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LIFE HISTORY, BEHAVIOR AND MORPHOLOGY OF THE IMMATURE STAGES OF *ENOCHRUS QUADRIPUNCTATUS* HERBST IN THE LABORATORY (COLEOPTERA: HYDROPHILIDAE) II- MORPHOLOGY

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Abstract

The life history, behavior and morphology of the immature stages of *Enochrus quadripunctatus* Herbst from Iran are studied. The results of the work on the life history and behavior of this species in the immature stages and as newly-emerged adults have been presented as part I. The morphology of the egg cases, larvae, pupae, and the male genitalia of the adult is covered in the present work. Measurements of the egg cases, larvae, pupae and adults are made and the average ratio of growth in the larval stages given.

Introduction

Aquatic Coleoptera, like other aquatic insects, are valuable in the study of different aspects of biology and ecology, both in the adult and immature stages [12, 13, 19, 31]. Morphological studies on the immature stages are also helpful in the works on taxonomy and phylogeny of their group as a whole [10, 11, 22, 23, 24, 25, 28, 29]. Nonetheless, despite the vast variety of these beetles and their abundance, not much work has been done on the life history or morphology of their immature stages. Most of the work done in this regard on Hydrophilidae is listed in the references [1-8, 15, 16, 18, 20, 21, 26, 27, 30, 32-35]. As is evident by such a short list, this type of work is very limited. Therefore, such a study on almost any of the water beetle species is necessary, because the results of such studies can be helpful in improving general knowledge about these insects and assisting further studies.

Keywords: *Enochrus quadripunctatus* Herbst; Hydrophilidae; Iran; Life history; Morphology

Enochrus quadripunctatus Herbst was selected for this study because it is obtained easily from some of its habitats near Shiraz (Fars, Iran), and because it has not been presented in the literature for such studies.

Details of the methods of rearing this species in the laboratory, its life history, and behavior in the immature stages have been presented previously [18]. In this study, the morphology of the egg cases, larvae, pupae, and male genitalia of the adult is presented. Measurements of the egg cases and the beetle in its immature stages were made (Plate I, Figs. 1-4) and the average ratio of growth according to Dyar's law [9,15] given. Some morphological characters of the adult (male and female) along with some of their measurements (Plate I, Figs. 5-8), which help in their identification, are also presented.

Materials and Methods

Adults were collected from Zarghan and Barm-e-Shur marshes near Shiraz [14,17]. Zarghan marshes are freshwater and Barm-e-Shur marshes are salty.

