ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

Fakulta životního prostředí

Katedra ekologie

Thermal Summation Models in Selected Species of Silphinae (Coleoptera)

Termálně sumační modely u vybraných druhů podčeledi Silphinae (Coleoptera)

Bakalářská práce

Autor bakalářské práce: Iana Ipatova

Vedoucí bakalářské práce: doc. Jan Růžička, Ph.D.

Praha 2019

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

BACHELOR THESIS ASSIGNMENT

Iana Ipatova

Applied Ecology

Thesis title

Thermal summation models in selected species of Silphinae (Coleoptera)

Objectives of thesis

Carrion beetles (Silphidae) are important group of necrophagous beetles with applications in forensic entomology. The Bc thesis will focus on general review of ecology of this group (with focus on the members of the subfamily Silphinae). Relevant information from forensic applications can complete the picture, incl. brief description of methods used for estimation of post mortem interval (PMI). The goal is to learn how to collect and process the data to find parameter estimates of the thermal summation models for the selected species. Student will also learn how to identify developmental stages of studied beetles and how to breed them under laboratory conditions.

Methodology

Practical part of the thesis will focus on developmental biology of selected carrion beetles (Silphidae: Silphinae). The adults will be collected in the field and placed in breeding colonies under laboratory conditions at three different constant temperatures and stable light regime. Emerging larvae of new generation will be separated and their individual development observed and recorded. Relationship between temperature and length of development will be calculated by linear regression to obtain parameters and they standard errors (lower developmental threshold and sum of effective temperatures) of thermal summation models for each developmental stage.

The proposed extent of the thesis

30 pages

Keywords

Coleoptera, Silphidae, Silphinae, immature stages, thermal summation model

Recommended information sources

JAKUBEC P. 2016: Thermal summation model and instar determination of all developmental stages of necrophagous beetle, Sciodrepoides watsoni (Spence) (Coleoptera: Leiodidae: Cholevinae). PeerJ, 4: e1944.

MATUSZEWSKI S. 2011: Estimating the pre-appearance interval from temperature in Necrodes littoralis L. (Coleoptera: Silphidae). Forensic Science International, 212: 180-188.

MIDGLEY J.M. & VILLET M.H. 2009: Development of Thanatophilus micans (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. International Journal of Legal Medicine, 123: 285-292.

RIDGEWAY J.A., MIDGLEY J.M., COLLET I.J. & VILLET M.H. 2014: Advantages of using development models of the carrion beetles Thanatophilus micans (Fabricius) and T. mutilatus (Castelneau) (Coleoptera: Silphidae) for estimating minimum post mortem intervals, verified with case data. International Journal of Legal Medicine, 128: 207-220.

RICHARDS C.S. & VILLET M.H. 2008: Factors affecting accuracy and precision of thermal summation models of insect development used to estimate post-mortem intervals. International Journal of Legal Medicine, 122: 401-408.

RŮŽIČKA J. & JAKUBEC P. 2016: Icones Insectorum Europae Centralis. Coleoptera: Agyrtidae, Silphidae. Folia Heyrovskiana, Series B, 26: 1-17.

RŮŽIČKA J. 2015: Silphidae, pp. 5, 291-304. In: LÖBL I. & LÖBL D. (eds): Catalogue of Palaearctic Coleoptera Volume 2/1. Hydrophiloidea – Staphylinoidea, Revised and Updated Edition. Brill, Leiden & Boston, xxvi + 900 pp.

SIKES D.S. 2005: Silphidae Latreille, 1807, pp. 288-296. In: BEUTEL R.G. & LESCHEN R.A.B. (eds): Handbook of Zoology, Volume IV: Arthropoda: Insecta, Part 38: Coleoptera, Beetles. Volume 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim). Walter de Gruyer, Berlin & New York, 632 pp.

SIKES D.S. 2008: Carrion beetles (Coleoptera: Silphidae), pp. 749-758. In: CAPINERA J.L. (ed.): Encyclopedia of Entomology. Springer, Berlin, 4346 pp.

VELÁSQUEZ Y. & VILORIA A.L. 2009: Effects of temperature on the development of the Neotropical carrion beetle Oxelytrum discicolle (Brullé, 1840) (Coleoptera: Silphidae). Forensic Science International, 185: 107-109.

Expected date of thesis defence
2018/19 SS – FES

The Bachelor Thesis Supervisor
doc. Mgr. Jan Růžička, Ph.D.

Supervising department
Department of Ecology

Advisor of thesis
ing. Pavel Jakubec

Electronic approval: 27. 3. 2018

doc. Ing. Jiří Vojar, Ph.D.

Head of department

Electronic approval: 27. 3. 2018

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 08. 04. 2019

DECLARATION

I hereby declare that this Bachelor's thesis is a presentation of my original research work, which was done under the guidance of Assoc. Prof. Jan Růžička, Ph.D. at the Czech University of Life Sciences Prague. Wherever contributions of others are involved, there is an acknowledgement of collaboration and every effort is made to indicate this clearly, with due reference to the used literature.

In Prague, 23 April 2019   _____

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis adviser Assoc. Prof. Jan Růžička, Ph.D. for his energy and competence and express my appreciation to him for his patience, calm and support in overcoming numerous obstacles I have been facing through writing my thesis. He always steered me in the right direction and kept my spirits up. I am also grateful to Ing. Pavel Jakubec, Ph.D. for his feedback, assist in the processing of the data, cooperation and the attentive attitude he lent to me. Without the assistance of my research supervisors and their involvement in every step throughout the process, this work would have never been accomplished. Thanks to them, It was a remarkable experience over the past two years. Their passion and love for their work really helped me get results of better quality. My sincere thanks also go to Petr Šípek and Dominik Vondráček, who collected adult specimens of *Diamesus osculans* and took them to the Czech Republic.

