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Prevalence of intestinal cestode infections of conventionally maintained laboratory (albino) and house mice in Kalar district / Sulaymaniyah province

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Abstract

Mice (*Mus musculus*), mammalian rodent animals belonging to the order Rodentia, family Muridae, represent one group of the most important reservoir hosts for human pathogens. In this study, a total of 127 mice were necropsied for intestinal cestode infections. Ten of them were conventionally maintained laboratory mice and the remaining 117 were house mice and collected between July 2009 and October 2016 in numerous storages of hay, rice, cereals, flour, as well as in houses from different regions of Kalar district. Six out of 10 laboratory mice were found infected with *Hymenolepis nana*, family Hymenolepididae, with the prevalence rate of 60%. Non-significant differences were found between both sexes. On the other hand, only one house mouse was found infected with *H. nana* with infection rate of 0.85%. In addition to this cestode, house mice were found infected with two unidentified cestodes from the family *Cataenotaenidae*, with the overall infection rate of 12.82% (15 out of 117 house mice). The overall prevalence rates of infections were 7.46% and 20% in male and female house mice; respectively. Significant differences were observed between overall prevalence of *Cataenotaenidae* species in infected male and female house mice. In addition, the results of this study showed that there were significant differences ($P < 0.01$) between overall infection of laboratory and house mice regarding cestode infections.

To sum up, laboratory mice were found infected with intestinal cestodes of public health concern and these might contaminate the environment, infect other animals and humans in the laboratory, and affect the accuracy of the experimental results. Therefore, an appropriate management and control of diseases in these animals, prior to the experiment, must be considered. Other cestodes were also identified belonging to the family *Cataenotaenidae* but it was not possible to identify them at species level due to lack of a proper morphological guide. Further study will be required to identify these parasites at species level using molecular methods.

Introduction

Mice (*Mus musculus*, Linnaeus, 1758) including house and conventionally maintained laboratory species, like other

mammalian animals represent one of the most important reservoir hosts for human pathogens including bacteria, virus and

parasitic organisms (Bonthius 2012; Gomes-Solecki et al. 2017; Webster 2007). Several species of helminth cestodes of veterinary and public health concern have been found in the small intestine of house and laboratory mice all over the world (Ahmad et al. 2014; CDC 2018; Chou et al. 1998; Pinto et al. 1994). The most common cestodes encountered in the majority of the researches were belonging to the species of the genus *Hymenolepis*, family Hymenolepididae including *Hymenolepis nana* (*H. nana*) and *Hymenolepis diminuta* (*H. diminuta*). In addition to mice and rats, these parasites were also found to infect human particularly children and young people (Craig and Ito 2007; Marangi et al. 2003). That may cause lethal cestodiasis (Olson et al. 2003). In addition, several other species of cestodes belonging to the family Cataenotaenidae were found to infect these rodents but not humans (Haukisalmi et al. 2018; Owen 1976).

Hymenolepis nana is small cestode ranging between 1.5-4 cm long with a scolex characterizes by armed rostellum, while the length of *H. diminuta* varies between 20- 60 cm long with an unarmed rostellum (CDC 2018). The life cycle of *H. nana* and *H. diminuta* are indirect, adult stages infect small intestine of different mammalian animal species including mice, rats and humans. Different species of arthropods including grain beetles, flour beetles, and fleas known to play critical role as intermediate hosts and transmit these parasites to the definitive hosts (John et al. 2006). However, the life cycle of *H. nana* could be indirect through ingestion of infective eggs. The eggs of this cestode are

infective once passed in stool and if ingested by the definitive host, the hexacanth embryo releases and become an adult in the small intestine (Baker 2008; CDC 2018). In addition, *H. nana* has the ability to cause reinfection of the infected host through a process known as autoinfection. The eggs hatch within the small intestine of the host and penetrate the intestinal villi and develop into cysticercus and ultimately an adult parasite (Baker 2008).

While species belonging to the family Cataenotaenidae are host specific and found to infect different rodent hosts but not humans (Haukisalmi et al. 2018). *Cataenotaenia pusilla* as an example, the worm's length reaches 3-16 cm long, the scolex is bearing four sucker and unarmed rostellum. The life cycle is indirect, mice are hosts for the adult tapeworm and different arthropod species (common grain mites, *Tyroglyphus farinae* and *Glyphogastus domesticus*) as intermediate hosts. In the body cavity of these arthropods, the embryo become cysticercoid (Owen 1976).

