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The Use of Model Groups of Necrobiont Beetles (*Coleoptera*) for the Diagnosis of Time and Place of Death

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ABSTRACT

The article considers the issues of determining the time and place of death. Methodological materials for conducting a forensic medical examination are provided. The material will be useful both to entomologists studying the issues of decomposition of cadaveric mass in nature, and to medical personnel for whom it is a question of establishing the exact time of death.

Keywords: *Forensic examination, Corpses, Time of death, Biotope of death, Necrobiontic Coleoptera, Ciscaucasia.*

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INTRODUCTION

Forensic entomology is one of the areas of entomology, as science has developed in recent decades. The task of the forensic entomology consists of observations of the fauna of insects and their succession (a successive change on the corpse, to determine the time of death). In many cases, corpses are found in the initial stages of decay. Insects, which represent the richest group in species, play several key roles [1]. Insects colonize a wide variety of substrates [2, 3]. They use these substrates as food source, their shelter and their place of development, mating or laying [4]. The presence of species of different taxonomic groups is the key to determine the time of death. A correlation between the stage of decomposition and the change of species was revealed; these patterns are observed in tropical and temperate regions [5, 6]. Foreign authors distinguish five consecutive stages of decomposition of a corpse: a new (fresh) stage, a swollen stage, a decay stage, a dry stage, and a residual stage [5-7]. Changing the corpse during decomposition causes the sequence of changes in species; so that, some prefer fresh dead tissue, and the others prefer the fabric which have undergone decay, and Step I - when are scraps of skin and bone [8, 9]. Larvae of flies dominate in the early stages of decomposition and are replaced by beetles in subsequent stages [5, 10-16]. The time of death can be precisely determined if the expert carefully collects samples from the corpse and fixes the collected material. If a species is found in the pupal stage, and development takes 30 days to develop from an egg to a pupa, then we can conclude that the sample was dead at least 30 days ago. Physical conditions around the body affect insects and their numbers on the corpse. Decomposition of a corpse largely depends on the environmental conditions including temperature, the number of insects feeding on the corpse, microclimate surrounding the corpse, and predatory vertebrate animals [17]. Forensic entomology is important in criminal investigations. Below we presented our model for determining the time of death. The development was carried out on the basis of our 15-year research with the corpses of animals weighing 45-150 kg.

MATERIAL AND METHOD

The work is mainly based on our own material collected in different regions of the Ciscaucasia during 20 field seasons (from 1998 to 2018) in the spring and winter. Over 20000 corpses of different taxonomic affiliation were examined. 50000 exemplars beetles, 19000 larvae, and 1500 pupae were collected. The most effective way to collect necrophages is to manually collect from the corpses.

Table 1. The structure of the coleopteran necrobiont complexes of terrestrial ecosystems of the Ciscaucasia.

Environmental Group	Family, genus	
I. Obligate or specialized necrophages	**I.1. Necrophages** **1.1.1.** Silphidae: *Nicrophorus* **I.1.2. Surface** Silphidae: *Necrodes*, *Oiceoptoma*	**Carrion Barking** **carrion beetles of** *Thanatophilus*
	I.2. Dermestids and keratophagi Dermestidae (L): *Dermestes*, *Anthrenus* Nitidulidae (L, I): *Omosita*, *Nitidula* Trogidae	
II. Facultative necrophages and necrobionts	**II.1. Necrophages** Silphidae: *Silpha* (L, I) Catopidae (i) catops Cleridae (I) Necrobia	Sciodrepoides
	II. 2. Caprophages (I) Hydrophilidae: *Cercyon* Leiodidae Staphylinidae: *Omalium*, *Oxytelus* Scarabaeidae: *Geotrupes*, *Aphodius*	
	II. 3. Zoophages (I) Histeridae: *Saprinus*, *Hister*, *Gnathoncus*, *Margarinotus* Staphylinidae: *Philonthus*, *Creophilus*, *Ntholestes*, *Tachinus*	
	II. 4. Parasites (L) Staphylinidae: *Aleochara*, *Atheta*	
III. Occasional visitors to corpses (zoophages, mycetophages, polyphages, etc.)	Carabidae, Cryptophagidae, Ptiliidae, mycetophilic Staphylinidae	

* L: larva; I: imago

At the same time, we used entomological sieve, spatula, knife, tweezers, and exhauster. All kinds of loose (soil) or semi-liquid (semi-decomposed corpses) substrates were disassembled on a plastic film (1 m^2). Barber traps (soil traps) were used to collect surface-carrion species. Traps were consumer plastic cups (0.5 L) with a fixing liquid (10-15% solution of acetic acid or 80% solution of ethanol). Small forms were collected using an exhausted, the rest were collected with tweezers. To account for pupae and eggs, soil excavations (using a spatula) and flotation (in a concentrated NaCl solution) were carried out. The soil samples were taken according to the procedure [18-20].