I would like to warmly thank my family and my friends for invaluable supporting me throughout encouragement my years of study, throughout writing this thesis and my life in general. You gave me even more than support, you listened to me when I needed it and I always have found the strength to move on because of you. Every time I was ready to give up when problems arose, you did not let me, and I shall always be grateful. Thanks for being there for me. You are wonderful people, and I love you.

Thank a lot you again, everybody. I am glad I spent it with you. Your presence in my life allowed me to ignore everything that was happening around me. Without all of you, I never would have made it.

ABSTRACT

Information regarding necrophagous beetles is under-represented in forensic entomology studies despite the fact that knowing about larval development rate of selected species could be used for the postmortem interval (PMI) determination. The theoretical part of my thesis can be divided into three main topics: a description of the family Silphidae and information about forensic entomology and thermal summation models. In the practical part of the thesis, information is presented about the genus *Diamesus* Hope 1840 in general and laboratory research conducted. The laboratory study was conducted on the species *Diamesus osculans* (Vigors 1825) within one month. Collected beetles were kept in climate chambers at constant temperature (20, 22 and 24 ◦C) and their development was regularly documented. In total, we had 180 first instar larvae to explore, although most of the larval specimens did not develop into 3. larval instars. The R statistical program was used for calculated parameters of thermal summation models and standard errors for 1st larval instar.

Keywords: Coleoptera, Forensic entomology, Larval development, Silphidae, Thermal summation models

ABSTRAKT

Nekrofágní brouci jsou stále nedoceněni ve forenzní entomologii i přesto, že znalost délky larválního vývoje jednotlivých brouků by mohla být použita pro stanovení postmortálního intervalu (PMI). Teoretickou část mé bakalářské práce lze rozdělit do tří základních témat: popis čeledi mrchožroutovití (Silphidae) a informace o forenzní entomologii i termálně sumačních modelech. V praktické části je představena obecná informace o rodu *Diamesus* Hope 1840 a o provedeném laboratorním výzkumu. Během jednoho měsíce byla provedena laboratorní práce, zaměřená na druh *Diamesus osculans* (Vigors 1825). Sebraní brouci byly chováni v klimatických komorách při konstantních teplotách (20, 22 a 24 ∘C) a jejich vývoj byl pravidelně dokumentovan. Celkem se povedlo vychovat 180 larev, ale větší množství pokusných jedinců se nevyvinulo ani do 3. larválního stádia. Pro následující analýzu a výpočet parametrů termálně sumačního modelu pro 1. larvální instar byl použít statistický program R.

Klíčová slova: Coleoptera, forenzní entomologie, larvální vývoj, Silphidae, termálně sumační modely

TABLE OF CONTENTS

1. INTRODUCTION

Recently, insects are increasingly used to solving crimes. Identification of insects on a corpse helps confirm the place of dead or crime scene, and study of the cycle of their larval development can help to estimate the time of death. In that regard, necrophagous beetles becoming more and more interesting to examine. Most of the research in forensic entomology is devoted to flies due to the fact that they are the first colonizers of corpses. However, the benefit of necrophagous beetles is that they are present on the corpse in the later stages of decomposition.

The subject of my thesis is the thermal summation models in selected species of large carrion beetles (Coleoptera: Silphidae: Silphinae). This topic is most relevant and deserves to be highlighted in relation to the potential application in forensic science. The family Silphidae, which is described in more detail in the theoretical section of my thesis, was selected as our focal for research. However, the principal subject of study is the species *Diamesus osculans* (Vigors 1825).

The purpose of the study is to identify the existence of a relationship between temperature and larval development rate and the creation of a thermal summation model. And an additional purpose would be to describe this relationship in as much as detail as possible. To achieve this goal, several tasks were defined. It is primarily laboratory work with the creation of the necessary conditions for data collection, the analysis of information received and comparison with other scientific works. The working hypothesis is that a relationship between temperature and larval development rate exists and larval development rate increases accordingly when the temperature increases.

My thesis work is divided into two parts. The first part included a literature review about the family Silphidae, forensic entomology and thermal summation models. The second part focused on the genus *Diamesus* Hope 1840, laboratory research, analysis of the results and discussion. The analysis of the case studies was held during the writing of this thesis. The uniqueness of my work consists in that similar studies had not been carried on *Diamesus osculans* and the genus *Diamesus* had not been studied in detail.

2. GOALS OF THESIS

1. Write a literature review of ecology of Silphidae with a focus on the members of the subfamily Silphinae.
2. Supplement a theoretical part by information on forensic entomology and thermal summation models.
3. Conduct a laboratory investigation and analyse findings.
4. Create and describe in detail a thermal summation model for *Diamesus osculans*.

3. SILPHIDAE
3.1. Systematics and phylogeny

The family Silphidae is a relatively small but diverse group of beetles, which include two subfamilies: Nicrophorinae, called burying beetles or sexton beetles, and Silphinae, called carrion beetles, which are morphologically and ecologically different (Sikes, 2005). The subfamily of Silphinae according to different sources is presented by 12–18 genera and Nicrophorinae, in turn, by 3 genera (*Nicrophorus* Fabricius 1775, *Eonecrophorus* Kurosawa 1985 and *Ptomascopus* Kraatz 1876) and 65 species (Sikes, 2005). This is a clear indication that Silphinae has higher generic diversity that Nicrophorinae. Currently, carrion beetles have composed by 9 genera and 45 species through Europe (Růžička et al., 2015).