In Iraq, there are many studies investigated the prevalence of infection with *Hymenolepis* species in humans. These studies found different rates of infections in humans in various provinces, for examples, in Najaf province 1.79% (Taher 2017), in Baghdad capital 9.82% and 6.67 % (Al-Kubaisy et al. 2014; Al-Marsome 2012), in the Hilla, Babylon Province 6% and 5.3% (AL-Khafaji and AL-Jiboury 2013; Al-Morshidy 2007), in Erbil province, the northern part of Iraq 4.04% (Al-Daoudy et al. 2017) and in Kalar district, Sulaymaniyah province 4.04% (Bajalan 2010).

On the other hand, other studies in Iraq have attempted to identify these cestodes from the reservoir hosts, mice and rats. These studies have found different prevalence rate of several cestode species in variety of rodent hosts. For instances, Taher (2017) has found *H. nana* in house mice in Najaf province. Another study in Baghdad, isolated *H. nana* and *H. diminuta* in house mice and rats (*Rattus norvegicus* and *Rattus norvegicus*) (Hasson 2010). In addition, *Hymenolepis* spp. have been found in conventionally maintained laboratory rats (Ahmed et al. 2012; Karim and Al-Salihi 2014).

Based on the presented information about the prevalence of cestodes in mice and rats in Iraq, no study has found other intestinal cestodes other than the species belonging to the family Hymenolepididae. To the best of my knowledge, no study has investigated the prevalence of the intestinal cestodes in Kalar district and Sulaymaniyah province. Therefore, this study aimed to investigate the prevalence of the intestinal cestodes infecting conventionally maintained laboratory mice, and house mice captured in different vicinities of Kalar district.

Material and methods

Geography of the studied area

Kalar district is located in the southeast of Sulaymaniyah province, and is belonging to the Garmian Administration, Kurdistan region of Iraq. The term (Garmian) is a Kurdish word which is used to denote a 'hot and dry area' indicating information about location and climate. Geographically, Kalar is located in between the latitude (34°37'45"N) and the longitude (45°19'20"E) of the eastern hemisphere. It

has a total area of 438,317 km². According to the official site of the general board of tourism of Kurdistan- Iraq in 2015, the total population of the central town of Garmian, Kalar, is about 250,000 residents (BOT 2015).

Sample collection and preparation

A total of 127 mice *Mus musculus* were necropsied for intestinal cestode infections. Ten of these were conventional laboratory (albino) mice used for researches and were obtained from an institutional animal house from Erbil province, Kurdistan region, Iraq. While the remaining 117 were house mice and collected between July 2009 and October 2016 by using trap and adhesive glue in many storages of hay, rice, cereals, flour, as well as in houses from different regions of Kalar district including the city center and the surrounding villages.

In case of laboratory mice, following euthanasia, they were alive and euthanized in a chamber containing a piece of cotton soaked with ether. While in case of the house mice, they arrived either alive or recently dead. In both types of the mice, they lied on their back and an incision was made in the midline of the abdominal wall. After that, the small and large intestine were removed and put in a clean petri dish containing warm normal saline and cut longitudinally very carefully to expose the contents (Fan 2005). Cestode helminthes were recovered alive from the intestine. Next, they allowed to relax completely in warm normal saline for 10 minutes. Then they were preserved in acidified formal alcohol (AFA) fixative for 48 hours. Finally, the worms were transferred into 70%

ethanol. The number of worms were counted and recorded for each mouse.

The isolated cestodes were stained by Kirkpatrick's carmalum, then dehydrated and mounted in Canada balsam (Drury and Wallington 1976). The worms were identified depending on the morphology of the proglottids, the presence and absence of hooks on the scolex, and the shape and size of the eggs from feces collected in rectum (CDC 2018; Marangi et al. 2003; Owen 1976; Pinto et al. 1994).

Statistical analysis

Chi-square (χ^2) test was used for statistical analysis of the study results using SPSS software program "SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.)" were used for all analyses. A *P*-value of <0.05 denoted a statistically significant difference.

