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Biology of the blind geobiont scarab beetle genus *Chaetonyx* Schaum, 1862 (Scarabaeidae: Orphninae) with new distribution records of *Ch. robustus* Schaum, 1862 from Bulgaria

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Abstract: Life cycle of the orphnine scarab beetles genus *Chaetonyx* was recorded for the first time based on the multiyear observations on the populations of its type species, *Ch. robustus* Schaum, 1862, in riverside habitats at Zemen Gorge region, South-Western Bulgaria. Our observations showed that this geobiont species is common in alluvial soils near Struma River, where it forms large colonies. At the Zemen Gorge, its preferable habitats are sparse riverside forests of *Alnus glutinosa* L. and *Populus nigra* L. neighboring grass communities. Larvae, adults and pupae were found up to 50 cm deep with most specimens inhabiting the upper soil layer from 0 to 20 cm deep. The highest recorded density was 89 adults and 581 larvae per 0.25 m^2. Oviposition, hatching of the eggs, pupation and emergence of the new adults occur during the summer. Eggs have been found in surface soil layer in July and August, the pupae – mainly in July and August. Larvae of different instars were found in soil during all months of the study period. Adults from the new generation first occur in the beginning of August. In August, September and October adults from different generations can be found together. The main part of the adults seems to die after the reproduction period, and the common lifespan of adults seems to be about a year only. The life cycle of *Ch. robustus* in investigated habitat seems to be perennial and includes two years as larval stage and one as adult. Tropical Orphninae occupy a number of ecological niches but lack colonial geobiont specialists similar to *Chaetonyx*.

Key words: Coleoptera, Orphninae, *Chaetonyx robustus*, biology, life cycle, insect ecology.

Introduction

Subfamily Orphninae is one of the poorly known groups within the hyper-diverse family Scarabaeidae. It includes 15 genera and 195 species, distributed mainly in the Afrotropical and Neotropical biogeographic regions (Paulian 1948, 1984; Frolov 2012). Habitat preferences, feeding and nesting behavior, and the life cycles are unknown for the

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majority of the orphnine taxa, and the data available are scarce and largely incomplete. The relatively complete account on the biology, including life cycles and biotope preferences is only available for *Hybalus cornifrons* (Brullé, 1832) (Palmà 1938).

In the Palearctic Region, the subfamily Orphninae is represented by two genera – *Hybalus* Brullé, 1834, and *Chaetonyx* Schaum, 1862, occurring in the Mediterranean. Of them, only *Chaetonyx* is relatively widely distributed in Europe including Bulgaria. *Chaetonyx* is a small genus comprised of three nominal species with one being divided into three subspecies (Mariani 1946). The differences between the species and subspecies are feeble and characters provided by Mariani (1946) are difficult to interpret unequivocally. Variability of these characters was not assessed and may call for the revision of the genus but the taxonomy of *Chaetonyx* is outside the scope of the present work. We consider all the specimens collected by us as conspecific and belonging to *Ch. robustus* Schaum, 1862.

Two species of *Chaetonyx* – *Ch. robustus*, and *Ch. schatzmayri* Mariani, 1949, – have been reported from Bulgaria (Král & Malý 1993). *Ch. robustus* was cited in several publications (Joakimov 1904, Nedelkov 1905, Zacharieva-Stoilova 1974, Mikšić 1957, Král & Malý 1993), while *C. schatzmayri* was reported without precise locality by Mikšić (1959), Král & Malý (1993) and Bunalski (2001) from the Eastern part of Bulgaria. The records made by the mentioned authors apparently resulted from occasional findings of the adult specimens without biological and ecological observations. *Ch. robustus* was reported from the region of Zemen Gorge (Gradinarov & Petrova 2012, Gradinarov 2014), mainly in respect to its suitability as a host of the entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae).

The purpose of the present study is to analyze the results of the multiyear observations on the *Ch. robustus* populations in the Zemen Gorge region of SW Bulgaria, to provide the first comprehensive account on the habitat preferences and life cycle of a member of the genus *Chaetonyx*, and to summarize the available data about the biology of the Orphninae.

Material and Methods

Material for the present study was collected from different localities at the Zemen Gorge. The investigated region is a part of the Struma River Valley, situated between Risha and Zemenska Planina Mts., SW Bulgaria. The climate of the region is subcontinental, with an average annual air temperature of about 8.8°C and average annual precipitation of about 600 mm. All the localities where *Ch. robustus* were found are situated on the lower river banks with sandy loam alluvial soils, at altitude of about 500–600 m a.s.l.

Data on the distribution are based on hand collection of adults from soil litter and soil upper layer up to 10–20 cm depth, mainly in the period from 2009–2014. Data from a single sampling in 2002 were also included into the list of distribution. The abbreviations of the names of collectors used in the list are as follow: DG – Denis Gradinarov, YP – Yana Petrova.