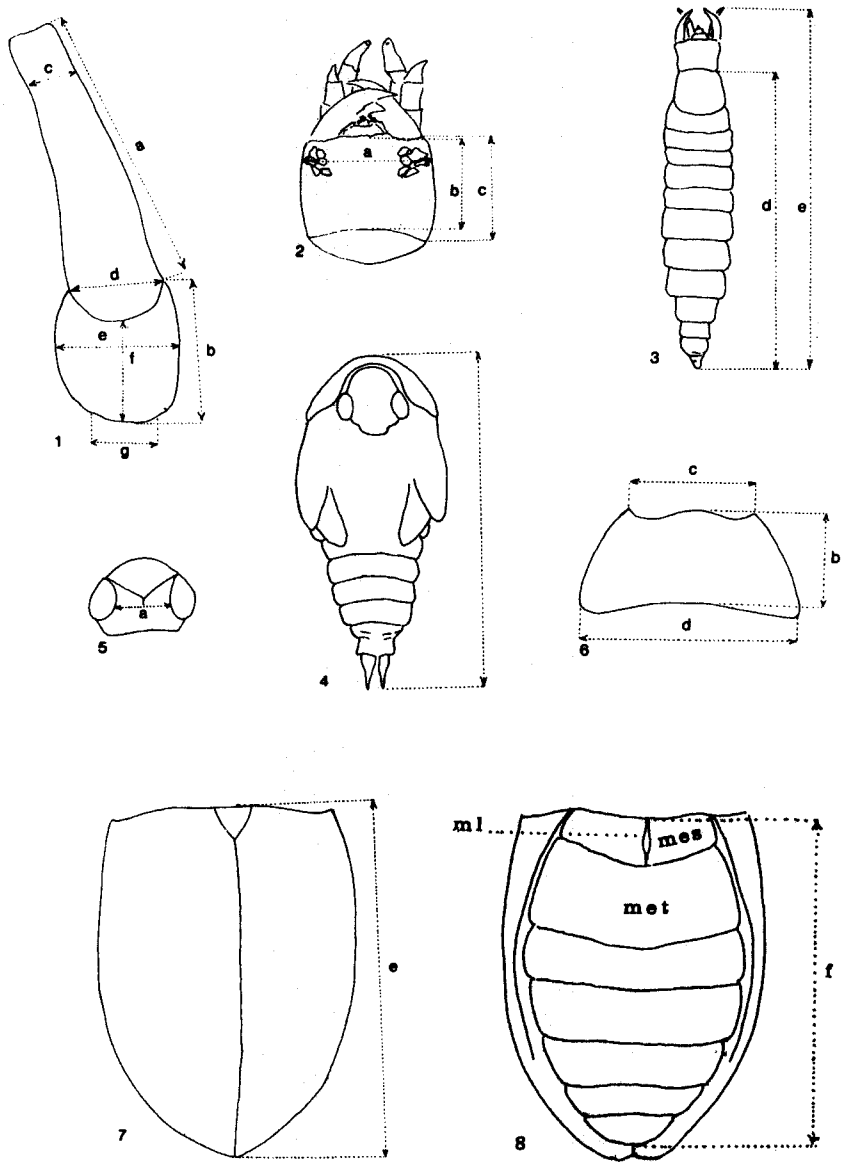


Plate I- Measurements of different parts of *Enochrus quadripunctatus* (see Tables 1-6)

Figure 1. Egg case (see Table 1)
2. Larva, head (dorsal view) (Tables 2-4)
3. Larva (dorsal view) (Tables 2-4)
4. Pupa (ventral view)
5. Adult, head (dorsal view)
6. Adult, pronotum (dorsal view)
7. Adult, elytra
8. Adult, mesothorax (mes), metathorax (met), mesothoracic lamina (ml), and abdomen

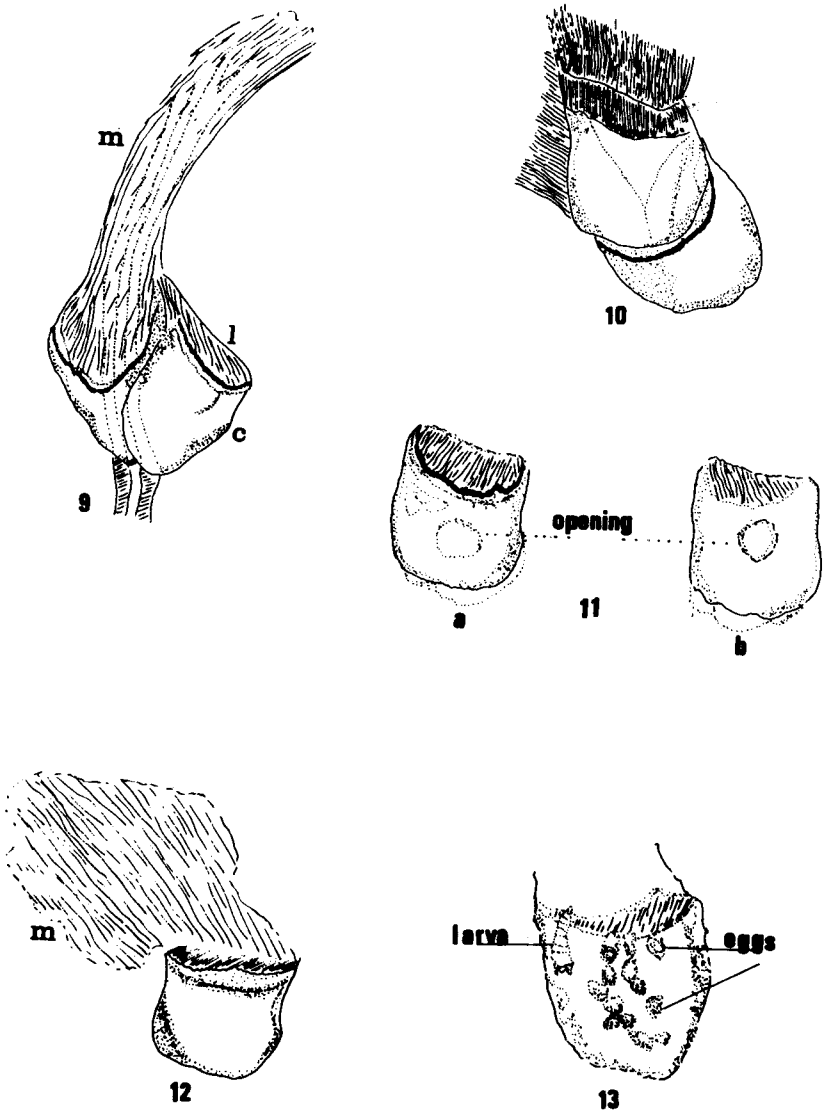


Plate II-Egg case of *E.q.p.*

Figure 9. Two egg cases together on a plant stem

10. Two egg cases together, one almost on top of the other

11. Opening through which the larvae must have crawled out of the case. (It is not the lid; see the lid in Plate I, Fig. 1-d)