Breeding issues were clarified by observing animals under natural conditions in different seasons. We set the time for mating and laying eggs, counted the masonry on and near the corpse, and also the number of larvae. To clarify the structure of populations, observations were made on changes in the number and density of species in different seasons. At the same time, the food specialization of the studied species was determined. For the classification of the families of the Coleoptera order, we used the classification proposed by Lawrence, Newton, 1985.

In describing the structure of the complex, we used the classification proposed by Braack [21], which characterizes the ecological connections of the imago of insect necrobionts with a corpse (trophic specialization of species and the preference of a corpse for development). Necrophilic Coleoptera are organisms closely connected by consortium bonds with a corpse (see table 1).

Among necrophilous beetles, the majorities are stenobionts and are confined to some types of sinusia, forming the corresponding ecological complexes. In the necrobiont complex, as in most others, three degrees of confinements can be distinguished, including necrobionts, necrophages, and necroxenes, however, the last group, which are guests, should not be confused with random species

[21-23]. The study area is shown in Figure 1.

Fig. 1. Map of stationary research sites.

RESEARCH RESULTS

Stages of Decomposition

The trophic specialization of necrobionts can be clearly seen through the interchangeability of taxonomic groups at different stages of the decomposition of a corpse. After analyzing the processes of decomposition of corpses of different taxonomic affiliations and masses, we highlighted some patterns (table 1). The appearance of necrophagous beetles of various species on corpses occurs at certain stages of the "life" of the corpse (= microsucession of the corpse), and it has a regular repeating character (Fig. 2).

The population of corpses with necrophages is due to the fact that at different stages of the decomposition of the corpse under the influence of bacteria, different gases including NH_3, H_2S, SO_2, NO, NO_2, CO_2, indole, skatol, and metocarpol are released. Observations on the attraction of various substances give reason to say that indole and NH_3 attract *Necrodes*, metocarpol attracts *Silpha*, and *Thanatophilus*, and finally, some species of Dermestidae prefer scatola [21, 25].

1. The stage of the beginning of the "life" of the corpse (0-3 days after death). The smell of a corpse on an organoleptic scale is 0-3 points. In the first stages flies and *Nicrophorus* appear. Small corpses (up to 400 gr.) are buried by grave diggers for laying eggs (at first wool, feathers, and hornworms are removed from the corpses), and larger ones serve as food. If the corpse (which is buried or not) is not suitable (damaged mechanically), they proceed to the next step (see fig. 3).

During this stage, complex proteins and carbohydrates begin to decompose. This stage lasts approximately 3-6 days in the summer (1). In spring and late autumn, it can last much longer [6, 7]. Usually, Calliphoridae is the first group that arrives and is the dominant group during the first hours (2; 3; 6; 7). Species of Sarcophagidae and Muscidae arrive on the first day [5]. The eggs of the flies are laid in the first 24 hours, and the larvae can appear after 24 hours under optimal conditions. However, the first larvae do not appear earlier than 2-3 days [26, 27]. The eggs of the flies are fed by ants (Hymenoptera: Formicidae), which are also found in this stage by representatives of staphylinids [28].

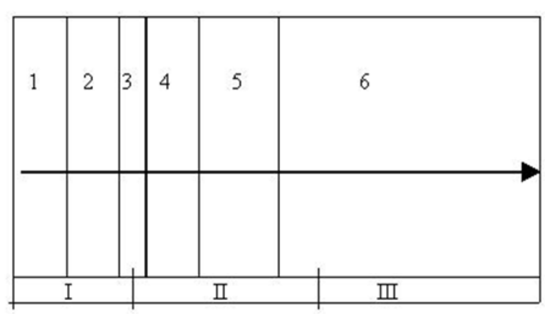

Fig. 2. Microcirculation scheme of necrobionts on the corpses of animals (averaged version): I, II, III-

stages of microcirculation; the direction of microsucession; The order of emergence of necrophage beetles: 1. *Nicrophorus*; 2. *Thanatophilus*, *Silpha*; 3. *Necrodes*; 4. *Oiceoptoma*; 5. *Phosphuga*; 6. *Dermestes*, *Attagenus*.