Density, vertical distribution in the soil and the biology of *Ch. robustus* were studied on a sampling site where the species have been detected several times in 2011 (Gradinarov & Petrova 2012). The site is situated on the first river terrace of the Struma River near the town of Zemen (42°27.80'N, 22°42.85'E). The overall dimensions of the studied area are 15 x 2 m, the long side being parallel to the river. The distances of the sampling points to the river were 3 to 5 m. The investigated habitat is an ecotone with moderately developed herbaceous vegetation between the sparse riverside forest with black alder (*Alnus glutinosa* (L.)), black poplar (*Populus nigra* L.), black locust (*Robinia pseudoacacia* L.), dogwood (*Cornus sanguinea* L.) and the abandoned orchard of cherry plum (*Prunus cerasifera* Ehrh.) and apple (*Malus domestica* Borkh.) (Fig. 1). Among the herbaceous species, *Aegopodium podagraria* L. (Apiaceae) predominated on the sampling site, in places in combination with several Poaceae species. The soil of the site is alluvial heavy sandy loam containing no stones or gravel particles and almost no remains of decaying wood fragments. Soil litter is not well developed.

The quantitative soil samples from the site were collected monthly during the periods of April–November 2012 and May–October 2013. The size of an individual sample was 0.25 m^2 (50 x 50 cm) at 50 cm depth. The soil from each sample was excavated, processed twice manually and the number of adults, larvae and pupae of the species was counted. Detected individuals were fixed in 70% alcohol during collecting or transferred live in perforated Eppendorf tubes with a little amount of the soil from the sample site for laboratory rearing. Each month a single sample from the sampling site was processed. Vertical distribution of *Ch. robustus* has been monitored from July to October 2013. In this period individuals were counted separately for 0–20 and 20–50 cm soil layers of each sample. Additional material of *Ch. robustus* from the sampling site, beside the quantitative samples, was collected in 2011–2014. The data obtained in these collections are included in the list of distribution and also used at the discussion of the species biology.

Fig. 1. Sampling site at the Zemen Gorge, SW Bulgaria.

Rearing experiments with larvae and eggs of *Chaetonyx* were conducted at room temperature (20–25°C). For pupation, third instar larvae were placed separately in 50 cm^3 tubes with soil from the sampling site. Eggs hatched in Petri dishes or in perforated Eppendorf tubes with soil from the site. The head capsules of the larvae were measured on the preserved in 70% alcohol material under a stereomicroscope Olympus SZ61 using an ocular-micrometer. The photographs (Figs. 2 and 4) were taken with a digital color camera Olympus Color View I and a stereomicroscope Olympus SZ61.

Identification of the adults and larvae was confirmed with Baraud (1992) and Barbero & Palestrini (1993), respectively. Conspecificity of the eggs, larvae and pupae with *Ch. robustus* was also confirmed by the laboratory rearing. The material used in this study is deposited in the collections of the Department of Zoology and Anthropology, Faculty of

Biology, Sofia University "St. Kliment Ohridski" and Zoological Institute RAS, Saint-Petersburg (ZIN).

Results

Distribution of *Ch. robustus* at the Zemen Gorge

Material examined (listed by localities): 1). Near town of Zemen, left bank of Struma Riv., 42°28.00'N, 22°43.22'E, 580 m a.s.l., poplar, alder vegetation, 11–IV–2009, 6 ♂♂, 1 ♀, beneath soil litter and in soil, leg. YP & DG; the same locality, 23–IX–2009, 2 ♀♀, in soil, leg. DG; the same locality, 14–V–2011, 1 ♂, in soil, leg. DG; the same locality, 03–X–2011, 1 ♂, in soil, leg. DG; 13–VII–2014, 1 ♂, 1 ♀, in soil, leg. DG; 2). Near town of Zemen, right bank of Struma Riv., 42°27.80'N, 22°42.85'E, 580 m a.s.l. (the sampling site), mixed riverside vegetation next to an orchard, 22–IV–2011, 3 ♂♂, 2 ♀♀, in soil, leg. DG; the same locality, 05–V–2011, 4 ♂♂, 6 ♀♀, in soil, leg. DG; the same locality, 03–X–2011, 7 ♂♂, 11 ♀♀, in soil, leg. YP & DG; the same locality, 27–V–2013, 2 ♂♂, 2 ♀♀, in soil, leg. DG; the same locality, 15–VI–2013, 2 ♂♂, 8 ♀♀, in soil, leg. DG; the same locality, 24–25–X–2013, 16 ♂♂, 15 ♀♀, in soil, leg. DG, Boyan Zlatkov & Ognyan Sivilov; the same locality, 13–VII–2014, 3 ♂♂, 3 ♀♀, in soil, leg. DG; the same locality, 03–VIII–2014, 9 ♂♂, 9 ♀♀, in soil, leg. DG; the same locality, 18–IX–2014, 4 ♂♂, 9 ♀♀, in soil, leg. DG; 3). Saraya Place, right bank of Struma Riv., 42°27.57'N, 22°42.60'E, 570 m a.s.l., poplar, alder vegetation, 23–VII–2002, 1 ♂, in soil, leg. DG; the same locality, 30–IV–2011, 1 ♀, in soil, leg. DG & Boyan Vagalinski; 4). Near Aydutsite Mahala, right bank of Struma Riv., 42°27.38'N, 22°42.79'E, 570 m a.s.l., poplar, alder vegetation, 03–VI–2012, 1 ♂, 1 ♀, in soil, leg. DG & Georgi Hristov; 5). Bliznatsite Place, left bank of Struma Riv., next to karst spring, 42°26.82'N, 22°42.73'E, 570 m alt., poplar, alder vegetation, 02–VI–2012, 1 ♀, in soil, leg. DG & Georgi Hristov; 6). Near Razhdavitsa Vill., right bank of Struma Riv., 42°24.11'N, 22°41.68'E, 520 m a.s.l., alder vegetation, 04–V–2009, 1 ♂, 4 ♀♀, beneath soil litter and in soil, leg. YP & DG; 7). Near Razhdavitsa Vill., left bank of Struma Riv., 42°23.78'N, 22°42.16'E, 510 m a.s.l., poplar, alder vegetation, 04–V–2009, 4 ♂♂, 1 ♀, beneath soil litter and in soil, leg. YP & DG.