12. An egg case with a wide "mast"

13. An egg case demonstrating the eggs within. A larva is also seen ready to crawl out (hatch)

c- case, cup or body

l- lid

m- "mast" or ribbon

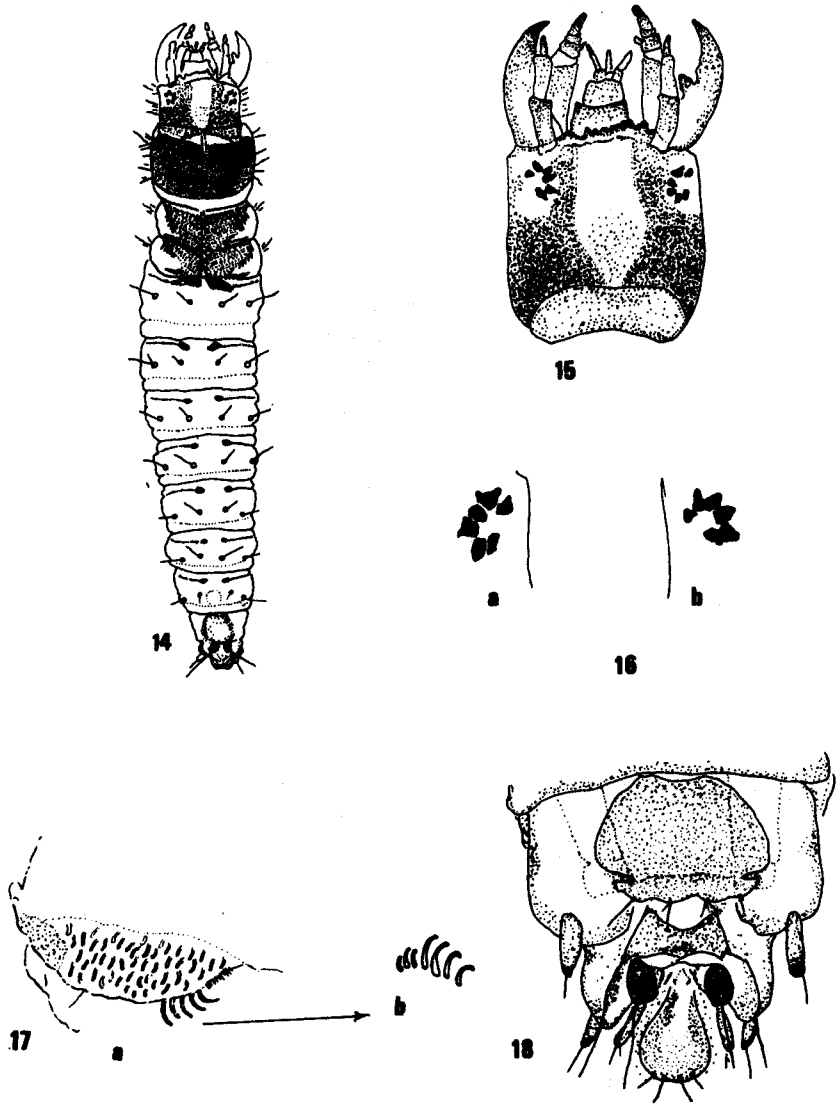


Plate III- Third instar larva of *E.q.p.*

Figure 14. Habitus

15. Head, showing the pigmentation pattern

16. The cluster of simple eyes (ocelli) (enlarged)

a. Right side b. Left side

17. a. A proleg and its hooklets

b. A few hooklets enlarged

18. Stigmatic atrium, dorsal (enlarged)

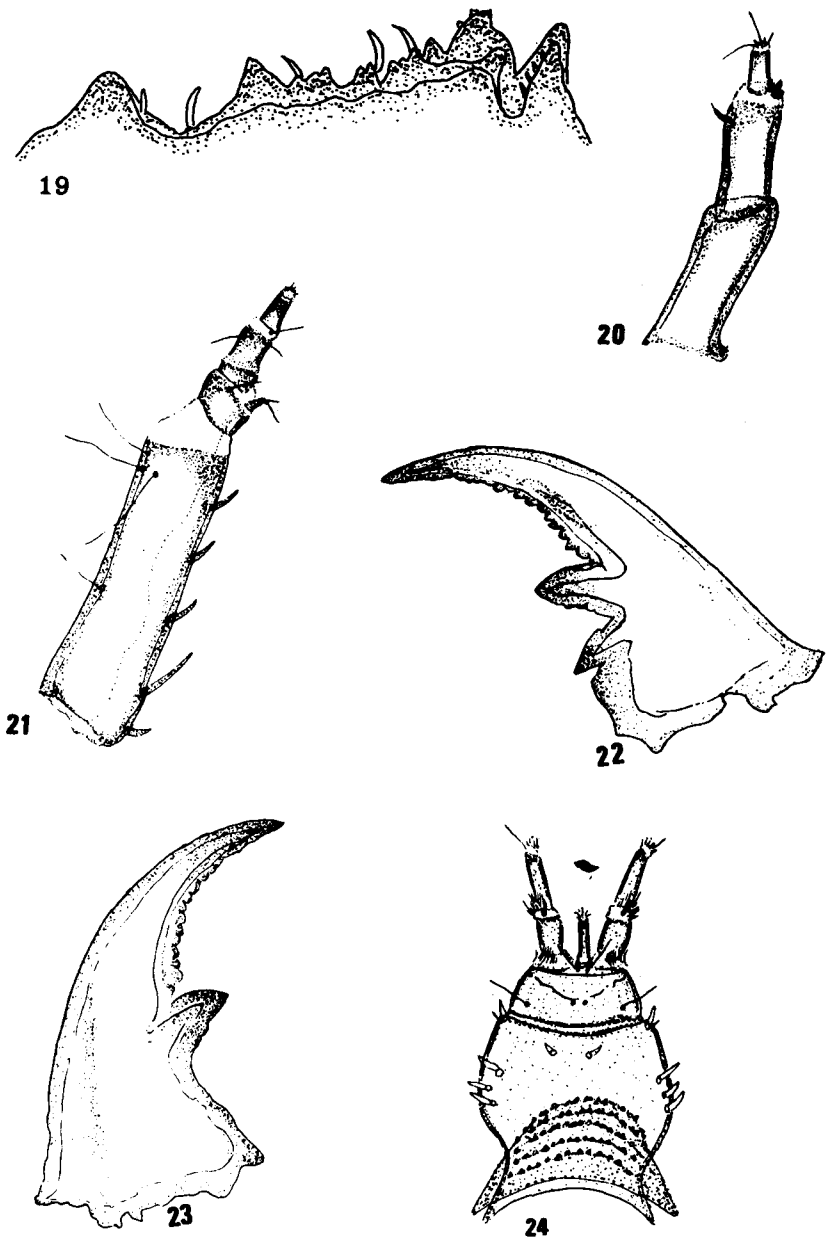


Plate IV- Third instar larva of *E.q.p.*

Figure 19. Labrum, dorsal
20. Antenna
21. Maxillary palpus
22. Right mandible
23. Left mandible
24. Labium, dorsal

A full account of the rearing procedure of this beetle has been given previously in Part I of this study [18]. What follows is a summary:

Males and females were separated soon after they were brought to the laboratory, and placed in containers. Some of the females, having mated in the field, constructed egg cases and laid eggs during this period. Then, several days to two or three weeks after the last egg case was constructed, mating was induced in the laboratory. One male and one female were put in the same container. Adult beetles were kept in 200-250 cc wax cups or glass beakers almost half-filled with tap water. Because the adults are scavengers, the food consisted of some vegetation, such as green algae and lettuce, with small pieces of dog food added, about once a week. The containers were covered with pieces of glass or cloth to prevent the adults from escaping.

Egg cases were constructed on the walls of the containers or under the vegetation. Egg cases were laid singly, or in small groups attached to each other (Plate II, Figs. 9, 10). Some of the egg cases did not contain viable eggs but of those which did an average number of 15 larvae hatched.

Larvae are carnivorous, and so they were separated immediately after hatching to prevent cannibalism. They were kept in 200-250 cc wax cups which were half-filled with tap water, some algae were added (for the larvae to cling onto) and covered (to prevent the larvae from escaping). The larvae were fed with mosquito larvae or ground meat. There are three larval instars, the last stadium ending with pupation.

