. There were accumulations of necrotic debris and giant cells within the granulomas (McAllister *et al.,* 1993).

The, gastro-parietal larval migration was demonstrated by the presence of chronic granulomatous lesions in all the gastric wall layers (including *muscularis externa* and serosa). According to this, the gastro-parietal migration of early immature forms of *S. contortus* seems to be necessary for nematode maturation. As in the current report, McAllister *et al.* (1993) noted granulomas which lacked nematodes, but otherwise had a typical morphology of a parasitic granuloma. Larvae of *Spiroxys* were found in various potential paratenic hosts, among them, snails, tadpoles and adults frogs and, larval and adult of newts (for a detailed list see Anderson, 2000). More recently, pancreatic granulomas were found associated with *S. microcephalus* in *T. scripta elegans* (Hidalgo-Vila et al. 2011).

Although rare in aquatic turtles, granulomas induced by spirurid nematodes are well known and described in other reptiles. However, it seems the histologic structure is similar with the present findings. Granulomas produced by *Ascarops* sp. in the stomach of the sagebrush lizard, *Sceloporus graciosus* were found in the submucosa within thin-walled granulomas which consisted of fibroblasts and macrophages (Goldberg & Bursey, 1989). In addition, similar granulomas containing larval *Ascarops* sp. were found in the liver of the western fence lizard, *Sceloporus occidentalis* (Goldberg & Bursey, 1988). In a long-term retrospective study, Jones (1995) described lesions induced by larval spirurids from family Physalopteridae in Australian snakes and lizards. In this case, the lesions consisted in cystic structures rather than granulomas. Their histologic examination revealed that the cystic wall was composed of collagen tissue, usually with little cellular infiltration. Interestingly, the location of the cysts varied according on the host species (within the muscle layer or subserosally). Severe granulomatous lesions induced by *Eustrongylides* sp. in multiple organs and tissues were also described in dice snakes, *Natrix tessellata* (Mihalca *et al.,* 2007).

Although lesions induced by *S. contortus* larvae (Nematoda: Spirurida) in European pond turtle, *E. orbicularis* are severe, little is known about how they can influence the health of the turtles. We strongly recommend full necropsy of all endangered vertebrates which are found dead. The increase in the number of such published reports will eventually facilitate the understanding of the potential negative effects of the pathogens associated with threatened fauna.

References

ANDERSON, R. C. (2000): *Nematode Parasites of Vertebrates: Their Development and Transmission*. 2nd Edition, Wallingford, England, CABI Publishing, 650 pp.

BAKER, M. R. (1987): *Synopsis of the nematode parasitic in amphibians and reptiles.* St. John's, Nfld., Canada, Memorial University of Newfoundland, Occasional papers in biology, no. 11, pp. 1 – 325

EDWARDS, S. J., KIRBY, M. D., CRAWLEY, L.R., RAYBURN, J. D., SHAW, J. H., WALKER, M. L. (1974): *Index-catalogue of Medical and Veterinary Zoology*. Animal Parasitology Institute, Agricultural Research Service, U.S. Government Printing Office, Washington

FRITZ, U., GUICKING, D., KAMI, H., ARAKELYAN, M., AUER, M., AYAZ, D., CÉSAR AYRES FERNÁNDEZ, C. A., BAKIEV, A. G., CELANI, A., DŽUKI´C, G., FAHD, S., HAVAŠ, P., JOGER, U., KHABIBULLIN, V. F., MAZANAEVA, L. F., ŠIROKÝ, P., TRIPEPI, S., VÉLEZ, A. V., ANTÓN, G. V., WINK, M. (2007): Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*) – an update. *Amphibia-Reptilia*, 28: 418 – 426. DOI: 10.1163/156853807781374737

GOLDBERG, S. H., BURSEY, C. R. (1988): Larval nematodes (*Ascarops* sp., Spirurida, Spirocercidae) in the liver granulomata of the western fence lizard, *Sceloporus occidentalis* (Iguanidae). *J. Wildl. Dis.*, 24: 568 – 571. DOI: 10.7589/0090-3558-24.3.568

GOLDBERG, S. H., BURSEY, C. R. (1989): Larval nematodes (*Ascarops* sp.) in stomach granulomas of the sagebrush lizard, *Sceloporus graciosus*. *J. Wildl. Dis.*, 25: 630 – 633. DOI: 10.7589/0090-3558-25.4.630

GONZALES, C. E., HAMANN, M. I. (2010): Larval nematodes found in amphibians from northeastern Argentina. *Braz. J. Biol.*, 70(4): 1089 – 1092. DOI: 10.1590/S1519-69842010000500026

