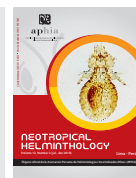




## Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

TEMNOCEPHALIDS ON *TRACHEMYS DORBIGNI* (DUMÉRIL & BIBRON, 1835) (TESTUDINES, EMYDIDAE): IMPLICATIONS OF ANTHROPOGENIC ENVIRONMENTS AND TURTLE'S GENDERS IN SYMBIOTIC RELATIONSHIPS

TEMNOCÉFALOS SOBRE *TRACHEMYS DORBIGNI* (DUMÉRIL & BIBRON, 1835) (TESTUDINES, EMYDIDAE): IMPLICANCIAS DE LOS AMBIENTES ANTROPOGÉNICOS Y LOS GÉNEROS DE LAS TORTUGAS EN LA RELACIÓN SIMBIÓTICA

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### ABSTRACT

We studied the association between epibiont helminths temnocephalids and the freshwater turtle *Trachemys dorbigni* (Duméril & Bibron, 1835), comparing urban and rural environments and turtle's genders. Sixty specimens of *T. dorbigni* were collected in urban canals and rural lakes at two locations in Rio Grande do Sul, southern Brazil. *Temnocephala pereirai* Volonterio, 2010 and *Temnocephala* sp. (Temnocephalidae) were found associated with *T. dorbigni* only in rural environments. There was a positive relationship between epibiont helminths and males of *T. dorbigni*, suggesting that the population dynamics of these helminths can be related to the behavior or reproductive condition of *T. dorbigni*.

**Keywords:** Brazil – freshwater turtles – rural area – *Temnocephala* – turtle's genders – urban area

### RESUMEN

Se estudió la asociación entre los helmintos epibiontes de Temnocephalidae y la tortuga de agua dulce *Trachemys dorbigni* (Duméril & Bibron, 1835), comparando los ambientes urbano y rural, y el género sexual de las tortugas. Sesenta especímenes de *T. dorbigni* fueron recolectadas en canales urbanos y lagos de la zona rural de dos localidades de Rio Grande do Sul, extremo sur de Brasil. Los epibiontes, *Temnocephala pereirai* Volonterio, 2010 y *Temnocephala* sp. (Temnocephalidae), fueron encontrados solamente en las tortugas de la zona rural. Se observó una relación positiva entre helmintos epibiontes y los machos de *T. dorbigni*, sugiriendo que la dinámica poblacional de los helmintos esté relacionada con el comportamiento y la condición reproductiva de *T. dorbigni*.

**Palabras clave:** Brasil – género sexual de las tortugas - *Temnocephala* - tortuga de agua dulce - zona rural - zona urbana

## INTRODUCTION

*Temnocephala* Blanchard, 1849 (Temnocephalida, Temnocephalidae) consists of 31 nominal endemic species of the Neotropical Region, associated with freshwater organisms such as crustaceans (Damborenea & Cannon, 2001; Amato *et al.*, 2003, 2006; Volonterio, 2007; Seixas *et al.*, 2011; Seixas *et al.*, 2018), insects (Damborenea & Cannon, 2001; Amato & Amato, 2005; Amato *et al.*, 2007, 2011), molluscs (Damborenea & Cannon, 2001; Damborenea & Bursa, 2008; Garcés *et al.*, 2013; Seixas *et al.*, 2015) and freshwater turtles (Damborenea & Cannon, 2001; Volonterio, 2010; Seixas *et al.*, 2014).

Most studies on temnocephalids associated with freshwater turtles are taxonomic studies. Temnocephalids have been reported mainly in turtles of the family Chelidae from South America. In Brazil, *Temnocephala brevicornis* (Monticelli, 1889) has been reported in the chelids *Acanthochelys spixii* (Duméril & Bibron, 1835) (Yuki *et al.*, 1993), *A. radiolata* (Mikan, 1820), *Mesoclemmys gibba* (Schweigger, 1812), *Hydromedusa tectifera* Cope, 1870 (Pereira & Cuocolo, 1940) and *H. maximiliani* (Mikan, 1820) (Pereira & Cuocolo, 1940; Novelli *et al.*, 2009). In addition, *Temnocephala* sp. has been reported from *H. tectifera* (Soares *et al.*, 2007). In Uruguay, *T. brevicornis*, *T. pereirai* Volonterio, 2010 and *T. cuocoloi* Volonterio, 2010 have been recorded in *H. tectifera* (Cordero, 1946; Volonterio, 2010). There has been only one record of *T. brevicornis* in *H. tectifera* (Brusa & Damborenea, 2000) in Argentina. In emydid turtles, there has been a report of association of *T. brevicornis* and *T. pereirai* with *Trachemys dorbigni* (Duméril & Bibron, 1835) in Rio Grande do Sul State, Brazil (Yuki *et al.*, 1993; Seixas *et al.*, 2014).