Larvae pupate in moist sand. Therefore, moist sand cups were prepared for the last instar larvae which had stopped feeding. After emergence, the teneral adult was transferred to a new container containing tap water and vegetation. There, after the first penetration of the surface film of water was made by the adult, it started swimming, mostly submerged.

Morphological studies were carried out using Zeiss stereo and compound microscopes, to both of which a camera lucida (drawing tube) was attached. Because detailed measurements are given in the tables, no size scales accompany the figures.

Results

1- Egg Case

The egg case (Plate II, Figs. 9-13) is small, cup-shaped, round or triangular in cross section, with a "mast" or ribbon that is usually long and rather narrow, but sometimes short, wide and flat (Plate II, Fig. 12). The case is mostly constructed below the surface of water, and the "mast" floats on the surface. The cup is covered by a "lid", which is woven to the top of the case, in a semicircular form, from one side of the base of the ribbon to the other

The texture of the case is silk-fine and beige in color. The texture of ribbon is much finer than the cup, mostly due to the fact that the ribbon is constructed more loosely than the rest of the case.

The average length of the mast is about 9 mm, with the narrowest width of 1.5 mm and widest of about 3 mm. The length of the cup proper is 2 to about 4 mm. (Table I).

The eggs are very tiny, elongate oval, with a length of about 0.1 mm, and the widest part, in the middle, much less, at almost half the length. They are beige in color, and fine in texture.

2-Larvae

All the larval stages are very similar, different mainly in size and a few minor morphological characters. Therefore, the third instar larva will be described below, with a few references to the first two.

Larva (Plate III, Fig. 14), long and rather narrow, about 7 mm long (Table 4) and about 2 mm wide in the widest part in the abdominal area. The first two larval stages are smaller (Tables 2 and 3). The total average ratio of growth of the larvae is 1.5 (Table 5). Head (Plate III, Fig. 15), dark yellow to light brown, with light yellow patches in the mid-dorsal area of the head and also below the ocelli.

Ocelli (Plate III, Fig. 16), six on each side of the head,

Table 1. Egg case measurements, in mm, in *Enochrus quadripunctatus* Herbst (see Plate I, Fig. 1)

	$\bar{X}$	Range	Standard deviation
"Mast"			
Length (a)	9.38	8.4-11.4	1.59
Width (c)	1.5	1-2	0.7
Case			
Length (b)	3.32	2.5-3.9	0.54
Width at lid (d)	3.1	2.4-4.2	0.6
Width in the middle (e)	3.1	2.7-3.6	0.3
Midline length (f)	2.13	1.2-2.5	0.28
Narrowest width (g)	1.64	0.7-2.1	0.54

Table 2. Measurements of the first instar larva, in mm, in *Enochrus quadripunctatus* Herbst (see Plate I, Figs. 2,3)

	$\bar{X}$	Range	Standard deviation
Head width (a)	0.41	0.4-0.43	0.072
Head length (b)	0.32	0.3-0.45	0.082
Head length (c)	0.31	0.3-0.32	0.066
Prothorax (Pronotum)-abdomen (stigmatic atrium)	2.1	1.7-3.3	0.505
Head (Maxillary palpus)-abdomen	2.6	2.3-3	0.11

Table 3. Measurements of the second instar larva, in mm, in *Enochrus quadripunctatus* Herbst (see Plate I, Figs. 2,3)

	$\bar{X}$	Range	Standard deviation
Head width (a)	0.56	0.38-0.68	0.08
Head length (b)	0.46	0.3-0.53	0.11
Head length (c)	0.48	0.33-0.6	0.11
Prothorax (Pronotum)-abdomen (stigmatic atrium)	0.54	3-6.6	1.24
Head (Maxillary palpus)-abdomen	5.3	3.6-7.2	1.28

Table 4. Measurements of the third instar larva, in mm, in *Enochrus quadripunctatus* Herbst (see Plate I, Figs. 2,3)

	$\bar{X}$	Range	Standard deviation
Head width (a)	0.73	0.6-0.87	0.25
Head length (b)	0.6	0.53-0.68	0.1
Head length (c)	0.6	0.53-0.63	0.1
Prothorax (Pronotum)-abdomen (stigmatic atrium)	5.7	4.5-6.9	1.7
Head (Maxillary palpus)-abdomen	6.9	5.4-8.4	2.19

Table 5. Larval average ratio of growth in *Enochrus quadripunctatus* Herbst (see Plate I, Figs. 2,3) (According to Dyar's Law*)

Head width (a)	Head length (b)	Head length (c)	Prothorax-abdomen (d)	Head-abdomen (e)	Total average
1.34	1.37	1.4	1.7	1.7	1.5

* The average ratio of growth is obtained when the average size measurements of each part or the whole body at each succeeding stage of development is divided to that at the previous stage, and the means for all the stages is calculated.

dark in color. The shape of the right and left ocelli does not seem to be exactly the same in the same larva, but the general pattern of their shape and distribution on the head is very similar.