Results

Six out of 10 laboratory mice were found infected with the *H. nana*, the only cestode identified with the prevalence rate of 60% (6 out of 10). Both male and female were found infected similarly, and no significant differences ($P= 1$) were found between both sexes (Table 1). On the other hand, only one house mouse was found infected with *H. nana* with infection rate of 0.85% (one out of 117). *Hymenolepis nana* was the only cestode species of the family Hymenolepididae isolated from both laboratory and house mice.

In addition to this cestode, house mice were found infected with one of the two different cestodes isolated from the family Cataenotaenidae, with the overall infection rate of 12.82% (15 out of 117 house mice) (Table 1). The overall prevalence rates of infections were 7.46% (5 out of 67 mice) and 20% (10 out of 50 mice) in male and female house mice;

respectively. Significant differences ($P= 0.045$) were observed between overall prevalence of Cataenotaenidae species in infected male and female house mice (Table 1 and 2).

The prevalence of infections with these cestodes were 13.46% (7 out of 52) in the city and 12.30% (8 out of 65) in the surrounding villages. There were no significant differences ($P= 0.853$) between infections in the city center and villages (Table 2).

In addition to all the statistical data mentioned above, the results of this study showed that there were significant differences ($P= 0.001$) between overall infection of laboratory and house mice regarding overall cestode infections and *H. nana* as well (Table 1).

The general morphology of the cestodes isolated from the intestine were studied (Table 3). All the cestodes isolated from the laboratory mice were morphologically similar to *H. nana* (Figure 1a-e); they had armed rostellum, their eggs were oval shaped, with an average size of 45 μm long by 37.5 μm wide. There were two poles on the inner membrane of the eggs, from which 4-8 polar filaments projected out and spread between the inner and outer membranes (CDC 2018). In addition, only one house mouse was also found infected with *H. nana* cestode and it was similar to those of the infected laboratory mice morphologically.

In house mice, other cestodes also were found, which were morphologically different from all known species of the Hymenolepididae family. These cestodes were of two morphologically distinct species, here referred to as species 1 and species 2 (Table 3, Fig 1f-i, and Fig 1j-m). Both of the cestode's scolexes were characterised by unarmed rostellum. Species 1 had square shaped gravid segments (Fig 1h), while the species 2 had elongated shape (Fig 1i). Eggs of species 1 (Fig 1i) were smaller than those of species 2 (Fig 1m) with an average length and width of 63 x 61 μm , and 78 x 52 μm , respectively. However, both eggs were morphologically very similar.

The average length of the isolated worms was 3.7 cm (range 1-8 cm) for the species 1 (Table 3) and 2.7 cm (range 1.3-4 cm) for the species 2 (Table 3).

Discussion

Due to apparent lack of studies investigating cestode infection in both conventionally laboratory mice use for experimental studies, and house mice in Kalar district, Kurdistan region of Iraq, this study aimed to study the prevalence of the intestinal cestodes infecting these rodents in Kalar district. A total of 127 mice (10 albino and 117 house mice) were necropsied and examined for infection with intestinal cestodes of veterinary and public health importance. An overall prevalence rate of infection with cestodes in albino (60%) and house mice (13.67%) were reported. A significant difference ($P= 0.001$) was observed between infections with cestodes in both types of the mice. An overall prevalence rate of infection with *H. nana* was 60% (6 out of 10) for albino mice, while only one out of 117 house mice were infected with this cestode with the prevalence rate of 0.85%. However, none of the mice were infected with *H. diminuta*.

The result of this study is not comparable with other studies conducted in central and southern Iraq in that they found higher infection rates from house mice. For examples, Taher (2017) has found 20% overall prevalence rate of infection with *H. nana* in house mice in Najaf province. Another study in Baghdad, reported 3.5% and 7.3% from house mice and rats, respectively (Hasson 2010). The difference in the prevalence rates could be due to the geographical divergences between north and

other parts of Iraq and public health crises following civil war since 2003 (Levy and Sidel 2013).