Ch. robustus was found in seven different localities along the Zemen Gorge. The total number of collected adult specimens is 143 (66 ♂♂ and 77 ♀♀), not including the data obtained within the monthly quantitative collections from the sampling site (the latter are presented in Table 1). In all cases the specimens were found in alluvial riverside soil (at a distance up to 15 m from the river), inside the soil or at the boundary between the soil and the soil litter. The tree vegetation in the habitats usually was represented by black poplar (*Populus nigra* L.) and black alder (*Alnus glutinosa* (L.)). The forests never have formed dense canopy and in the most cases the habitat was situated next to herbaceous communities.

Density of the population of *Ch. robustus* in soil

Fourteen quantitative soil samples from the sampling site were processed during the period of investigation (Table 1). A total of 412 adults, 66 pupae and 2320 larvae of *Ch. robustus* have been excavated from the samples. Both larvae and adults have been found during all months of the study. The observed density of the adults and larvae for all the months was on average 29.4 ± 6.44 (from 2 to 89) and 165.7 ± 41.30 (from 19 to 581) individuals per 0.25 m^2, respectively. The maximal recorded density of the pupae was 38 specimens per 0.25 m^2.

Table 1. Occurrence of *Chaetonyx robustus* in the soil samples (leg. & obs. D. Gradinarov). In the sample marked with asterix, not all the individuals were sexed.

Date	Larvae	Adults				Pupae
		Males	Females	Total	Dark individuals (%)	
30–IV–2012	70	10	5	15	6 (40)	0
11–V–2012	88	11	10	21	21 (100)	0
02–VI–2012	116	9	6	15	15 (100)	0
01–VII–2012	63	14	14	28	28 (100)	3
04–VIII–2012	101	16	14	30	8 (26.7)	1
02–IX–2012	80	21	16	37	17 (45.9)	0
06–X–2012	108	6	8	14	5 (35.7)	0
03–XI–2012	156	5	4	9	5 (55.6)	0
02–V–2013	19	3	3	6	2 (33.3)	0
05–VI–2013	39	0	2	2	2 (100)	0
06–VII–2013*	346	4	2	33	33 (100)	20
03–VIII–2013	286	25	29	54	38 (70.4)	38
01–IX–2013	267	40	49	89	15 (16.9)	4
24–25–X–2013	581	29	30	59	4 (6.8)	0
Total	2320	193	192	412	199 (48.3)	66

Adults and oviposition

From late April to early August, adults have been found mainly in the soil layer from 0 to 20 cm deep (Table 2). In this period, the findings of adults at greater depths were considerably rarer. Since the beginning of August to October–November the adults showed a more uniform distribution in the studied depth range, reaching up to 50 cm depth in the soil. Adults with varying intensity of coloration have been observed throughout the different months in the both years of the survey (Table 1). In April and May, dark-brown adults and lighter ones, reddish-brown in color, have been excavated. In June and July all the collected individuals were uniformly dark-brown. In August, except for dark-brown individuals, yellowish or orange adults were also observed (Fig. 2). Such "pale" individuals were found at different depths, and constituted the major part of the adults under 0–20 cm soil layer (Table 2). In the sample of August 2013, most of these adults were yellowish, with soft and easy to deform exoskeleton. In the corresponding sample of the previous year, such slightly sclerotized adults were rarer. From August to November 2012, the proportion of dark-brown beetles increased (Fig. 3A, B), and ranged from 26.6% to 55.6% of all collected beetles (Table 1). In contrast, during the same period of 2013 this proportion showed clear decrease, reaching 6.8% at the end of October. In additional samples in 2014, only dark beetles were presented in the collections from July and August, while in the collection from September, 12 of all 13 found individuals were clearly lighter in color. No mating individuals were observed during the study period. In July 2012 and August 2013, dead intact dark-brown adults were found in the soil of the sampling site.

Table 2. Vertical distribution of *Chaetonyx robustus* in the soil in summer and autumn of 2013.