Species richness in the urban-rural gradient has been increasingly studied to assess the impact of urbanization on biodiversity. Typically, urban landscapes have presented less biological diversity than rural environments (Mckinney, 2002; Kowarik, 2011). The loss of biological diversity has been linked to several direct and indirect human actions (Hero & Ridgway, 2006). Thus, increased urbanization and agricultural activity

cause modification or loss of habitats (Mckinney, 2002; Hero & Ridgway, 2006). These factors are considered the main threats to biodiversity (Brooks *et al.*, 2002). In southern Brazil, for example, wetland ecosystems are undergoing rapid changes and significant area loss due mainly to agricultural activities and actions driven by urbanization, such as landfills and garbage and sewage dumps (Carvalho & Ozório, 2007).

*Trachemys dorbigni* (Duméril & Bibron, 1835) occurs in Brazil, Uruguay and Argentina (Van Djik *et al.*, 2014). In Brazil, it is one of the most abundant freshwater turtles in Rio Grande do Sul State (Bujes *et al.*, 2011). *Trachemys dorbigni* often be found in heavily modified urban environments, such as sewage systems, and in agricultural environments such as rice field drainage canals with pesticide residues (Bujes & Verrastro, 2008; Fagundes *et al.*, 2010). This species is neither on the IUCN red list (IUCN, 2018) nor on the list of endangered species of Brazilian fauna (ICMBio, 2016).

Studies of epibiont helminths associated with freshwater turtles contribute to the knowledge of the biology of the hosts and their habitats, contributing to the conservation of species and of the ecosystems that support these invaluable relationships. In this context, this study aims to analyze the association between epibiont helminths (Temnocephalidae) and the freshwater turtle, *T. dorbigni*, in relation both urban and rural environments and turtle's gender.

## MATERIAL AND METHODS

Turtles were collected between July 2010 and December 2012 in two distinct areas, one urban and one rural, in the State of Rio Grande do Sul, Brazil (Fig. 1). The sampling period encompassed spring and summer months in the southern hemisphere. Only two male hosts were sampled in July 2010.

Sixty adult *T. dorbigni* were manually collected with nets, and transported in plastic containers (56 liter) to the laboratory, where they were examined.

Twenty-eight turtles (13 males, 15 females) were collected in rural areas from four ponds at the Centro Agropecuário da Palma of the Universidade Federal de Pelotas (UFPEl), located in Capão do Leão county (31°48'01.1" S, 52°30'48.6"W) (Fig. 1A and Figs. 2A – B). Another 32 specimens (14 males, 18 females) were collected in canals in the urban area of the city of Pelotas (31°46'16.9" S, 52°18'45.9" W) (Fig. 1B and Figs. 2C – D).

The carapace and plastron, and skin-carapace and skin-plastron junctions of each turtle were examined for external helminths. Posteriorly, the turtles were then sacrificed for study of internal parasitic helminths. Turtle's genders were determined during necropsy. This study was licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (23196-ICMBio) and was approved by the Ethics and Animal Experimentation Commission (3026 - CEEA/UFPEl).

Epibionts were prepared according to Amato *et al.* (1991), and were identified based on morphological characteristics described by Volonterio (2010) and Seixas *et al.* (2014). Vouchers were deposited in the Coleção de Helminthos do Laboratório de Parasitologia de Animal Silvestres (545–568 CHLAPASIL-UFPEl). Some specimens were deposited in the Coleção Helminológica do Instituto Oswaldo Cruz and in the Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia as vouchers of the morphological study carried out by Seixas *et al.* (2014).