The prevalence of the current study was also lower than other study conducted over all the world (Chou et al. 1998; Sharma et al. 2013). In a study conducted in house mice in Taiwan, reported that 63% of them were infected with *H. nana* (Chou et al. 1998). Other studies were also reported high prevalence, 11.42% (house mice) and 39.53% (rats) in Uttarakhand, India (Sharma et al. 2013), 50% and 83% for house mice and rats respectively in Ahvaz, South-West of Iran (Rahdar et al. 2017). The low prevalence of *H. nana* among house mice population in Kalar district might indicate that this rodent would not play critical role in the transmission of the parasite and potentially would not pose public health threat. It is also may be due to the role of regular rodent control using rodenticide by the health authority in the district This might reduce the number of the carrier mice and hence decrease the infection rate within their population. Furthermore, it could be due to the effect of the hot weather in summer which reduces the infectivity of the parasite eggs and also diminish the activity of the intermediate hosts (Selstad Utaaker and Robertson 2015). A larger population study in different seasons, will be recommended to confirm this.

The present study has not reported infection with *H. diminuta*, which was different from other studies in that they reported infection with this parasite with an overall prevalence rate of 7.3%, 12.5% and 4.4%, in house mice, *Rattus norvegicus* and *Rattus rattus*, respectively in Baghdad (Hasson 2010), an

overall of infection 5.71% and 44.18% in house mice and rats in Uttarakhand, India (Sharma et al. 2013), 0.5% and 14.2% in house mice and black rats in Yucatan, Mexico (Panti-May et al. 2017), 10% and 17% for house mice and rats respectively in Ahvaz, Iran (Rahdar et al. 2017). In addition, Karim and Al-Salihi (2014) have found that 75% (3 out of 4) of the laboratory rats necropsied infected with *H. diminuta*. Furthermore, another study on conventionally maintained laboratory rats, overall infection rate with *Hymenolepis* spp. was 4% (Ahmed et al. 2012). The absence of *H. diminuta* in the examined mice could be due to the fact that this worm is specific to rat species (rat tapeworm) (length range between 20-60 cm) (CDC 2018). In addition, it could also be due to differences in the habitats of these animal species, rats usually dwell sewage tunnel and live far away from human activities than mice which prefer to inhabit houses (Feng and Himsworth 2013).

Albino mice were found infected with *H. nana* with an overall prevalence rate of 60%. This result is comparable with the reports of a study conducted to investigate the range of intestinal parasites in conventionally maintained laboratory mice from different laboratory animal houses (Pinto et al. 1994). The study reported overall prevalence rates from 29.15% to 88% in the mice from different suppliers (Pinto et al. 1994). High prevalence of laboratory mice with a zoonotic helminth *H. nana* could be due to the life cycle of the parasite which develop in two ways, monoxenous (direct without involving intermediate host) and heteroxenous (involving grain beetles as

intermediate host) (CDC 2018). In both ways of the life cycle, a proper management is required to control this helminth and prevent transmission to other animals as well as humans working in the laboratory (Ngongeh et al. 2011). It is worth to mention that presence of parasites within the small intestine of laboratory mice may lead to misinterpretation of some experimental results (Pinto et al. 1994).

Based on the information presented about the prevalence of cestodes in mice and rats in Iraq, no study has found intestinal cestodes other than the species belonging to the family Hymenolepididae. In this study, depending on observing the morphological characteristics, it has been reported two of unidentified species of cestodes from small intestine of house mice in Kalar district/ Iraq with the overall prevalence rate of 12.82 (15 out of 117). These species were potentially linked to the family Cataenotaenidae Spasskii, 1950 (Haukisalmi et al. 2018; Owen 1976). It is a family of cyclophyllidean cestodes, which involves 37 species belonging to seven genera and they are parasites of rodent (host specific) and were not found to infect humans, reviewed in Haukisalmi et al. (2018). Significant differences were observed in female than in male ($P= 0.045$), could be related to suppressed immune response of female during pregnancy (Engels et al. 2017).

There is an apparent lack of morphological guides for the diagnoses of species belonging to this family (Haukisalmi et al. 2018). Therefore, it was very difficult to explicitly identify the exact species based on the morphological characteristics of the isolated cestodes. Therefore, Haukisalmi et

al. (2018) were studied species belong to the family Cataenotaenidae using molecular biology to identify these cestodes.