2. The stage of "prosperity" (putrification + buterification fermentation). By the time it is about 5-10 days the putrification and after 9-16 days the buterification fermentation occur. The smell of a corpse is 4-5-4 points. Considering that most beetles feel the smell of falling at a distance of 200 meters or more from the corpse, under favorable conditions, a medium-sized corpse can collect necrophages from an area of more than 5.5 hectares. *Nicrophorus* feeds on large corpses, at the same time *Necrodes* appears (prefers large corpses). At this stage, the development of microorganisms takes place most intensively; they cause the release of various odors by the corpse. Many types of necrophage beetles appear. Contradictory relations develop between species: adults and the larvae of many species maintain the "purity" of the corpse from flies. We noted that necrobionic sylphides and dermestids kill the larva, but do not feed on it. By reducing the kinds of food stocks, first the transfer to predation between the families, and then to cannibalism occur (Fig. 2).

The putrification stage is characterized by rotting, during which the gases produced by anaerobic bacteria inflate the stomach. The internal temperature of the corpse rises above ambient temperature due to the activity of larvae and bacteria (1; 2). The stage lasts 5 days in the summer, and about 7 in the spring and late autumn. It ends when the gases cease to be released. According to [6], coleopterans begin to fly in large numbers at the end of the stage. They include carnivores (Silphidae), staphylinids (Staphylinidae), and hysterids (Histeridae).

The buteric fermentation stage begins when the corpse becomes dry [6]. During this stage, the integrity of the skin is disturbed by insect larvae. This provides anaerobic bacterial decomposition [5, 6]. The internal temperature of the corpse during the stage reaches 30.5 °C, and always more than 20 °C. Larvae migrate a considerable distance from the body before pupation [26]. At the end of the stage, the larvae of the flies leave the corpse, and the beetles dominate the corpse [6]. Carpet beetles (Dermestidae), which are necrophages, eat skin and dry parts of a corpse. On dry corpses with a lot of subcutaneous fat, species of the family Nitidulidae develop [9, 29-34].

3. The stage of "decline". In time it is about 18-23 days, the smell is on the organoleptic scale of 3-0 points. By this time, the bones and parts of the corpse (skin, hair, nails), that were not eaten by the necrophages, remain from the corpse. At this time, many species of Dermestidae appear (Fig. 2, 3).

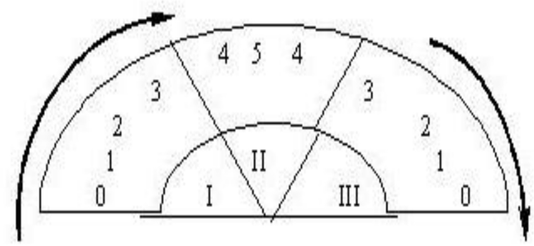

Fig. 3. The intensity of the cadaveric smell (according to the organoleptic scale) at different stages of decomposition of the corpse (0-5-0). I, II, and III: stages of decomposition of a corpse.

Table 2. Groups of necrophagous beetles at different stages of corpse decomposition (I-finding less than 25%, II-within 35-70%, and III-over 70%).

Genus	Corpse decomposition stage		
	1	2	3
Nicrophorus	W	II	-
Silpha	W	II	-
Oiceoptoma	II	III	-
Thanatophilus	I	III	I
Necrodes	-	III	I
Phosphuga	-	III	-
Attagenus	-	I	III
Anthrenus	-	I	I
Dermestes	-	I	III

The stage begins when the corpse dries up and dry skin, hair, cartilage, and bone remains from it. The temperature of the corpse is equal to the ambient temperature. Towards the end of the stage, bones and hair remain. It lasts about 13 days in the summer, 27 in the spring and more than 25 in the fall and winter. Skin tanners dominate throughout the entire stage [5, 21, 22, 25, 28]. Predatory beetles feed on insect larvae especially Cleridae and Scarabaeidae. Necrobiont species of Scarabaeidae appear in large quantities; 10-12 days are required for the development of their larvae. Dead-eating larvae reach their maximum size, burrow into the ground near the corpse, create pupal chambers and pupate. Pupa free, pupal stage lasts an average of 10-25 days. By the end of the stage, a skeleton with scraps of skin remains. Trogidae, the last representatives of insects, develop on the corpse and dominate the corpses at this time. Dermestids complete development and leave the corpse [35]. However, they can still be found in skeletal clefts, especially inside the skull. At the very end, soil acarimorphic mites such as *Oligota* spp. appear [5].