Labrum (Plate IV, Fig. 19) with several large and small teeth on its border. Some of the large teeth carry several smaller ones on their inner border. Labrum bears four or five setae on the dorsal side, projecting mostly from the small teeth. The labrum is oblique, declining from the right side of the head to the left. The margin of the labrum is dark, the teeth, particularly the smaller ones, are very dark and hard.

Antennae (Plate IV, Fig. 20), rather stout, three-segmented, carrying large setae on the second segment, and two long and some shorter and finer ones on the last segment; tip and edges usually dark, the rest yellow in color.

Mandibles (Plate IV, Figs. 22, 23), distal teeth present and pointed on both mandibles, a few smaller teeth on the underside of each distal tooth. Right mandible with proximal tooth, but this tooth is lacking on the left mandible. Both mandibles have about nine large teeth, and several small projections or denticles on their inner border. The pointed tip, the distal teeth, and the proximal tooth (of the right mandible), are all darker than the rest of the mandibles.

Maxillary palpi (Plate IV, Fig. 21), longer than antennae, five-segmented. Segment two bears a "palpule" carrying fine setae or spines. The maxillary palpi carry large setae (spines) on one side and longer and finer ones on the other side. In the distal segment, the fine setae are in the form of short, clustered hairs; tips and edges dark, the rest of the palpi yellow in color.

Labium (Plate IV, Fig. 24), submentum almost conical and bulky, with rows of small teeth arranged in a rather semicircular manner on the lower portion of the dorsal side. Several rows of sense cones are arranged on the dorsal side of the submentum, a row of three on the mid-lateral side, one on each distal end, and two below the distal edge. Mentum conical to straight, with two long, fine spines on each proximal end. Ligula slender, shorter than labial palpi. The palpi two-segmented, proximal segment shorter and bulkier than the distal one; clusters of spines, short and long, present on different parts of the palpi. The membranous area between the two palpal segments, besides bearing clusters of setae, carries a sense cone. The tips of the ligula and palpi, as well as the edges of the labium, and the submental teeth, are all darker than the rest of the labium.

Thoracic segments (Plate III, Fig. 14) with specific pigmentation pattern, the prothorax different in its pattern from the other two segments, which are almost similar.

Eight complete abdominal segments present (Plate III, Fig. 14), the ninth and tenth reduced and transformed to stigmatic atrium [20], (Plate III, Fig. 18). The abdominal

segments are separated by true folds, but false foldings on almost every one of them, except the last two, are present. The whole body is covered by setae, although their arrangement and size are different on the three body regions.

On the ventral side of the abdomen prolegs are present on all the segments, except the last two. The first two abdominal segments are supplied with much smaller and weaker ones, but on the following abdominal segments (from third to seventh) the prolegs get stronger and more prominent. These prolegs represent the developed parasternal areas [4, 5]. There are two series of hooklets on the prolegs and a series of spines and setae (Plate III, Fig. 17). The hooklets on the external border are longer than the ones inside (Plate III, Fig. 17-a), and a pack of hooklets cover almost the entire surface of each proleg. The hooklets are bent (Plate III, Fig. 17-b) and they seem to be different in shape in different species, thus of taxonomic value [5].

3- Pupa (Plate V)

Whitish in color with red eyes. Pronotum with a total of about 22 styli, a row of six on the proximal border, two longer ones in the middle, the other two pairs arranged distally from the first, the rest arranged symmetrically on the surface of pronotum. Most of the abdominal tergites with six styli, four medially, and one on each side. The first two tergites have a different number of styli, and the last one has only two.

Abdominal cerci long, ending in thread-like projections. Male and female pupae can be distinguished on the basis of the shape of the last abdominal segments (Plate V, Fig. 27a, b).

4-Adult

Emerging from the pupal chamber is light-brown, with appendages on the head whitish and the body brownish. As is noted in some other species [15], the eyes get dark (black) first, and then the rest of the body changes color, elytra first, head and thorax later and more slowly.