Month of sampling	Soil layer (cm)	Larvae (%)	Adults (%)			Pupae (%)
			Total	Dark	Light	
July	0–20	273 (78.7)	29 (87.9)	29 (87.9)	0 (0)	15 (75.0)
	20–50	74 (21.3)	4 (12.1)	4 (12.1)	0 (0)	5 (25.0)
August	0–20	171 (69.8)	50 (92.6)	38 (100)	12 (75.0)	30 (78.9)
	20–50	74 (30.2)	4 (7.4)	0 (0)	4 (25.0)	8 (21.1)
September	0–20	233 (87.3)	69 (77.5)	15 (100)	54 (73.0)	3 (75.0)
	20–50	34 (12.7)	20 (22.5)	0 (0)	20 (27.0)	1 (25.0)
October	0–20	384 (66.1)	35 (57.4)	3 (75.0)	32 (58.2)	0 (0)
	20–50	197 (33.9)	26 (42.6)	1 (25.0)	23 (41.8)	0 (0)

Numerous eggs of *Ch. robustus* have been observed in the upper soil layer of the sampling site (0–20 cm deep) at the beginning of July 2013 and in early August of 2014. In August 2013, only one egg was found in the sample. The eggs were relatively large, with a length of 1.20 ± 0.05 mm (1.13–1.28 mm) and a width of 0.93 ± 0.02 mm (0.9–0.96 mm) (n = 11). Hatching of the eggs in the laboratory continued during a week after collection of the eggs. One egg hatched in July 2013 and three eggs in August 2014 (Fig. 4A). In some of the eggs, collected on 03–VIII–2014, the developing embryos were observed (Fig. 4B).

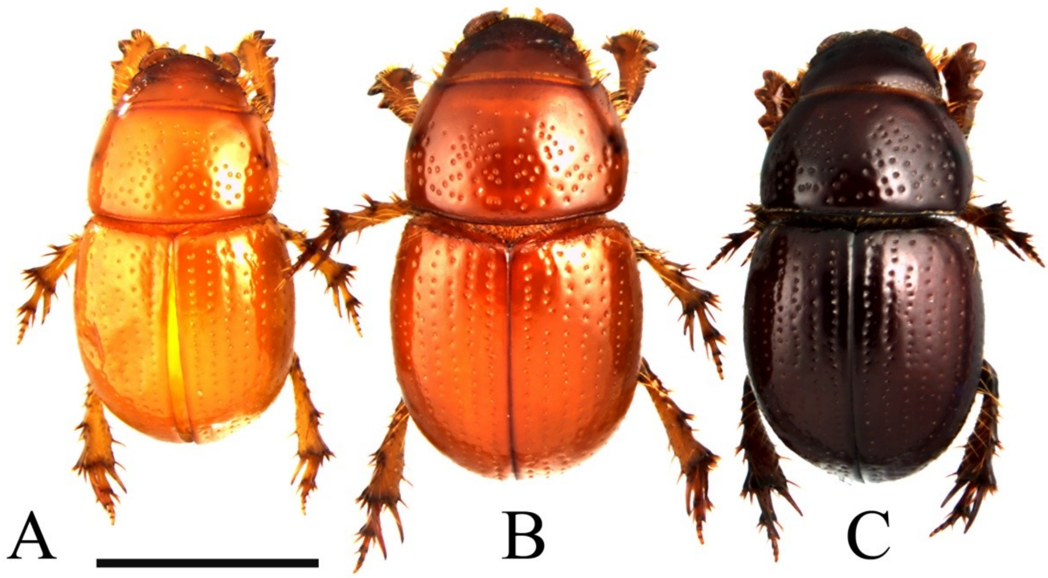

Fig. 2. Adults of *Chaetonyx robustus* with different degrees of sclerotization, collected on 04–VIII–2012 from the sampling site. A: Slightly sclerotized, just emerged from the pupae; B: Moderate sclerotization; C: Fully sclerotized, adult from previous generation. Scale bar: 2.5 mm.

Fig. 3. Adults of *Chaetonyx robustus*, excavated from the site near town of Zemen (in situ imaging). A: sampling on 04–VIII–2012; B: sampling on 02–IX–2012.

Larvae

Along with the eggs, small larvae, apparently first instar, were detected in samples from July and August of 2013. Some of these larvae in the samples taken during these two months had light abdomen with little or no food particles in the gut. The maximal width of the head capsule of these larvae was 0.65 ± 0.015 mm (0.62–0.68 mm, n = 38). Small larvae with width of the head capsule up to 0.70 mm have been found in samples of September, October and November, as well as in May 2013. Visibly larger larvae, probably of both second and third instars, have been found in the soil during all months of the study. The larvae preparing to pupate (prepupae) were found in the soil of the site in early June to early August of both 2012 and 2013. These larvae were significantly less mobile, with bright and partially transparent abdomen. The maximal width of the head capsule of the prepupae was 1.28 ± 0.07 mm (1.18–1.45 mm, n = 16). No prepupae were observed before June and after August. However, a significant amount of larger larvae were presented in the soil during September, October and November, along with the group of small larvae. Moulting of such larvae was repeatedly observed in August and September of both 2012 and 2013 in the soil samples during collection and during laboratory rearing.