To analyze the association between epibiont helminths and turtles, parasitological indices of Bush *et al.* (1997) were adapted and interpreted as ecological parameters of symbiotic interaction. The mean intensity of infection was treated as Mean Intensity (MI) of the non-parasitic association between epibionts and turtles. Prevalence (P%) and Mean Abundance (MA) were used in the sense of Bush *et al.* (1997). These measures can be used to represent population patterns applicable to free-living populations; such as population density and values of minimum and maximum occurrence. These indices were calculated for all sampled turtles, as well as for turtle's genders (Male: M; Female: F). Thus, P% and MI were compared between genders, whereas

the P% values was compared by using the chi-square test ( $\chi^2$ ) ( $p < 0.05$ ) in the software Quantitative Parasitology (QP 3.0) (Reiczigel & Rózsa, 2005), the MI values were compared by using the Mann-Whitney test ( $p < 0.05$ ) in "Paleontological Statistics – PAST 2.17" (Zar, 1999).

## RESULTS AND DISCUSSION

Among all examined turtles ( $N = 60$ ), 25% were positive for epibiont helminths, which belong to *Temnocephala* (Temnocephalidae), *T. pererai* (Fig. 3) and *Temnocephala* sp. The association occurred only in turtles from the rural area, where 53.6% ( $N = 15/28$ ) were positive (Table 1). A total of 2401 (6-1434) temnocephalids were collected on freshwater turtles from rural area, unlike the turtles of the urban area, where there was no association between the epibionts and *T. dorbigni*. Helminths were found on the skin of the neck, axillary, inguinal and perianal areas, as well as the posterior plastron lobe (Figs. 4A – E).

There was no significant difference in the prevalence of epibionts among males and females of the turtles ( $\chi^2$ ,  $p = 0.122$ ) (Table 1). However, temnocephalids showed significant differences in their association with males and females measured as to MI, which was higher in males (MI = 256.78 epibionts) than in females (MI = 15) (Mann-Whitney  $U$  test,  $z = -3.126$ ,  $p = 0.001$ ). The amplitude of epibionts on male turtles was 46 – 1434, whereas female turtles the amplitude was 6 – 29 epibionts (Table 1).

Degradation of freshwater environments may influence the diversity of species occurring in these environments as well as the population dynamics of organisms living at different trophic levels (Collier *et al.*, 2016). Figure 2 shows the aquatic environments of the rural and urban area where the specimens of *T. dorbigni* were collected. The rural ponds were ecosystems little impacted by human intervention (Figs. 2A – B). In contrast, the urban canals were extremely degraded by human activities, such as deposition of trash and sewage (Figs. 2C – D). The characteristics of the aquatic environment in natural ecosystems have not been covered by most studies of temnocephalids, which

are largely of a taxonomic nature. It has been reported that low levels of eutrophication are important for the maintenance of *T. brevicornis* in tanks (Pereira & Cuocolo, 1940). In this context, the absence of temnocephalids in turtles from urban area may be related to the degradation of urban aquatic environments, negatively influencing the occurrence of temnocephalids and organisms that are part of the food web, in which temnocephalids can be considered predators.

The majority of temnocephalids species feed on small organisms such as bacteria, diatoms, protists, rotifers, and nematodes found in, or around, the host and, although they are mobile, they do not normally leave their hosts. If experimentally removed from their host, some individuals of temnocephalids species die promptly, whereas others survive for weeks (Goater *et al.*, 2014). Pereira & Cuocolo (1940) have reported the consumption of small oligochaete, as well as the inability of the temnocephalids to capture other invertebrates such as copepod crustaceans. These predatory characteristics are probably related to displacement of the temnocephalids, which need a solid surface to get around. Dioni (1967) classified *Temnocephala* associated with *Aegla* spp. as epibiont predators that feed directly on polychaetes (*Stratiodrillus*), protozoa, oligochaetes, rotifers and crustacean-associated remains. Thus, this author classified temnocephalids as belonging to the top of the epibiont organism food web.