Aedeagus is shown from both ventral and dorsal views, (Plate VI, Figs. 30, 31), showing the detail structure of the male genitalia. Male and female adults are somewhat different in total size, the male being less bulkier than the female (Table 6). There are also other adult parts and structures on the basis of which the two sexes can be distinguished from each other. More prominent examples of such structures are antennae (Plate VI, Figs. 28a, 29a), maxillary palpi (Plate VI, Figs. 28b, 29b), protarsus and claws (Plate VI, Figs. 28c, 29c), and mesothoracic lamina (Plate VI, Figs. 28d, 29d).

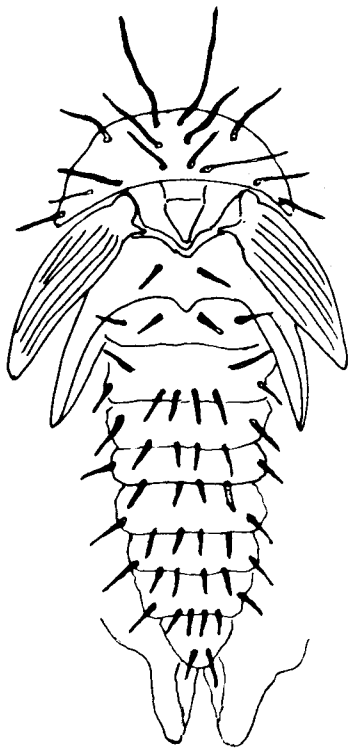
Discussion

Comparing the results of studies on the morphology and size measurements of *Enochrus quadripunctatus* with similar studies on other hydrophilids, basic similarities are revealed along with obvious differences, as is to be expected. However, the literature on the life history and biology of the aquatic beetles shows that a thorough study in this area on any one species is lacking. The literature on the subject, being scanty in itself, mostly includes brief reports on a stage or two, with short accounts of some morphological characters added. Few drawings, if any, accompany the text, and measurements of different life stages are scant or totally missing. There are a few exceptions, but even in these works a thorough study of one particular species, as that done for some Nearctic [15, 16] or Palearctic species [18, and the present study] is not presented.

In recent years, some works on the details of the morphological characters of some immature stages have been done, but these works do not consider or present details of the life history or rearing of the beetles in the laboratory. Most of these studies have been carried out on some larval or pupal stages brought into the laboratory from the field. Attempts to rear the aquatic Coleoptera in the laboratory have been very limited. This may be due to the fact that providing laboratory conditions, not very different from the natural environment of these beetles, is not easy. Also, the larvae, being carnivorous in many families, usually need to be fed by hand, that is food given by forceps. Under laboratory conditions, this ensures that

Table 6. Measurements of the adult, in mm, in *Enochrus quadripunctatus* Herbst (see Plate I, Figs. 5,6,7,8)

	$\bar{X}$	Range	Standard deviation
Dorsal			
Between eyes (a)			
female	1.24	1.2-1.35	0.1
male	1.1	0.98-1.2	3
Pronotum length (b)			
female	1.44	1.2-1.62	0.14
male	1.44	1.2-1.56	3.5
Pronotum width (c)			
female	1.92	1.8-2.1	0.17
male	1.83	1.8-2.1	5.01
Pronotum width (d)			
female	3.1	2.88-3.3	0.2
male	3.03	2.7-3.3	8.2
Elyteral length (e)			
female	4.61	4.44-4.8	0.14
male	4.64	4.2-5.7	12.6
Ventral			
Thorax-abdomen (f)			
female	4.38	4.2-4.62	0.17
male	4.34	3.9-5.52	11.8



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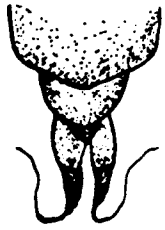
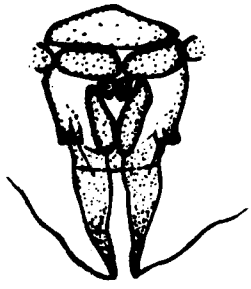