Dobler & Müller (2000) deduced the phylogenetic relationships of nine genera of the Silphinae and two of the Nicrophorinae using sequences of the genes COI and COII. The results of their work are different from the conclusions of all prior workers of this family which placed the genera *Necrodes* Leach 1815 and *Diamesus* Hope 1840 sister to all remaining the Silphinae or as a distinct tribe Necrodini. Dobler & Müller (2000) placed *Ptomaphila* Kirby & Spence 1828 as the sister genus to *Necrodes* Leach 1815 fully two major branching events from the base of the subfamily. If *Necrodes* and *Diamesus* are not the most basal Silphinae, and *Ptomaphila* and *Oxelytrum* Gistel 1848 are, then the evolution of different characters should be reviewed. Such as *Necrodes* and *Diamesus* have truncate elytra, and although of quite different forms, this state is shared in general with the Nicrophorinae (Sikes, 2005).

3.2. Distribution

The distribution of the family Silphidae is relatively well studied. The family Silphidae is distributed in almost all continents, but mostly in the northern hemisphere, in the temperate zone; that is, the family has a Holarctic distribution (Anderson & Peck, 1985; Sikes, 2005). Antarctica is the only continent where they do not live (Sikes, 2005). It is fair to say that the Palearctic region, which together with the Nearctic forms the Holarctic biogeographic region, is a major location of family Silphidae because there are the most genera and the highest number of species of Silphidae (Anderson & Peck, 1985; Růžička et al., 2015). Additionally, it should be noted that Silphinae are more widely distributed than Nicrophorinae, and take place in Australia, although both silphid subfamilies are not reported from the main Pacific islands such as New Zealand, New Caledonia or Fiji (Peck, 2001; Sikes, 2005).

3.2.1. Silphinae

Silphinae are represented by the four species in Australia and New Guinea (*Ptomaphila*, 3 endemic species; *Diamesus,* 1 species) and a larger radiation in South America than is seen in Nicrophorinae (*Oxelytrum*, 8 species) (Sikes, 2008). In addition, there are three silphine species in South Africa (*Thanatophilus* Leach 1815, 2 species; *Silpha* Linnaeus 1758, 1 species) and a separate silphine genus (*Heterotemna* Wollaston 1864, 3 species) endemic to the Canary Islands off the northwest coast of Africa (Sikes, 2008). Most species of the Silphinae inhabit the Northern Hemisphere, but they are more tolerant of warm habitats than the Nicrophorinae (Sikes, 2008).

3.2.2. Nicrophorinae

Nicrophorus species are inhabiting mainly the temperate northern hemisphere and are missing in Antarctica, sub-Saharan Africa and Australia (Sikes, 2005). Generally, they are rare in warmer climates, such as lowland tropical forests, and practically not present from dry climates like deserts (Sikes, 2008). Nicrophorines are less tolerant of warm habitat than members of subfamily Silphinae. *Nicrophorus* species requires

small carcasses that can be buried and defended, and their preference of colder habitat is due to competition with the ants and carrion-associated scarab beetles that are more abundant in warmer habitat (Sikes, 2008). Surely these ecological constraints have limited their distribution in places like Africa, Australia and Tibet (Sikes, 2008). For the Western Palearctic, there is only one genus of Nicrophorine: *Nicrophorus* (Růžička et al., 2015). A small number of Nicrophorinae species are endemic to south-east Asia, New Guinea and central and southern America (King et al., 2015). Nicrophorinae were thought to be absent from the Indian subcontinent south of the Himalayas, but there may be a population of species *Nicrophorus sausai* Růžička, Háva & Schneider 2000 in north-eastern India (Sikes, 2008).

3.2.3. Silphidae species' protection

Despite the ubiquity of the family Silphidae, there are species in federally endangered of extinction, such as *Nicrophorus americanus* Olivier 1790, which lives in the United States and Canada (Backlund et al., 2008). Previously, this species was inhabited the eastern 35 U.S. States from Canada to Texas but it was listed as a federally endangered species in 1989 with remaining distribution in only six U.S. States (Jenkins et al., 2018). In the Czech Republic, there are three vulnerable species of genus *Nicrophorus*, such as *Nicrophorus antennatus* (Reitter 1885), *Nicrophorus germanicus* (Linnaeus 1758) and *Nicrophorus vestigator* Herschel 1807 and one near threatened species (*Nicrophorus sepultor* Charpentier 1825) (Růžička & Jakubec, 2017).

Regarding the species of the subfamily Silphinae listed in the Czech Red list, there are three vulnerable species (*Ablattaria laevigata* (Fabricius 1775), *Aclypea undata* (O. F. Müller 1776), *Silpha tyrolensis* Laicharting 1781) and one regionally extinct (*Thanatophilus dispar* (Herbst 1793)) (Růžička & Jakubec, 2017). Data on species *Aclypea souverbii* (Fairmaire, 1848), which was considered regionally extinct, is deficient (Růžička & Jakubec, 2017).