Organisms that make up the temnocephalids diet have been reported in association with *T. scripta* (Schoepff, 1792) and *Pseudemys concinna* (LeConte, 1830) from a North American (Tumilson & Clark, 1996). The authors mentioned Cyanobacteria, Chlorophyceae, Xanthophyceae, Bascillariophyceae, Protozoa, Turbellaria, Gastrotricha, Rotatoria, Nematoda, Oligochaeta, Crustacea (Cladocera, Copepoda, Ostracoda) and Insecta (chironomids) associated with the carapace and plastron of turtles. Unlike turtles collected from urban canals in the city of Pelotas, some rural specimens showed algae and nematodes associated with the carapace and plastron, pointing to an eventual creation of a micro-habitat favoring an epibiont food web for temnocephalids. The absence of temnocephalids in urban turtles seems to be linked to the degradation of the aquatic environment. This degradation might act as a

limiting factor on the population dynamics of these helminths that live on the turtle in direct contact with the surrounding water and also because they feed on other organisms associated with the turtle carapace.

Although ecological indices suggest that these helminths should occur on males and females, a significant difference in MI of the epibiont helminths between turtle's genders suggests a closer association with males. It is likely that the lower rate of association with females is related to the behavior of females during the nesting period. During this period, females leave the water in search of suitable land for laying eggs causing probably the death or detachment of temnocephalids from their body surface.

Various aspects of reproductive biology of *T. dorbigni* have been studied by Krause *et al.* (1982) and Bager *et al.* (2007) at the Estação Ecológica do Taim in Rio Grande do Sul State, Brazil, where the nesting season extends from September to February. The females start the nesting process by leaving the water to search for a suitable place to construct their nest; after a site has been chosen, it is excavated with the hind limbs, followed by egg laying and nest closing and then finally return to water (Krause *et al.*, 1982; Bager *et al.*, 2007). Krause *et al.* (1982) observed that the distance between the nest and water ranged from 1-400 m depending on soil characteristics; they also observed that on their return to the water the females made 10 to 60 min stops. Bager (2003) did not directly estimate nesting time, but hypothesized that it should last between two and three hours. Furthermore, some *T. dorbigni* females at Estação Ecológica do Taim had three nesting cycles at 15 to 20 day intervals in the same reproductive season (Bager *et al.*, 2007).

The reproductive characteristics of *T. dorbigni* suggest that female behavior can act negatively on the association with temnocephalids. The lengthy nesting process would cause the death of helminths by friction with the ground during the opening and closing of the nest, and the possibility of more than one nesting cycle in the reproductive period.

Lengthy walking activity of female freshwater turtles the search of suitable egg-laying sites have been reported for *Phrynops geoffroanus*

**Table 1.** Prevalence (P%), Mean intensity (MI), Mean abundance (MA) and Amplitude (A) of temnocephalids associated with males and females of *Trachemys dorbigni* in a rural environment of Rio Grande do Sul, Brazil.

Ecological indices	N=28	Turtle's genders	
		Male (N=13)	Female (N=15)
P%	53.6	69.2	40.0
MI ( $\pm$ SD)	160.06 ( $\pm$ 357.93)	256.78 <sup>a</sup> ( $\pm$ 444.82)	15 <sup>b</sup> ( $\pm$ 8.07)
MA ( $\pm$ SD)	85.75 ( $\pm$ 270.26)	177.77 ( $\pm$ 383.56)	6 ( $\pm$ 9.01)
A	6 - 1434	46 - 1434	6 - 29