27-a



27-b

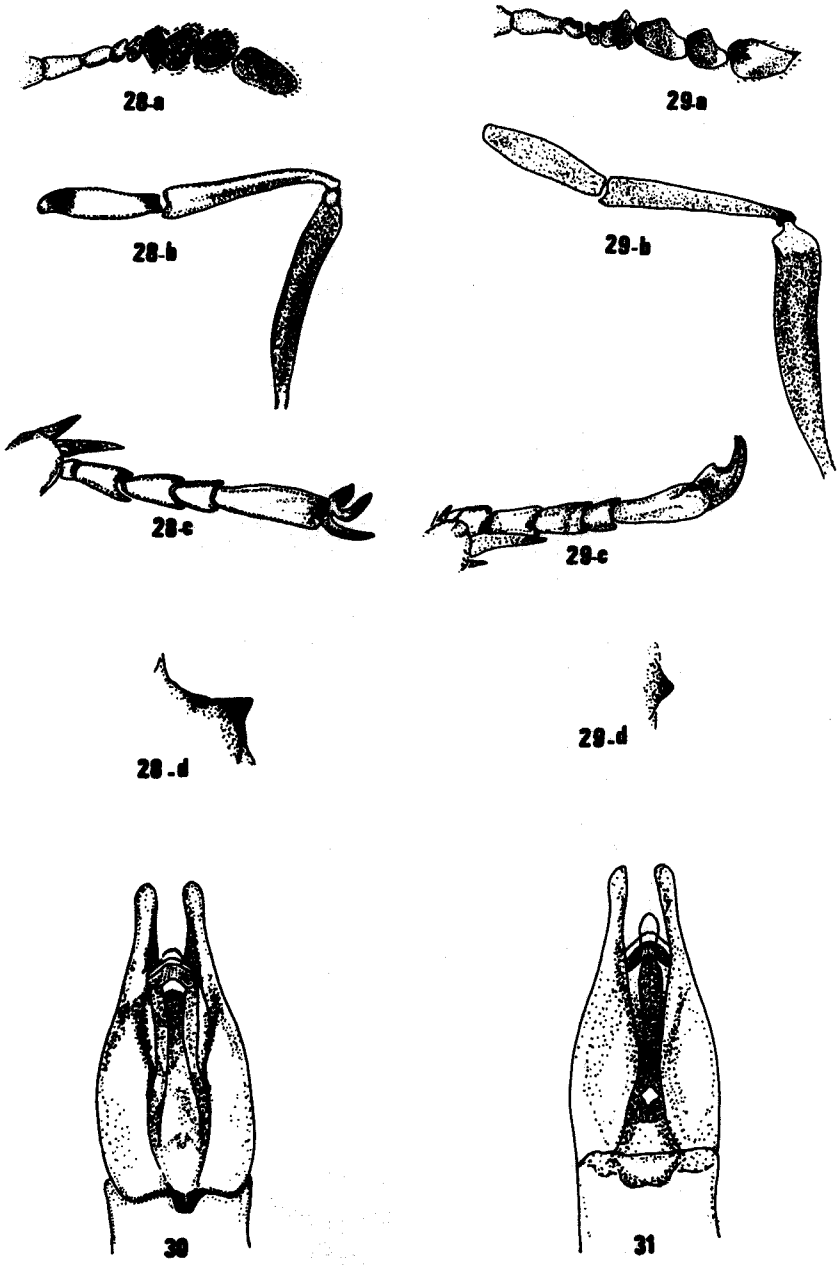
Plate V- Pupa of *E.q.p.*

Figure 25. Dorsal surface

26. Ventral surface

27. End of the abdomen and its cerci, in

a. male b. female



PlateVI- Adult of *E.q.p.*

Figure 28. Male
- a. Right antenna
- b. Right maxillary palpus
- c. Right protarsus and its claws
- d. Mesothoracic lamina, lateral (see Plate I, Fig. 8)

29. Female
- a. Right antenna
- b. Right maxillary palpus
- c. Right protarsus and its claws
- d. Mesothoracic lamina, lateral (see Plate I, Fig. 8)

30. Aedeagus, ventral

31. Aedeagus, dorsal

the larva gets its food, but this is very time consuming, particularly considering the fact that, for statistical reasons, the number of the larvae should be high.

To get a better idea about the life history of any one species it would be better to compare the immature stages of those in the laboratory with those caught in the field, not only for their morphology, which should not be very different, but especially to compare the duration of each stage under laboratory and field conditions. While this would most certainly give more insight into the life history and biology of the species, it would also demand a good deal of manpower, time and other facilities.

The present study constitutes the first thorough investigation carried out into the life history and morphology of an Iranian species. Nonetheless, this should only be considered as a preliminary step. Running cultures in the laboratory for some years, acquiring several generations, and comparing the results with those obtained in the field, would certainly reveal more information on the life history and biology of this species, and any other species, hence confirming the results.

Meanwhile, the need for such thorough studies is constantly increasing, because the taxonomy, or rather biosystematic studies, are being more oriented, or based, on the numerical taxonomy. In other words, the more data gathered and presented about any one taxon, the more accurately its systematic status can be decided upon, both for the present and its past history or phylogeny. Life history studies offer a good deal of information for biosystematic works.

Acknowledgements

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