3.3. Biology and ecology
3.3.1. Morphology of beetles

Members of the family Silphidae are very variable in their shapes and sizes. Adults are dorsoventrally flattened beetles, which have the largest body among staphylinoids (Sikes, 2005). In general, they are medium or large beetles and their length ranges from 7 to 45 mm (usually from 12 to 20 mm) (Ratcliffe, 1996; Sikes, 2005). Carrion beetles have 11 antennomeres, although *Nicrophorus* species have a greatly reduced pedicel that is fused to the scape making them appear to have a 10 antennomeres (Sikes, 2005). Members of the Silphinae do not have a frontoclypeal (epistomal) suture (Sikes, 2005). In turn, beetles in the subfamily Nicrophorinae have a pair of stridulatory files on the 5th abdominal tergite (just under the apex of the elytra) (Sikes, 2005). Silphids are usually dark in color, but some of them (e.g., *Necrophila* Kirby & Spence 1828 sensu lato and *Nicrophorus*) have bright orange coloring on their prothorax, which may be a warning to other predators (Ratcliffe, 1996).

The larvae of Silphidae are flat and mobile (Newton, 1991). They can be characterized as follows: mandible without a molar lobe; maxilla with broad, apically cleft mala bearing dense setal brush on outer lobe; and articulated urogomphi present and usually 2-segmented (Newton, 1991). Mature larvae are large enough: about 12 to 40 mm long (Newton, 1991). But it is necessary to notice that the larvae differ between the subfamilies, with larvae of Silphinae bearing a cluster of 6 stemmata (ocelli) on each side of the head, and larvae of Nicrophorinae bearing only a single stemma on each side (Sikes, 2005). Moreover, the silphine larvae are strongly and widely sclerotized while the nicrophorine larvae (especially *Nicrophorus*) are weakly and sparsely sclerotized (Sikes, 2005).

3.3.2. Parental care

The reproductive biology of carrion beetles is divided into two types: the ancestral condition of no parental care in which eggs are deposited and abandoned on or the soil around primarily large (> 300 g) carcasses (Silphinae) and the derived condition in which small carcasses (< 100 g) are buried and guarded within a brood chamber with biparental care provided throughout larval development (*Nicrophorus*) (Sikes, 2005).

It is likely that parental care dates back to the Early Cretaceous and it has to do with competition between Silphids and flies and ants (Cai et al., 2014). One example is the burying beetle, *Nicrophorus orbicollis* Say 1825, which normally exhibits biparental care (Smith et al., 2017). Despite this fact, however, each parent of this species is capable of rearing offspring alone (Scott, 1989). The members of Silphinae, in contrast to the *Nicrophorus*, have no parental care. Adult beetles and larvae of Silphinae are usually noted feeding in groups at a larger carcass, often at various decomposition stages, with no maternal care (Ratcliffe, 1996). The larvae of Silphinae feed on the carrion themselves until development is complete (Pukowski, 1933; Newton, 1991).

3.3.3. Feeding preferences

Feeding habits of the family Silphidae are widely varied. For the most taxa, silphids are necrophagous as adult and larvae (Sikes, 2005). Most flight-capable species in the family Silphinae feed primarily on vertebrate carcasses, although flightless or flight-dimorphic species feed primarily on soil invertebrates which means that they are not fully necrophagous but rather predatory (Ikeda et al., 2007). In case a carcass is infested with maggots of flies, members of the family Silphidae will feed on these maggots (Byrd & Castner, 2009). As decomposition proceeds, silphid larvae become the predominant taxon on the corpse and feed on the remaining skin, connective tissue, sinew and flesh by scraping and gnawing with their mandibles (Ratcliffe, 1996). In this case, we are talking about actually necrophagous beetles which include the subfamily Nicrophorinae entirely, and the subfamily Silphinae, in part: Necrodini and some genera of Silphini (*Necrophila* Kirby & Spence 1828, *Oiceoptoma* Leach 1815, *Oxelytrum* Gistel 1848, *Ptomaphila* Kirby & Spence 1828, *Thanatophilus* Leach 1815) (Dekeirsschieter et al., 2011). But for example, *Phosphuga* Leach 1817, *Ablattaria* Reitter 1884 and *Dendroxena* Motschulsky 1858 are predators of small invertebrates (Newton, 1991; Sikes, 2005). *Aclypea* Reitter 1885 is phytophagous, however, in some cases, can be pestiferous on plants (*Beta vulgaris*) (Newton, 1991; Sikes, 2005). The members of the family Silphidae render the basic ecosystem function of contributing to the breakdown and recycling of organic matter within the terrestrial ecosystem and their feeding activity on carrion may also destroy some pathogenic bacteria (Peck, 2001; Sikes, 2008).

3.3.4. The burial of carrion

Burying beetles are named so because the adults of most species of this subfamily bury a dead body of small vertebrate, upon which their larvae later on feed. The adult beetles of *Nicrophorus* species find a small dead animals and bury them (Pukowski, 1933; Newton, 1991). The *Nicrophorus* have a keen sense of smell for a decomposing corpse. If they smell the dead animal, they can fly several kilometres away (Petruška, 1975). To do that they use specialized chemoreceptors on their antennae, which are adapted to detect carcasses (Ernst, 1969; 1972; Evans & Hogue, 2004). Through sensiles located apically on the ultimate palpomeres, they detect carcasses at small distances (McGavin & Lewington, 2001). They may find carcasses at a long distance and are one of the first invertebrates to colonize at a fresh corpse. In *Nicrophorus* all species bury carcasses; moreover, in some species a few individuals will work together in the burial, this is called "joint nesting" or "communal breeding" (Eggert & Müller, 2000), and then fight to decide which one will lay her eggs on the dead body. A significant fact is that burying beetles are unable to burying a whole human corpse but can inter bits if it has been dismembered (Gunn, 2009). In addition, they can chew out hunks of tissue, which are then buried (Gunn, 2009). After burying the dead body or body part, the female beetle lays her eggs above the corpse and then covers it with her hind gut secretions to inhibit the growth of bacteria and fungi (Pukowski, 1933).