Special mention should be made for keratophagy, as a special case of necrophagy. Keratin-containing substances include hair, wool, feathers, hooves, horns, claws, horny scales of fish, reptiles, and birds. These substances are not consumed by most of the necrophage beetles, and are not processed by the enzymes of dipterous larvae since these substances contain very little water (less than 14% by weight). So, the species that feed on them have the ability to produce metabolic water. This is a rare property in nature, and species possessing it are especially valuable. Among the species of our region, these include representatives of the genera *Anthrenus*, and *Choleva* [36]. Besides, in Trogidae and Catopidae, diet keratin predominates over other parts of the corpse. In addition, species of the genus *Dermestes* are able to accumulate a very large amount of sulfur in their body. It is formed when the keratin molecule breaks down into 2 cysteine molecules [19, 20].

The population of corpses with necrophages is due to the fact that at different stages of the decomposition of the corpse various gases (NH_3, H_2S, SO_2, NO, NO_2, CO_2, indole, skatol, and metocarpol) are released. Observations on the involvement of different gases, give grounds to say that indole and NH_3 attracts *Necrodes*; metocarpol attracts *Silpha* and *Thanatophilus*; H_2S and SO_2 attract *Catops* and *Choleva*; NO and NO_2 attract Dermestidae; and Skatol attracts *Trox*.

The time for the disappearance of corpses from the earth's surface is different. For small corpses (reptiles, birds, and mammals) up to 350 gr buried by grave digger bugs (*Nicrophorus*) it takes 1-2 days. If, for a number of reasons, the burial did not occur, then they are eaten in 6-7 days. For corpses weighing 1000-2000 gr this time is up to 15 days, for 2500-3000 gr is 1.5 months, for 5000-7000 gr is 2.5-3 months, and for 60,000 gr is 4-6 months. The burial process depends on many factors (as well as its fauna) including the place where the corpse is located, the composition of the soil, the condition of the corpse, and microclimatic factors. In our studies, we used corpses of animals such as dogs, sheep, and calves weighing 40-120 kg.

According to our calculations proportion of necrophage beetles (in general "work" decomposers) is about 9.5% at equilibrium ecosystem, and the proportion of one necrophage beetle in the total number of necrophages of Ciscaucasia is about 45%. Microsucession of necrobionts on a corpse is the “imprint of the evolution of the animal world”. Animals came out of the aquatic environment. Therefore, hygrophils (*Nicrophorus*) appear at the 1st stage of decomposition of the corpse; Stage 2 is the mapping of the mesophilic stage in the evolution of the animal world (during this period, most species of necrophagous beetles appear); Stage 3 is the imprint of the arid stage of evolution (Trogidae, Dermestidae), and these are xerophiles. Thus, the construction of models for the development of microsucessions of necrophage beetles on corpses is of not only practical, but also theoretical interest, as it allows one to understand the evolution of detritophages as one of the most important components of the group of reducers.

Based on the obtained data, we compiled Figure 3.

Biotopic Distribution of Beetles under Concases

Geographical position and landscape division is given in [23, 24]. The main land biotopes are forest, steppe, semi-desert, near-water, and urbanized (city and village) biotopes. The results of the analysis of static confinement are given in table 3.

From table 1, it can be seen that along with eurybiontic species (found in three or more biotopes), there are stenotopic species living in the same biotope. Theyshould be served as the key to determine the exact place of death. When laying eggs, one can diagnose a place (biotope) with sufficient confidence.

Influence of Climatic Factors on Decomposition of the Corpse:

Temperature.

Corpses are consumed faster in the summer than in other seasons of the year due to high temperatures. We found that the corpses of dogs (weighting 50 kg) in summer (at a temperature of about 28 °C) and spring (at a temperature of 15 °C) complete 3 stages of decomposition in 30 and 60 days, respectively. In cold weather of below 10 °C, the activity of species of necrobionts is limited. However, eggs and larvae do not die even at air temperatures below 5 °C, since decomposition generates heat and positive entropy is constantly observed. According to our data, it is more difficult to determine the exact date of death if the corpse was affected by significant temperature extremes above 26 °C. With such extreme differences, even in the spring-summer period, we observed the death of the pre-imaginal stages of development.