The highest larval density was found in the soil layer from 0 to 20 cm, but significant number of larvae was detected also deeper up to the maximal excavated depth of 50 cm (Table 2). During July–October 2013, the percentage of the larvae found below 20 cm ranged from 7.3% to 33.9%. Some differences in vertical distribution, depending on the larval instar, were observed during the investigation. First instar larvae were observed only up to 20 cm depth, while the prepupae occurred in both the upper and the deep layers, up to 50 cm deep.

Pupae and pupation

Pupae of *Ch. robustus* (Fig. 4C) have been found in the soil of the sampling site in July and August 2012 and in July, August and September 2013 (Table 1). During additional sampling in July 2014, pupae have not been found. Most of the pupae have been found in the soil layer from 0 to 20 cm deep (Table 2), but about 25% have been found deeper, up to 50 cm deep. In July 2013, all pupae were white in color. In August of the same year, some pupae were darkened before adult emergence. All four pupae found in early September 2013 were darkened. Both white and darkened pupae were found in early August 2014. At the laboratory, ten larvae, collected in early May 2013 from the soil of the site, built cocoons from soil particles in the end of the same month. The cocoons were fixed to the bottom of the experimental tubes and in the middle of June four adults and four pupae were obtained.

The cocoons had smooth interior surface, thin walls and were very fragile. One prepupa in similar cocoon was observed in the soil of the site in July 2013.

At the beginning of July of the both years of the quantitative sampling, adults, eggs, larvae of the all three instars, and pupae were observed together in the soil of the sampling site (Fig. 4D).

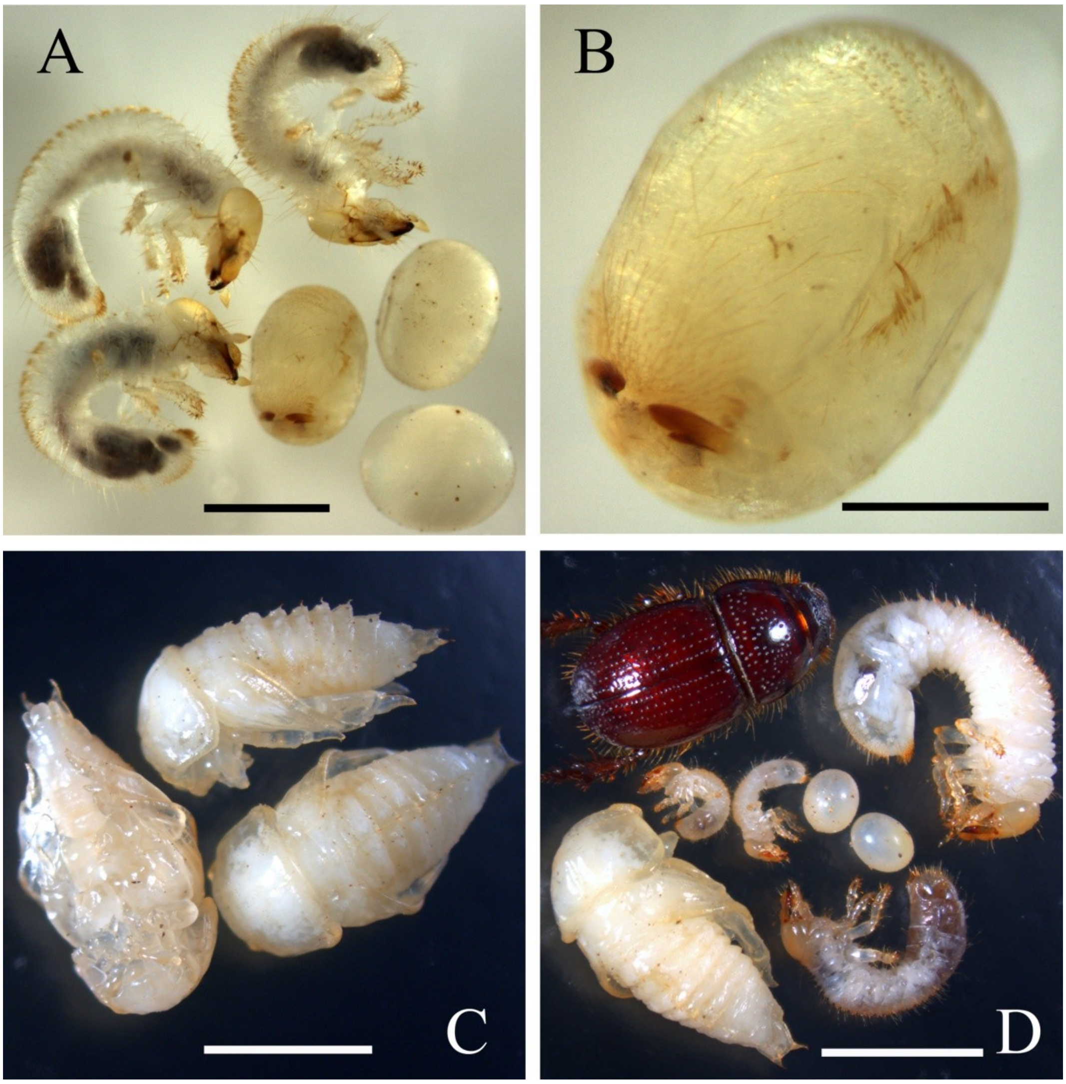

Fig. 4. Different stages of *Chaetonyx robustus*. A, B: Eggs and first instar larvae (laboratory rearing, eggs collected on 03–VIII–2014); C: Pupae, collected on 07–VII–2013 (in vivo imaging); D: Adult, pupa, larvae from the three instars and eggs (in vivo imaging, material was collected on 07–VII–2013). Scale bars: A: 1 mm; B: 0.5 mm; C, D: 2.5 mm.