4. FORENSIC ENTOMOLOGY
4.1. General description

Currently, forensic entomology is a rather forward-looking and a rapidly developing area of science (Midgley et al., 2010). This branch of science is accepted in many countries as a significant tool for legal investigations (Sharma et al., 2015), but it is primarily related to death enquiries (Grisendi et al., 2015). The postmortem time interval (PMI) could be interpreted as a time since death or the length of time between death and the discovery of a corpse (Richards et al., 2008). Proper evaluation of the post mortem interval is one of the most crucial aspects of legal medicine (Sharma et al., 2015). The most significant kind of forensic entomology is used in the investigation of death, abuse and neglect cases (Benecke & Lessig, 2001), including cases of homicide, suicide, or accidental death (Amendt et al., 2011).

In The Washing Away of Wrongs, the first documented forensic entomology case is indicated (Benecke, 2001). And this case is dated 1235 A.D. (Benecke, 2001). However, one of the first contemporaries who used forensic entomology to estimate time since death was the French doctor Bergeret in the 19th century (Sharma et al., 2015). And one of the first successful cases of the use of forensic entomology was the case of murderer Buck Ruxton in 1935 in the UK (Lee Goff, 2000).

Date of death can be determined by the rate of development of insects found on a corpse (Buchan & Anderson, 2001). The ages of insect immature stages found on a corpse can allow proof for the estimation of a minimum PMI (PMImin) ranging from 1 day up to more than 1 month, according to the insect species engaged and the climatic conditions at the death scene (Grisendi et al., 2015). So for instance, the age of the oldest immature insect specimen collected from a dead body, under of expected arrival time of adult females, lends a pointer of the minimum time that the decomposing remains were accessible for insect colonization and therefore PMImin (Matuszewski et al., 2016).

4.2. The stages of decomposition

The colonization time, development time, and departure time of the various insect species occupying carcass are closely connected to the progression of carcass decomposition (Fuller, 1934). Different groups of insects colonize a corpse not at the same time. Normally, insects having the shortest development period colonize a corpse first and species with longer development period colonize it later (Chaika, 2003). For the first time, a more or less predictable chronological sequence, known as insect succession, was proposed by Mégnin (1894). Based on current research the decomposition process can be divided into five stages related to physical appearance of carcasses, internal temperatures and characteristic insect populations (Joseph et al., 2011):

1. Fresh stage (Days 1-2), which begins at the moment of death and ends when the bloating of the carcass is observed. The first insects to arrive are usually Calliphoridae (blow flies), Sarcophagidae (flesh flies), Muscidae and adult ants may also be seen at a carcass during the fresh stage.

2. Bloated stage (Days 2-7), which is characterized by the beginning of putrefaction and the greatest numbers of adult Diptera on the carcasses. Ants stay to feed on the eggs and larvae of flies (Grassberger & Frank, 2004). And the first species of Coleoptera arrive during this stage, including members of the families Staphylinidae, Silphidae, Cleridae and Histeridae (Grassberger & Frank, 2004).

3. Decay stage (Days 5-13), in which a conversion of carcass biomass to dipteran larval biomass. The larvae subsequently depart from the carcass to pupate. Feeding larvae of Calliphoridae are the main insect group, but adult of Calliphoridae and Muscidae decrease in numbers in this stage (Payne, 1965; Anderson & Van Laerhoven, 1996). Coleoptera becomes dominant adult insects. Also, the first members of Sepsidae arrive during the decay stage.

4. Post-decay stage (Days 10-23), which begins when most of the Diptera larvae leave the carcass, leaving behind bones, cartilage, hair, small portions of tissue, and a large amount of wet, viscous material known as byproducts of decay (BOD). Dermestidae arrives at the carcass (Grassberger & Frank, 2004).

5. Remains stage (Days 18-90+), which characterized by bones with little cartilage remaining and the BOD has dried up. A few adults of Calliphoridae are attracted to the carcass (Payne, 1965) and Piophilidae emerges (Anderson & Van Laerhoven, 1996). Members of Dermestidae leave the carcass.

4.3. Characteristics of insects used in forensic entomology

Decomposing carcass submits a transitory habitat and food resource chance for many insect species (Wells & Greenberg, 1992). Some species of insects play an indispensable ecological role in the decomposition of organic matter. Smith (1986) presented four distinct ecological categories of insects that may be found on corpses:

1. Necrophagous species which feed on the dead tissue;
2. Predators and parasites of insects and other arthropods;
3. Omnivorous species which are not obligate necrophages, but will use the corpse as a food source if available;
4. Adventive species which are specific to the habitat of the scene of a crime and merely use the body as an extension of that habitat, e.g., for cover.

Forensic entomologists primarily use the first two groups of insects for estimating the PMImin (Amendt et al., 2011).

4.3.1. Diptera

Related to the assumption that Diptera locates corpses faster and may provide the better estimate of minimum Post Mortem Interval (PMImin), the majority of studies in forensic entomology is concentrated on flies. Typically, the most common arthropod inhabitants of decomposing human casrcasses and animal carrion are the larvae of flies (Diptera), especially the following families: Calliphoridae, Sarcophagidae, Muscidae and Piophilidae (Smith, 1986). Blowflies have the greatest role in the early decomposition process. The members of families Calliphoridae and Sarcophagidae are the first colonizers on human dead body (Smith, 1986; Erhan & Beyarslan, 2013). Experimental studies indicated that members of dipteran families Calliphoridae are attracted to the carrion in the first minutes (Watson & Carlton 2003). For this reason, they are the basic and most correct forensic indicators of time of death and their development rates are needed to allow more precise PMI estimates (Grassberger & Reiter, 2001).