In conclusion, the intestinal cestodes of zoonotic importance infesting laboratory mice may contaminate the environmental niche where other mammals including humans could be infected. Also, infection of the laboratory mice can potentially have negative impact on any physiological or pharmacological results obtained experimentally. Therefore, strict control measures to confirm this parasite should be taken into consideration prior to any experimental design. In addition, further studies on rats and house mice habitats in the area and study these worms will uncover the prevalence differences between infections with *H. nana* and *H. diminuta*.

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Furthermore, this study has discovered two unidentified species of cestodes belonging to the family Cataenotaenidae in house mice using morphological tools. However, it is recommended using molecular methods and phylogenetic analysis to identify the species of these worms. In addition, it is recommended to further study these cestodes in rats and other rodents in the area to understand the epidemiology of these parasites.

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Table 1: Distribution of cestode infections in both types of mice according to sexes.

| Sex | Laboratory mice/ Number = 10 Total no. infected with cestodes = 60% | | House mice/ Number = 117 Total no. infected with cestodes = 13.67% | | |
|--------|--|--------------------------------------|---|--------------------------------------|---|
| | Examined | Infected No. (%) with <i>H. nana</i> | Examined | Infected No. (%) with <i>H. nana</i> | Infected No. (%) with species of Cataenotaenidae family |
| Male | 5 | 3 (60) | 67 | 1 (1.49) | 5 (7.46) |
| Female | 5 | 3 (60) | 50 | 0 (0) | 10 (20)* |
| Total | 10 | 6 (60)** | 117 | 1 (0.85) | 15 (12.82) |

* Statistically significant (P= 0.045)

** Statistically significant (P= 0.001)

Table 2: Distribution of infections with the species of Cataenotaenidae in the house mice according to both sexes and localities.

| Sex | House mice | | | | | |
|--------|-------------|------------------|----------|------------------|----------|------------------|
| | city center | | villages | | Overall | |
| | Examined | Infected No. (%) | Examined | Infected No. (%) | Examined | Infected No. (%) |
| Male | 25 | 1 (4) | 42 | 4 (9.52) | 67 | 5 (7.46) |
| Female | 27 | 6 (22.22) | 23 | 4 (17.39) | 50 | 10 (20) |
| Total | 52 | 7 (13.46) | 65 | 8 (12.30) | 117 | 15 (12.82) |

Table (3): Represent morphological data of *H. nana* and cataenotaenidae species.

| Parameters | Morphological parameters | | |
|---|--|---------------------------------------|---|
| | <i>H. nana</i> (µm) Present | Species 1 Fig 1f-i (µm)* Absent | Species 2 Fig 1j-m (µm)* Absent |
| Hooks | | | |
| Immature segments (SD)** (length x width) | 95 (10) x 313 (25) (4 segments) | 248 (45) x 804 (61) (7 segments) | 213 (14) x 273 (12) (7 segments) |
| Mature segments (SD) (length x width) | 138 (31) x 326 (64) (6 segments) | 542 (31) x 1235 (52) (7 segments) | 370 (34) x 217 (8) (6 segments) |
| Gravid segments (SD) (length x width) | 130 (24) x 360 (31) (4 segments) | 1009 (68) x 1368 (64) (8 segments) | 2590 (522) x 1035 (272) (5 segments) |
| Eggs (SD) (length x width) | 45 (2) by 37.5 (2.2) (8 eggs) | 63 (4) x 61 (3) (8 eggs) | 78 (5) x 52 (4) (3 eggs) |
| Average length of whole worm (SD) | 2.06 cm/ 16 worms (range 0.5-6 cm) (1.6) | 3.7 cm/ 24 worms (range 1-8 cm) (2.5) | 2.7 cm/ 2 worms (range 1.3-4 cm) (1.9) |
| Number of worms / mice (minimum-maximum) | 1-8 | 1-17 | 1-5 |

* These two species belong to the family Cataenotaenidae.