Discussion

Distribution of *Ch. robustus* in Bulgaria

The areal of *Ch. robustus* includes mainly the Balkan and the Apennine peninsulas, as the species is recorded from Albania, Bulgaria, Greece, Italy, Macedonia, Romania, Serbia, Montenegro, Turkey and Hungary (López-Colón 2006). There are several records from Bulgaria, mainly from the mountain areas. For Stara Planina Mts., the species was reported from the regions of Murgash Peak (Joakimov 1904), Gorna Malina Vill. (Zacharieva-Stoilova 1974) and Vrachanska Planina Mts. (Petrova *et al.* in press), as well as without a specified locality by Nedelkov (1905). For Rila Mts., the species was listed by Mikšić (1957), with denotation of the locality as „Rilo-Kalinin“ (possibly the area of Kalin Peak, NW Rila Mts.) and Král & Malý (1993) from the vicinity of Trestenik Hut. The latter authors also reported the species for Pirin Mts., Liljanovo Vill. (Král & Malý 1993). Additionally, Joakimov (1904) reported the species from Kurubaglar (currently known as the Lozenets district of Sofia city).

Our data shows that the species is common and locally abundant in riverside habitats in the region of Zemen Gorge. The most of the old records of the species from Bulgaria did not include quantitative data (Joakimov 1904, Nedelkov 1905, Mikšić 1957). The data of Král & Malý (1993) on *Ch. robustus* are based on a total of four specimens, found under stones (a routine method often used by entomologists, in contrast to soil excavations). According to Král & Malý (1993), the rarity of *Chaetonyx* species is likely due to their cryptic habitats. However, Zacharieva-Stoilova (1974) reported repeated findings of *Ch. robustus* (a total of 40 specimens) in the soil samples up to 30 cm deep. Our research confirms the assumption that *Ch. robustus* is geobiont species and can inhabit considerable soil depths (up to 50 cm at least). Probably the findings of the *Chaetonyx* specimens under stones are rather accidental, and have led to the false conclusion about their rarity. Soil excavations are obviously more suitable for detection of the *Chaetonyx*, which is confirmed also by the other studies (Zacharieva-Stoilova 1974, Petrova *et al.* in press).

Habitat preference of *Ch. robustus* in Bulgaria

Published data about habitat preferences as well as other observations on the ecology of *Ch. robustus* in Bulgaria are scarce. However, the species has been reported from different types of habitats, including typical mountain ones. Joakimov (1904) indicated collecting «under fallen damp foliage», probably in beech forest, in regard to the locality of Stara Planina Mts. Zacharieva-Stoilova (1974) and Král & Malý (1993) found the species in pastures (at about 500–700 m a.s.l. in Stara Planina Mts. and 1700 m a.s.l. in Rila Mts., respectively). Recently, the species was detected in the soil of xerotherm oak-hornbeam forest at 500 m a.s.l. in Vrachanska Planina Mts. (Petrova *et al.* in press). During the present research, *Ch. robustus* was commonly found in the sandy alluvial soils of sparse river valley forests, at 500–600 m a.s.l. The old records lack information about the soil type in the species habitats and about the proximity to rivers or other water bodies. The locality at Vrachanska Planina Mts., mentioned above, is situated relatively close to Iskar River (approximately 200 m from the shore), but 100 m higher due to very steep riverbank (Petrova *et al.* in press). The soil of this habitat is quite different from that at the localities in Zemen Gorge: it is of brown forest type, stony, close to rendzinas. It seems that the sandy soil and the close proximity to rivers or other water bodies are not essential for the presence of populations of the species. Taking into account the results obtained in the present research and the reports of the earlier authors, it can be assumed that in Bulgaria *Ch. robustus* has significant environmental plasticity, at least in regard to the altitude, soil type and the type of vegetation community.