4.3.2. Beetles

Silphidae and other beetle families (such as Dermestidae, Trogidae, Histeridae, Nitidulidae, Staphylinidae etc.) are important for forensic entomology, since finding their larvae or imago on the corpse can help to define the postmortem time interval (PMI) and possible manners of death (Watson & Carlton, 2005). It is known that there are completed thermal summation models for some members of the family Silphidae. For example, Guerroudj and Berchi (2016) studied the life cycle of the carrion beetle *Thanatophilus ruficoruis* (Küster 1851) and this information would be useful to determine the postmortem interval (PMI) in Algeria (Guerroudj & Berchi, 2016). It should be noted that species *Silpha rugosa* (Linnaeus 1758) appears in their article. This species shall be named as *Thanatophilus rugosus* (Linnaeus 1758). However, the main mistake that the species was wrongly identified. Their study dealt with the above-mentioned *Thanatophilus ruficoruis* (Küster 1851). The life cycle of *Oxelytrum*

discicolle (Brullé 1840) was also studied. And this information could be used in forensic entomology in Venezuela and the Neotropical region (Velásquez & Viloria, 2009). Another example is a model for estimating post-mortem interval for *Thanatophilus micans* (Fabricius 1794), which is the most widespread species of the Silphidae in Africa (Midgley & Villet, 2009). Recent observations (Midgley & Villet, 2009) indicate that *Thanatophilus micans* can detect corpses start breeding within 24 h of death. Accordingly, it follows that *Thanatophilus micans* has the same potential utility of estimates as flies. In addition, members of the family Silphidae can be found in shallow or very shallow graves (Amendt et al., 2010) and they can be useful to estimate PMImin not only during early decomposition, but also in later stages of decomposition (Midgley & Villet, 2009). They are present in later stages of decomposition because they have a much longer larval development than flies (Midgley & Villet, 2009).

4.4. Estimating the minimum post-mortem interval

As stated above, the post-mortem interval (PMI) represents the time between the death and finding of a corpse (Catts, 1992). It should be noted that there are a few natural processes related to decomposition, such as rigour mortis or livor mortis, that can be used to estimate the PMI (Campobasso et al., 2001). Rigour mortis is the process that incites the muscles in the body to stiffen thereby in rigidity due to a range of chemical changes in the muscle structure (Rattenbury, 2018). Livor mortis is the gravitational pooling of blood in the dependent parts of the body, both externally in the skin capillaries and venules but also in the internal organs (Hayman & Oxenham, 2016) and it begins right away after the cessation of blood circulation (Bisker & Ralebitso-Senior, 2018). However, many of these processes are reciprocal functions and immediately turn incorrect in the application (Bourel et al., 2003). Moreover, these processes are restricted to the first 72h after death (Campobasso et al., 2001). After 72h, the body starts to bloat, blood-containing foam leaks from the mouth and nose, the body ceases to regulate its internal temperature and the internal temperature begins to approximate the ambient temperature, and it can be difficult to obtain an estimate the minimum time since death by traditional methods (Amendt et al., 2011). At the same time, since that 72h and later, insects can be useful for estimating the minimum

time since death (Amendt et al., 2011). Decomposition as a result of insect activity in and on the corpse is an ongoing process that can be measured, and it permits correct minimum PMI estimates during a few months after death (Campobasso et al., 2001). Forensics often have to resort to the methodology on estimates of retrospective determination of the beginning of insect development on a corpse (Marchenko, 1987). Specially for this purpose larvae extracted from a dead body are grown to imago stage at a constant temperature and in appropriate conditions. It offers the opportunity to identify pace of their development. And furthermore, there are methods for converting data on the rate of development of particular species from data obtained at one temperature into the data with other temperatures. The insect development rate primarily depends on temperature and differs between different species (Richards et al., 2009). It is, therefore, necessary to resort to a three-phase process (Amendt et al., 2011):

1. Clear identification of the species found on a corpse and their instar;
2. Reconstructing crime scene temperatures;
3. Modelling the developmental rate of the immature insects found on a corpse.

Let's look at every phase in detail. With regard to identification of the species found on a corpse, it is to be noted that this procedure should always be carried out by a specialist in insect taxonomy. Identification keys on forensically important insects can be found in Zumpt (1965), Smith (1986) and Szpila (2010). Modelling of crime scene temperatures is as follows: first and foremost, a data of ambient temperatures is recorded from the closest weather station to the body discovery site and at the crime scene for a few days, and then a regression relationship between these temperatures is derived (Amendt et al., 2007). That is because there may be a serious difference between the ambient temperatures of the weather station and the crime scene, for example in relation to experience different exposure to identical weather conditions (Archer, 2004). As a result, a derived equation is used to correct the weather station temperature records to ambient site temperature for the period that corpse was thought to be at the crime scene (Archer, 2004). The final stage is modelling insect development and ultimately an estimate of PMImin using all data received. Developmental data are data indicating the development duration of immature stages notified at various temperatures (Amendt et al., 2011). Then these data summarized in one or more of three developmental models, that is isomorphen diagrams (Grassberger

& Reiter, 2001), isomegalen diagrams (Reiter, 1984) and thermal summation models which are the most sophisticated of the three specified models (Amendt et al., 2011). In isomegalen or isomorphen diagrams, the lengths and the developmental stage of the larvae are combined as a function of time and mean ambient temperature in a single diagram (Madea et al., 2014). This method is appropriate only in a case when the body and the larvae were not undergoing fluctuating temperatures, that is, the temperature was nearly constant (Madea et al., 2014).