The influence of microclimatic factors on the decomposition of a corpse

Table 3. Biotopic distribution of carnivores and skin eaters on the territory of the Ciscaucasia.

Species	Biotope				
	Forest	Steppe	Semi desert	About-water	Urbanization rounded
Family Silphidae					
Necrodes littoralis	+			+	+
Oiceoptoma toracicum	[+]				
Thanatophilus rugosus	+			+	
T. sinuatus		+			+
T. dispar	[+]				
T. ferrugatus		+	+		
T. terminatus			+		
Silpha carinata		+	+		+
S. obscura		+			+
Nicrophorus humator	+			+	+
N. germanicus		+	+		
N. morio			[+]		
N. satanas			[+]		
N. vespillo	+	+	+	+	
N. vestigator		[+]			
N. antennatus		+	+		+
N. fossor		+	+		
N. vespilloides	+		+		
N. sepultor		+	+		
Family Dermestidae					
Dermestes maculatus		+	+		
D. frischi		+	+		+
D. dimidiatus		[+]			
D. undulatus			[+]		

D. murinus	[+]				
D. gyllenhali				[+]	
D. laniarius		+			
D. coronatus					
D. kaszabi			+		
D. lardarius					[+]
Family Trogidae					
Troxhispidus niger		+	+		+
Family Catopidae					
Catops tristis	+				
Sciodripoides watsoni	[+]	+			
Family Cholevidae					
Choleva oblonga	+				+

* stenotopic species are taken in [] brackets.

In a damp environment (swamps and shores of water bodies) the corpse will decompose faster than in dry places (steppe and semi-desert). Wet stations are favorable for the development of hygro- and mesophilic groups of necrobionts. Clothing on a corpse will accelerate decomposition, protecting larvae and eggs from sunlight [17]. Rainy and windy weather reduces the activity of adults and larvae. In the steppe biotopes and dry winds lead to the dominance of skin eaters (xerophilic necrobionts) on the corpses. Corpses found in water are difficult to identify. The presence of soil species or their larvae on the corpses indicates that they have laid on the ground for some time. However, larvae can develop on corpses that swim in water for 5 days [17]. Aquatic insects of chironomids (Diptera: Chironomidae) and Trichoptera are found on submerged bodies [37].

Corpse condition

The physical state of the body at the time of death is a factor influencing decomposition [17]. External trauma to the body accelerates decomposition, because insects are quickly attracted to open wounds and lay eggs in them. Cadaveric hoscus is attracted by *Creophilus* [28, 38]. With extensive penetrating wounds, they easily enter the body.

Procedures in Judicial Entemology

Estimation of the age of insects (adults and larvae)

The age of the preimaginal stages can be estimated by comparing the size, length, and stage of the life cycle [26]. Larvae of flies go through 3 stages before the flight of adults. The larval cycle lasts from 4 to 6 days, depending on the species. Assessing the age of the larvae is to measure their size, and ability to shrink when irritated, and stained.

The stages of the dead-eating, carnivore, and other necrobiontic Coleoptera larvae are basically similar, and the differences in species are in size and color. The average duration of larval stages is 24-31 days. In skin eaters, the larval development takes 30-45 days.

COLLECTION AND DETERMINATION OF INSECTS

For accurate diagnosis, it is necessary to collect the maximum possible number of adults and larvae. Collection should be carried out from different parts of the body, from under the corpse and soil [17]. The largest larvae should be collected and fixed in 70% solution of ethanol or 4-5% solution of formalin. If no larvae are found, then eggs are collected, they can be found in the nasal passages, ears, under the eyelids, and wounds, if they are present on the body. Clothing should be carefully examined to find pupae and puparia. If the corpse is highly decomposed, it is necessary to take soil samples from a depth of up to 20 cm. Half of the collected specimens must be stored as a collection, and the other (larvae and eggs) are kept in cages to complete the development cycle. This will help ensure that species are correctly identified. For laboratory egg maintenance, Koch cups with a substrate of river sand and humus are used. Larvae are kept in plastic cages measuring 25 × 25 ×15 cm.

CONCLUSION

Knowledge on insects on corpses is important in determining the time of death. Static confinement of species of carnivores and skin-eaters can help in determining the place of death. However, for reliable determination of the place of death and time, in addition to collecting material, the laboratory studies are necessary. Therefore, for correct interpretations in each case, organizations involved in these issues should be provided with appropriate methodological literature.

Conflict of interest.
The work was carried out by the authors on their own without the use of any support.

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