Density and vertical distribution in the soil

The average density of the species in all samples during our study is 117.6 adults and 662.8 larvae per m^2. The differences in the number of larvae and adults between samples most probably results from the random selection of sampling sites rather than seasonal dynamics of the population. However, these data show that the population of *Ch. robustus* in the riverbank soils in the Zemen Gorge is impressively dense. Higher abundance of *Chaetonyx* larvae has been observed in the upper soil layer (up to 20 cm deep), although the larvae have been found also at greater depth (Table 2). In the upper soil layer, roots of *Aegopodium podagraria* and other herbaceous plants, which decaying parts could be used as a food source by the larvae, are developed. On the other hand, this layer is warmed more intensely and earlier in the spring, which is favorable both for the larval growth and pupation. The spreading of the larvae on greater soil depths probably is an adaptation for more complete use of the food resources and for reducing the intraspecific competition. Such more even distribution of the larvae particularly leads to the decrease of the antagonistic relationships between them within population. If placed together in a little amount of soil, live larvae often injure each other, causing significant mortality. The pupation occurs both in the warmer upper soil layer and in greater depths. In July and August 2013, the percentage of the pupae found in the soil layer of 20–50 cm deep is comparable with that of the larvae. This suggests that the larvae do not migrate for pupation. Young adults remain close to the places of emergence for a certain time, which results in an increase of the proportion of adults found in greater soil depths in September–October. At the same time, oviposition and hatching takes place in the upper soil layer, which implies migration of the adults to the surface for reproduction and migration of a part of the young larvae to deeper layers during their subsequent development.

Development and life cycle of *Ch. robustus*

The research on the local population at the Zemen Gorge indicates that the whole development of *Ch. robustus* occurs in the same habitat. Due to its aptery, the species is colonial and can reach high local abundance in the soil of suitable habitats. The studies of biology and life cycle of the species, conducted on the sampling site, covered three consecutive years – quantitative surveys during 2012 and 2013 and additional collections in 2014. It should be noted that the latter two years – 2013 and especially 2014 were characterized by the uncommonly, for the region, cool and wet summers. This apparently affected the beginning and duration of the different stages of the life cycle of the species in these years.

Pupation, adult emergence, reproduction and lifespan

Both pupation and emergence of the adults of *Ch. robustus* are asynchronous within population and in the investigated habitat they occurred during all summer months. First pupae were observed in the quantitative samples in early July and it seems that the pupation under favorable conditions begins in late June. Finding of the pupae in early September 2013, as well as their delayed occurrence in 2014, were probably due to unfavorable temperatures during both years. At the laboratory, pupation was observed about one month earlier than in the field. It seems that at least a part of the third instar larvae are ready for pupation after overwintering and the process is initiated by the raise of the soil temperature. Lower temperatures at soil depth additionally delays the pupation and subsequent adult emergence when larval development takes place in deeper soil layers, increasing asynchronicity in the life cycle of the species.

Under favorable conditions, adult emergence probably begins in late July. In 2014, the emergence occurred later in accordance with the delayed pupation and the lower temperatures. Lighter colored beetles, that have been found at the beginning of August in both years, as well as in September 2014, belonged to the new adult generation. The slightly sclerotized yellowish adults in the sample from the early August 2013 obviously have had just emerged. In the following months, the slow progress of sclerotization of the exoskeleton of the young adults occurred, resulted in gradual darkening of the beetles. In some individuals, this process completed after wintering which explains the presence of lighter, reddish-brown beetles in the spring. In June and July, before and at the time of oviposition, only dark-brown adults have been found.

In the literature, there is no information on the lifespan of the adult orphnines. Data from this study indicates that adults of *Ch. robustus* from different generations coexist in the population at least for a few mounts. In early August to early September, they are recognizable by the degree of sclerotization. With the developing of the sclerotization of the young adults in the autumn of 2012, the differences between young and older beetles decreased (Fig. 3). In 2013, because of the later adult emergence, all young beetles did not complete their sclerotization by the end of October and even the darkest of them were easily distinguished from those belonging to the older generation. The percentage of the completely sclerotized dark-brown adults in August to October in this case clearly indicate the proportion of the old individuals in the population (Table 1). This proportion showed a clear decline trend towards to the autumn and it seems that the new adult generation displaces the previous. Thus, in the sample from October 2013 only four of 59 excavated adults belonged to the older generation. Some of the old adults die after oviposition, as dead dark-brown beetles have been found in samples from July and August during our study. It is possible that some of the older adults can overwinter and even reproduce next summer. It is unlikely, however, that these adults normally represent significant part of the population. It also seems improbable that adults can survive more than two years. The common lifespan of adults in studied population appears to be about one year, as they overwinter and reproduce only once. Reproduction begins before emergence of the adults from the new generation, during the period of pupation of the previous generation, and the new adults do not reproduce in the same year. Oviposition takes place at the warmer upper layer of the soil, where the dark-brown adults are gathering before and during reproduction. In late July 2014, in the colony of *Ch. robustus* in Vrachanska Planina Mts. we have observed adults, eggs, larvae of different instars, and a single pupa (Petrova *et al.* in press). This suggests that both reproduction and pupation occur during the summer also in the other regions and habitat types in Bulgaria.

Larval development

Larvae of apparently different instars have been observed during all months of the study. This, along with the life span of the adults, suggests at least two generations overlap. It appears that both larvae and adults overwinter. It is however unclear how many times the specimens overwinter in their life cycle and whether the number of overwinterings is constant in different environmental conditions. It seems that all three larval instars are able to overwinter. Apparently most of the first instar larvae that hatch in the summer overwinter as second instar, while the rest overwinter as first. The overwintering first larvae likely molt in the spring which resulted in their absence in the soil in June. Prepupae have not been found in the soil after the beginning of August, and it seems that not all of the older larvae pupate in the same summer. Some of these larvae apparently overwinter again as second or third instar. In summary, the life cycle of *Ch. robustus* seems to be perennial and in studied habitat may include two overwinterings as larval stage and one as adult (Fig. 5).