5. THERMAL SUMMATION MODELS
5.1. Degree days/hours (ADD/ADH)

Scientists were always interested in a link between ambient temperature and the development of exothermic organisms. As a result of his research, the French entomologist Réaumur (1735, 1736) came to the conclusion that the development of organisms stops at certain temperatures. It is called a threshold temperature and no development occurs when temperatures are below that level. He have also discovered that there is a constant sum of temperatures necessary for further development. It is a degree days, sometimes called heat units. The degree day or hour theory suppose that the developmental rate is proportional to the temperature within a certain species-specific temperature range (Sharma et al., 2015). The relationship between temperature and the development rate is normally curvilinear at high and low temperatures and linear only in between (Sharma et al., 2015). The threshold and maximum temperatures for development of an insect are used to calculate the number of degree days for a specific day. One degree day results when the average temperature for a day is one degree over the threshold temperature. The easiest way to calculate degree days for a specific date is the standard method using simple averaging (Fraisse & Paula-moraes, 2018).

One of the methods of calculating the post mortem interval is an estimation of the accumulated degree days or hours (ADD or ADH). ADH values constitute a particular number of "energy hours" that are required for the development of insect larvae (Sharma et al., 2015).

The formula for calculating ADH is as follows (Higley et al., 2006):

$$ADH = T \cdot (\theta - \theta°)$$

where T is the development time, θ is the ambient temperature, $\theta°$ is the lower developmental threshold, a species specific value, the so-called development zero, which is the x intercept, and is calculated by the linear approximation method.

But it must be noted that the ADH method offers suitable results solely when the larvae have been exposed to temperatures similar to those used for generating the reference value applied in the post mortem interval calculation (Sharma et al., 2015). And furthermore, application of ADH in post mortem interval estimations has imperfections, especially during the winter period where low temperatures are implicated or where there is an abrupt summer cold period during the development time (Sharma et al., 2015).

5.2. Relation of temperature to the duration of the insect development

Insects are exotermal animals and temperature plays a major role in their growth and development. One of the most important forms of dependence of ectothermal organisms on the thermal habitat conditions is the effect of temperature on the growth rate and duration of development. This form of the phenotypic plasticity is characterized by the temperature reaction norms (Groeters, 1992). Phenotypic plasticity can be defined as the capacity of an individual organism to alter its behavior in direct response to changing environmental conditions (Pfennig & Levis, 2017).

The sum of effective temperatures is used for assessing the impact of temperature on insect development. The count is conducted from the pre-calculated values of the lower developmental threshold. The development is inhibited at lower temperatures. The duration of development decreases with rising temperature and the rate of development increases within the tolerance limits for each species (Zakchvatkin, 2001). It is submitted that, insect development occurs faster at higher temperatures, because higher temperatures increase the metabolic rate (Boukal et al., 2015). There is also maximum temperature above which development stops. However, it should be noted that the development of each insect species can occur only in a limited temperature range (Dixon et al., 2009).

14

Within temperatures that are favorable for vital activity, the duration of insect development and other ectothermal organisms has approximately hyperbolic dependence on temperature (Campbell et al., 1975). The main equation (Blunck, 1923) is as follows:
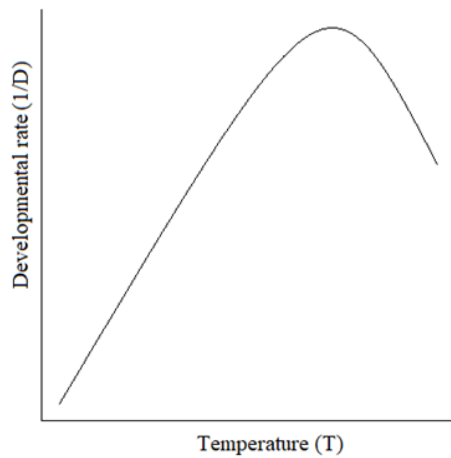
$$D = \frac{K}{T - T_0}$$

where D is the duration of development in days (hours, etc.), T is the temperature at which development occurs, T_0 is the lower developmental threshold, and K is the thermal summation constant which displays the sum of effective temperature. The equation actually reflects the temperature norm of the reaction of individuals of the species, population, family, etc., over the duration of development. Under it, the duration of development hyperbolically increases with decreasing temperature and becomes infinitely large when the temperature reaches T_0. Consequently, the development does not occur at the lower developmental threshold.

5.3. Thermal summation models

Driven by technological advances, various new methods have been developed by scientists that allow the data to be used with confidence while estimating the time since death. There are many methods for estimation of minimum post-mortem interval (PMImin) at the present time. One of these methods are thermal summation models. It is the most common method used to calculate PMI (Richards et al., 2008). This model is built on the theory that the development of immature stages is linear (Pedigo et al., 1986) and a relationship between temperature and development are positive (Amendt et al., 2011). Thermal summation models are able to handle size and developmental data and temperature fluctuations (Amendt et al., 2011). Insect development can be calculated at close intervals over a temperature range, and where the development rate (calculated as reciprocals of development time, 1/D) is plotted contrary to temperature, a sigmoid-shaped (Fig.1) curve arises (Wagner et al., 1984).