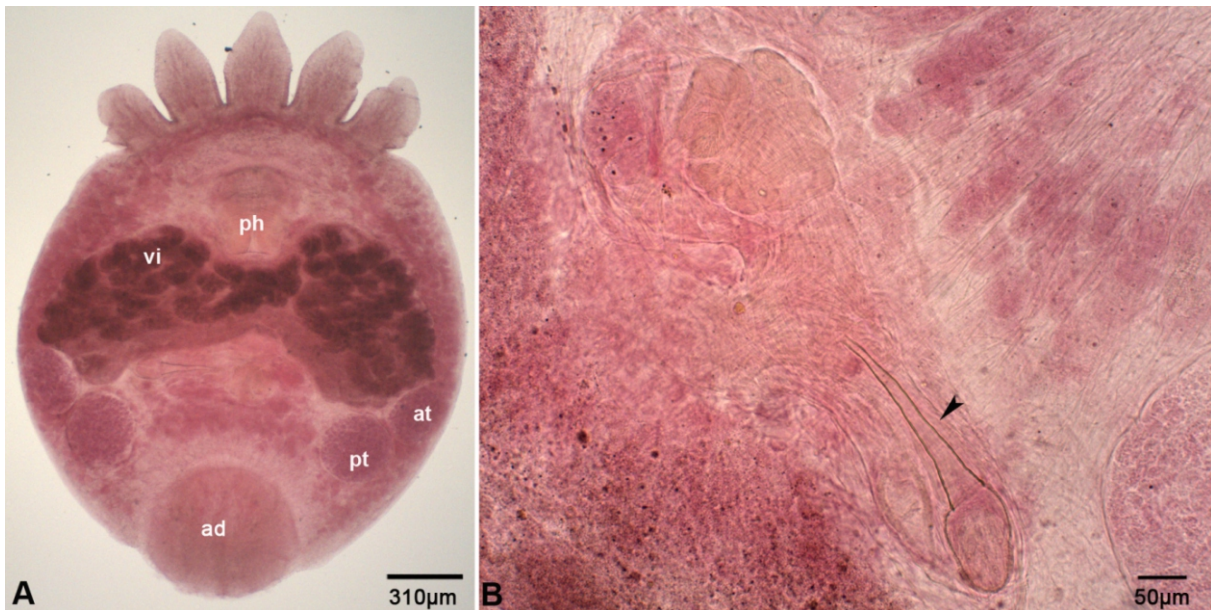
N – number of turtles examined; SD – standard deviation; a, b indicate differences between turtle's gender (Mann-Whitney U test,  $z = -3.126$ ,  $p = 0.001$ )



**Figure 1.** Overall layout of the collection environments in the study of epibionts associated with *f Trachemys dorbigni* in southern Brazil. A – Detail of the Centro Agropecuário da Palma (UFPel), rural area of Capão do Leão, State of Rio Grande do Sul. B – Detail of the urban area of Pelotas, Rio Grande do Sul. Source: Extracted and modified the site Google® Earth (©2014 Google – Images ©2014 Digital Globe).



**Figure 2.** Aquatic environments where the specimens of *Trachemys dorbigni* were collected in southern Brazil. **A–B.** Ponds in a rural area of Capão do Leão, Rio Grande do Sul State, Brazil. **C–D.** Canals of the urban area of Pelotas, Rio Grande do Sul, Brazil.



**Figure 3.** *Temnocephala pererai* stained with carmine Langeron. **A.** ph – pharynx, vi – vitellarium, at – anterior testes, pt – posterior testes, ad – adhesive disc. **B.** The arrow shows cirrus.