HEDRICK, L. H. (1935): The life history and morphology of *Spiroxys contortus* (Hudolphi); Nematoda: Spiruridae. *T. Am. Microsc. Soc.*, 54: 307 – 335

HIDALGO-VILA, J., DÍAZ-PANIAGUA, C., RIBAS, A., FLORENCIO, M., PÉREZ-SANTIGOSA, N., CASANOVA, J. C. (2009): Helminth communities of the exotic introduced turtle, *Trachemys scripta elegans* in southwestern Spain: Transmission from native turtles. *Res. Vet. Sci.*, 86: 463 – 465. DOI:10.1016/j.rvsc.2008.08.003

HIDALGO-VILA, J., MARTÍNEZ-SILVESTRE, A., RIBAS, A., CASANOVA, J. C., PÉREZ-SANTIGOSA, N., DÍAZ-PANIAGUA, C. (2011): Pancreatitis associated with the helminth *Serpinema microcephalus* (Nematoda: Camallanidae) in exotic red-eared slider turtles (*Trachemys scripta elegans*). *J. Wildl. Dis.*, 47(1): 201 – 205. DOI: 10.7589/0090-3558-47.1.201

JONES, H. I. (1995): Pathology associated with Physalopterid larvae (Nematoda: Spirurida) in the gastric tissues of Australian reptiles. *J. Wildl. Dis.*, 31(3): 299 – 306. DOI: 10.7589/0090-3558-31.3.299

MADER, D. R. (2006): *Reptile Medicine and Surgery*. 2nd Edition, Saunders Elsevier, St. Louis, Missouri, pp. 343 – 364

MCALLISTER, C. T., GOLDBERG, S. R., HOLSHUH, H. J. (1993): *Spiroxys contorta* (Nematoda: Spirurida) in gastric granulomas of *Apalone spinifera pallida* (Reptilia: Testudines). *J. Wildl. Dis.*, 29(3): 509 – 511. DOI: 10.7589/0090-3558-29.3.509

MICLĂUS, V., MIHALCA, A., OANA, L., RUS, V., CADAR, D. (2008): Gastric parasitism with full-aged *Spiroxys contortus* of the European pond turtle (*Emys orbicularis*). *Bulletin UASVM, Veterinary Medicine*, 65(2): 84 – 86.

MICLĂUȘ, V., MIHALCA, A. D., GAL A. F., CĂTOI C. (2013): Mesothelial metaplasia in European pond turtle, *Emys orbicularis* (Testudines: Emydidae) infected with *Spiroxys contortus* (Nematoda: Spirurida). *Helminthologia*, 50(2): 104 – 107. DOI: 10.2478/s11687-013-0116-4

MICLĂUȘ, V., MIHALCA, A. D., OGNEAN, L., JOANTĂ, A., RUS, V., DAMIAN, A., CADAR, D. (2006): Involvement of *Spiroxys contortus* larvae in etiopathogenesis of gastric and intestinal affections in European pond turtle (*Emys orbicularis*). *Annals of the SRBC*, 11: 46 – 50

MIHALCA, A. D. (2007): *Parasitic fauna of free-ranging European pond turtle (Emys orbicularis), sand lizard (Lacerta agilis) and grass snake (Natrix natrix) in Romania.* PhD thesis, Romania, Cluj-Napoca: University of Agricultural Sciences and Veterinary Medicine

MIHALCA, A. D., FICTUM, P., SKORIC, M., SLOBODA, M., KÄRVEMO, S., GHIRA, I., CARLSSON, M., MODRY, D. (2007): Severe granulomatous lesions in several organs from Eustrongylides larvae in a free-ranging dice snake, *Natrix tessellata*. *Vet. Pathol.*, 44(1): 103 – 105. DOI: 10.1354/vp.44-1-103

PLATT, T. R. (2000): Helminth parasites of the western painted turtle, *Chrysemys picta belii* (Gray), including *Neopolystoma elizabethae* n. sp. (Monogenea: Polystomatidae), a parasite of the conjunctival sac. *J. Parasitol.*, 86(4): 815 – 818. DOI: 10.1645/0022-3395(2000)086[0815:HPOTWP]2.0.CO;2

SHARPILO, V. P. (1976): *Parasitic worms of the reptilian fauna of the USSR*. Izdat. Naukova Dumka, Kiev, USSR, pp. 287 (In Russian)

SOMMER, R. S., PERSSON, A., WIESEKEC, N., FRITZ, U. (2007): Holocene recolonization and extinction of the pond turtle, *Emys orbicularis* (L., 1758), in Europe. *Quaternary Sci. Rev.*, 26: 3099 – 3107. DOI: 10.1016/j.quascirev.2007.07.009

RECEIVED MARCH 26, 2014

ACCEPTED JUNE 15, 2014