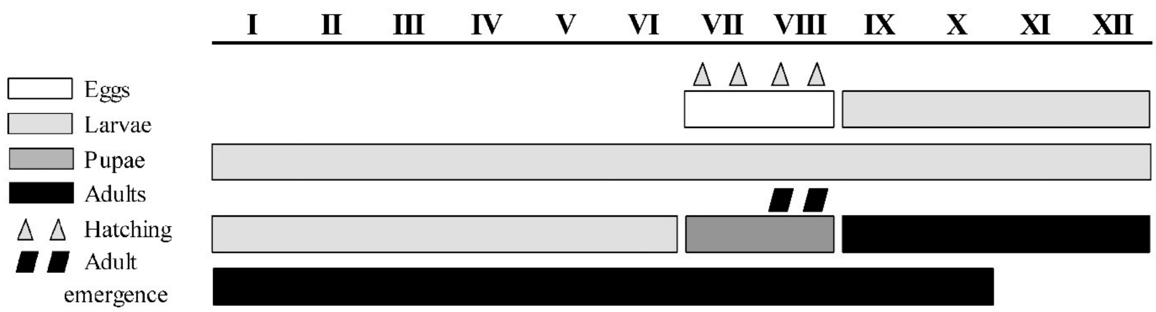

Fig. 5. Life cycle of *Chaetonyx robustus*. Diagram is constructed based on the investigation of the local population in riverside habitat at the Zemen Gorge, SW Bulgaria, in April 2012 to November 2014.

Biology of the Orphninae

Although little information is available on the biology of the other Orphninae genera, the habitat preferences and life cycle of *Chaetonyx* is probably unique among the orphnines and reflect its morphological and behavioral adaptations to subterranean life in temperate regions. *Chaetonyx* comprises the smallest orphnines possessing no head or pronotum armature – the character of allometric sexual dimorphism found in the majority of the Orphninae genera. All *Chaetonyx* are apterous, with fused elytra, and almost blind with the eyes reduced to only a few facettes. These are the adaptation to the subterranean life style and the species apparently do not leave soil at all stages of their life cycle. Due to the aptery and low dispersal potential, *Chaetonyx* live in large colonies. The ecological niche occupied by *Chaetonyx* apparently did not allow it for reasonable diversification in comparison to another geophilous genus, *Hybalus*.

The only other orphnine genus, which biology was studied in some detail, is *Hybalus*. This genus comprises over 30 species distributed mostly in the Atlas Mountains of North Africa (Baraud 1991). Similar to *Chaetonyx*, *Hybalus* are apterous and their elytra are fused, although the two genera are probably not closely related and the aptery was gained by them independently (Frolov, unpublished data). *Hybalus* are geophilous but not so much adapted to subterranean life as *Chaetonyx*. They are larger (body length 6.0–11.5 mm) and with small but well developed eyes. Although rarely encountered on soil surface, the beetles may come out on rainy days (Keith 2005). According to Gourvès (1988), who studied biology of *H. rotroui* Petrovitz, in the eastern Middle Atlas, the adults were found in rainy season, mainly from November to March in soil 3 to 5 cm deep. Mating took place in soil and the 2-instar larvae diapaused during the dry season. Larvae fed on grass roots and resumed feeding activity with the beginning of the rainy season in October to quickly finish development, pupate, and give new generation of adults in November. Palmà (1938) described the similar, annual life cycle of *H. cornifrons* (Brullé) in Sicily and noted reasonable damage to wheat crops caused by the beetles in some years (probably this record refers to *H. benoitii* Tournier, since *H. cornifrons* doesn't occur in Sicily according to Baraud, 1991).

Biology of the tropical Orphninae, especially their life cycles and nesting behavior, is virtually unknown. The data from the collection specimens labels suggest that imago of most species are litter dwellers inhabiting forest and savanna biotopes. In Afrotropical Region and, especially, in Madagascar, adult orphnines were collected in pitfall traps mostly baited with fish (Frolov & Montreuil 2006, 2009). The larvae of *Triodontus nitidulus* (Guérin-Méneville, 1844) were found in upland rice plantations in Central Madagascar (Randriamanantsoa *et al.* 2010). Although it is unclear if the larvae of this species cause any damage to the crops, the adaptation to the development on plantations might explain the relative abundance of *T. nitidulus* throughout the island. The immature stages of only one Neotropical species, *Aegidium cribratum* Bates, were descried (Morón 1991). The larvae and pupa of this species were found in association with adults in rotten logs, which suggest mycetophagy rather than detritophagy at least at the larval stages. An unexpected association of the Orphninae with epiphytes was recently discovered in the Andean cloud forest in Ecuador: the adults of an undescribed taxon were collected from the dead plant materials accumulated between epiphytes and tree bark up to 16 m high (Frolov & Vaz-de-Mello 2015). Available data, although scanty, suggest that the tropical Orphninae occupy a number of rather distinct ecological niches but apparently lack colonial geobiont specialists similar to *Chaetonyx*.

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