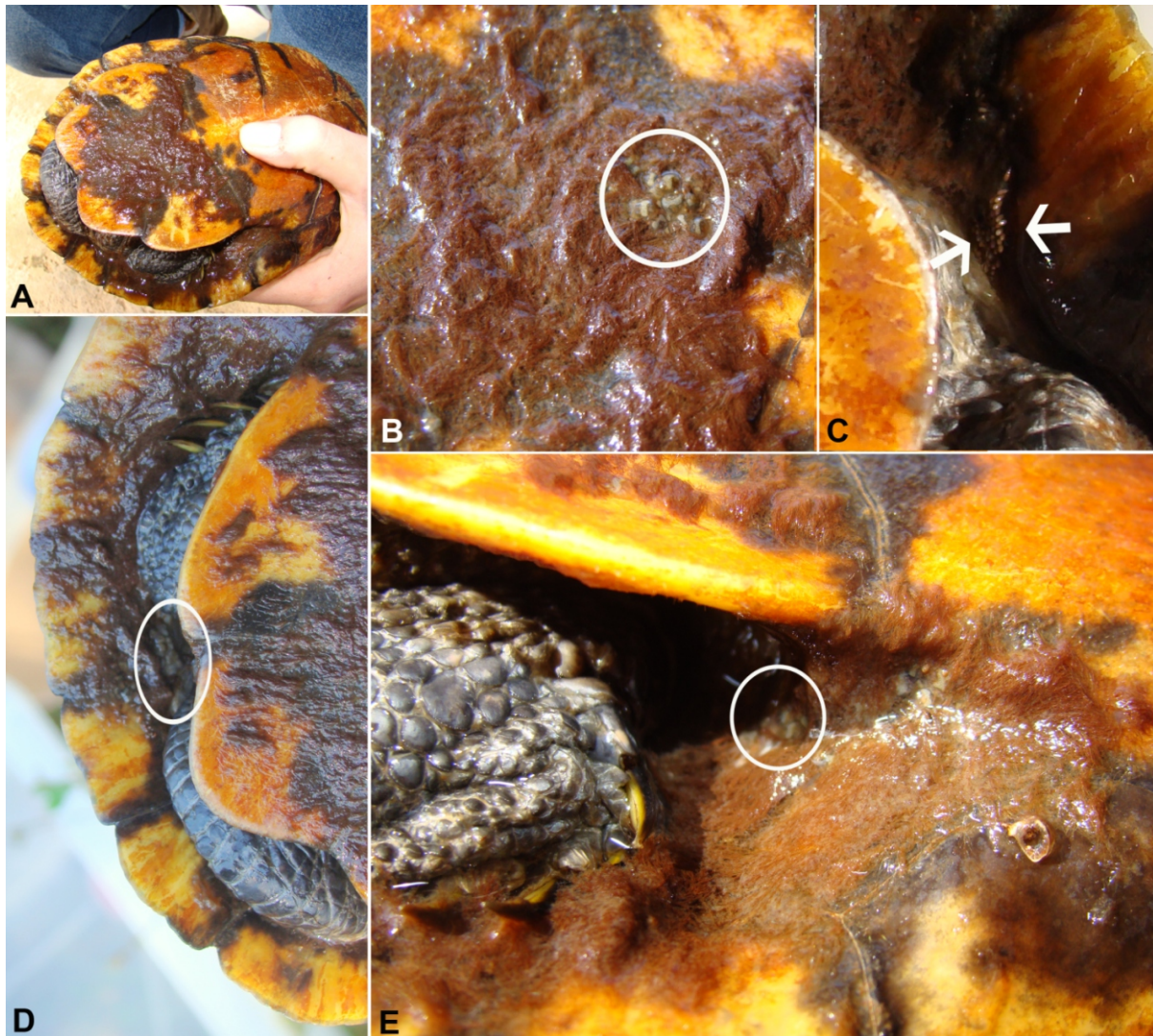
(Schweigger, 1812) (Guix *et al.*, 1989). This chelid species can repeat this process several times, abandoning sites considered inappropriate and looking for new ones, returning to the water body and leaving it repeatedly during both pre and post laying periods (Guix *et al.*, 1989). These authors reported that the nesting time (from the moment the female leaves water until its return) can last up to 90 min.

A field observation made during turtle collection that may strengthen our argument on the relationship between the turtle's genders and epibionts is the fact that many *T. dorbigni* males had their shell covered with algae (Figs. 4A – E), unlike the females. This suggests that males remain in aquatic habitat and these behaviors allow a more positive association with temnocephalids and their epibiont prey community. These aspects may be reflected in P%, MI and MA values in this study. Bager (2003) commented that males probably do not frequently leave the water when they are in favorable environments with respect to food availability, sun exposure areas, and water volume. It seems likely that recolonization of female *T. dorbigni* by temnocephalids occurs at the end of the nesting season through direct contact with males (in copulation), and/or the through the viable eggs

hatching deposited by epibionts on female turtles. These processes that occur after nesting could provide continuity of the association between temnocephalids and *T. dorbigni* females. Although the freshwater turtles in this study were captured during the reproductive period of the species, it is difficult to determine how much influence of the behavior and reproductive condition have on the epibiont helminths population dynamics.

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**Figure 4.** A - Ventral view of male *Trachemys dorbigni* with temnocephalids. B - Posterior lobe of plastron with epibionts (circle). C - Region between the carapace and plastron near the tail with eggs of temnocephalids (arrows). D - Region between the carapace and plastron with helminths near the tail (circle). E - Region between the carapace and plastron with temnocephalids near inguinal area (circle).

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