** Standard deviation

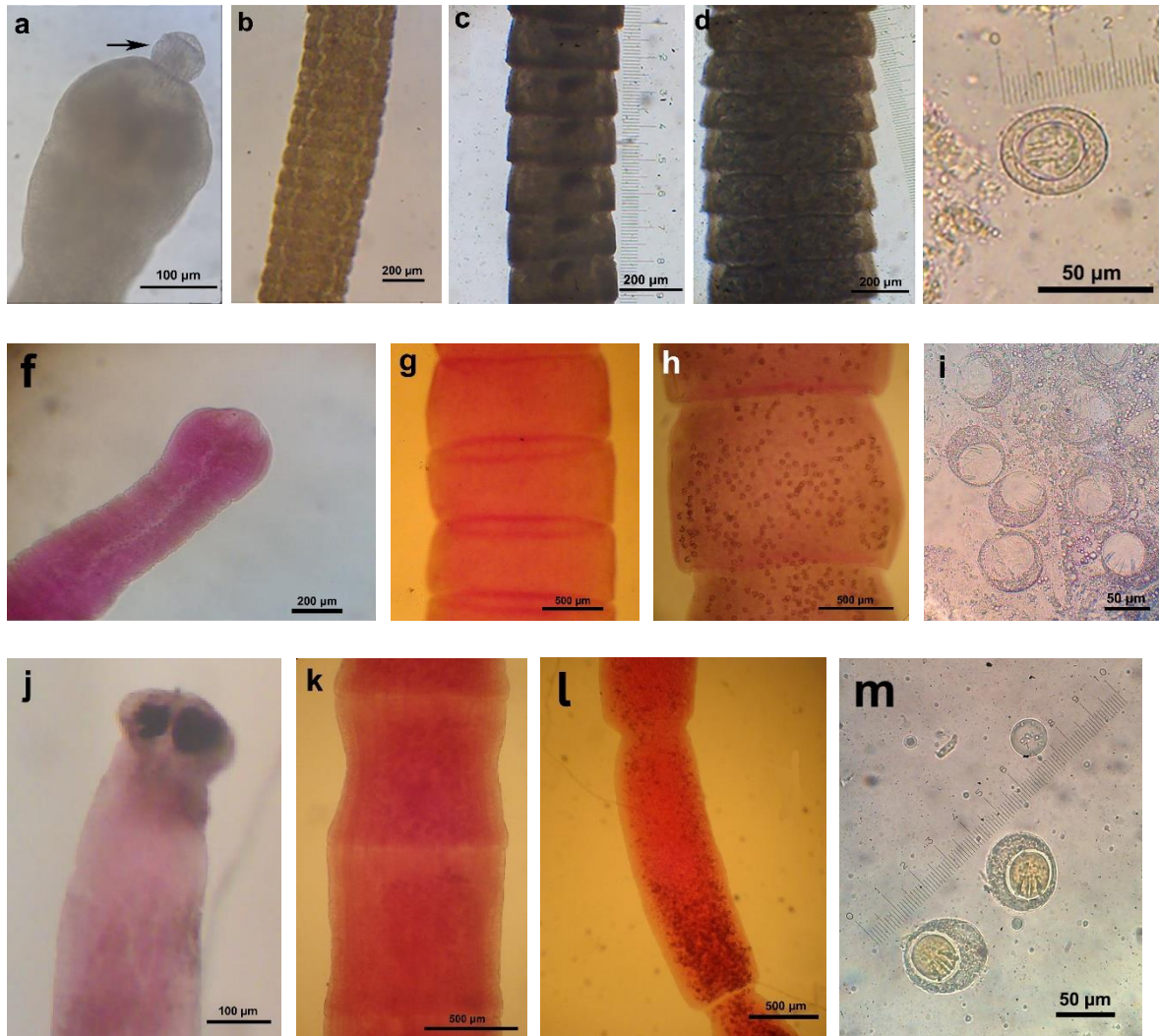


Fig 1. Isolated intestinal cestodes from conventionally maintained laboratory and house mice. *Hymenolepis nana*, No.: 7 infected mice (unstained, a: scolex (armed rostellum (arrow)); b: immature segments; c: mature segments and e: egg) and two unidentified species belong to the family Cataenotaenidae, No.: 15 infected mice (species 1 and species 2) (stained with carmine stain, f and j: scolexes; g and k: immature segments; h and l: gravid segments; i and m: eggs).