At temperature extremes, insect development is slowed or entirely stopped in accordance with an upper and lower developmental threshold (Campbell et al., 1974). As a rule, the correlation between temperature and development is linear between the lower developmental threshold and optimum temperature (Harvey et al., 2016). In this

regard, it could be concluded that linear regression may be used to decide an x-intercept (lower developmental threshold, TL) and an inverse of the slope of the linear regression (thermal summation constant, K) which permit prognosis of development time from the thermal history of a specimen (Amendt et al., 2011). Therefore, if the development rate (R) is defined as the feedback values of duration (R = 1/D), then the above main equation can be converted to a linear form (Kipyatkov & Lopatina, 2010):

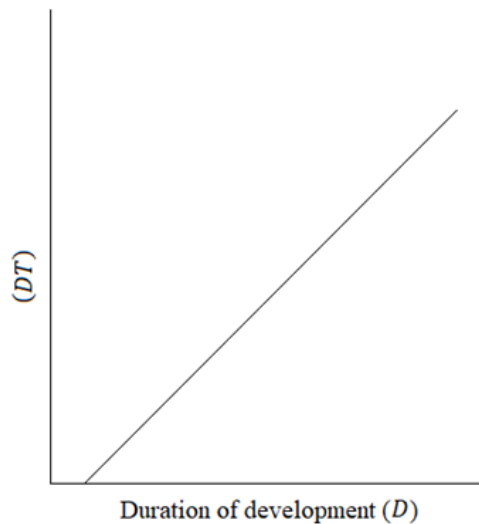**Figure 1:** A generalized insect developmental rate curve

$$R = a + bT$$

where a is a constant and b is a linear regression coefficient of the development rate to temperature. The graph of the equation (Fig. 2) shows the temperature norm of the reaction of individuals of a population, family, species etc., according to the development rate. It is a straight line that intersects the *x*-axis (the abscissa) at T_0. Thus, the lower developmental threshold is temperature at which development rate is zero.

In this linear regression model, development is quantified as physiological time with units of
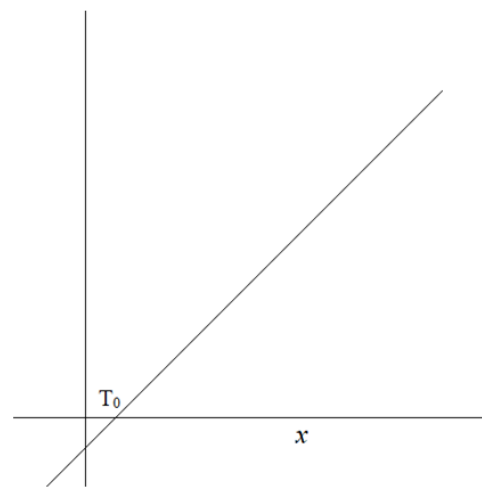
Figure 2: The graph of the linear equation (R = a + bT)

"degree days" or "degree hours", where one degree day is equivalent to one degree above the lower developmental threshold over 24 hours or 1 hour, accordingly (Harvey et al., 2016). Every stage in the life cycle (egg, first, second and third instar, or pupa) need a specific number of accumulated degree hours to move to the next phase and finished development equating to K (Campbell et al., 1974).

The major concern is linear models do not include the nonlinearity experienced in insect development at low and high temperatures, although they have the advantage of mathematical simplicity and permit estimation of lower developmental thresholds and thermal summation constants (Briere et al., 1999). A big difference between the upper and lower developmental temperature extremes is endangering the accuracy of the estimation PMImin (Amendt et al., 2011).

Figure 3: The graph of a new linearized formula $((DT) = k + tD)$

But there is a revised regression model submitted by Ikemoto & Takai (2000) which identifies the point nearest the extremes of the linear approximation that deviate significantly from it, which results in the calculation of more precise thermal summation parameters. Ikemoto & Takai (2000) proposed a new linearized formula and a method of estimation of parameters, which is based on a revision of the main drawbacks in the ordinary method. A new linearized formula is as follows (Ikemoto & Takai, 2000):

$$(DT) = k + tD$$

where D is the duration of development in days, T is a temperature in Celsius degrees, k is an effective cumulative temperature and t is an estimated developmental zero temperature. This equation (Fig. 3) looks like a straight line with x = D and y = DT (Ikemoto & Takai, 2000).

5.4. Strengths and weaknesses of the thermal summation models

Widespread use of the linear model is determined by what the dependence of the development rate on temperature for many insect species practically does not differ from linear in a favourable for the development temperatures (Campbell et al., 1975).

The main advantages of a linear model (Kipyatkov & Lopatina, 2010):

1. Mathematical simplicity;
2. Ease of calculating all parameters using regression analysis;
3. It is not necessary to conduct experiments at many temperatures, especially too low or high (3–5 values are enough within the optimal zone);
4. Ability to calculate statistical errors for all basic parameters;
5. Understandable adaptive ecological meaning of thermal constants;
6. Existence of the materials for comparative studies (currently accumulated data on hundreds of insect species).

Through these and other features, a linear model is used very broadly in studies on the ecology of insects (Campbell et al., 1975). The rule of the sum of effective temperatures is appropriate in most cases (Ceryngier & Hodek, 1996).

The use of a linear model and thermal constants in comparative studies of the insect life cycles is particularly promising. The regression coefficient of the development rate by temperature and return value (the sum of degree-days) allow comparison of species according to their thermal requirements and the degree of thermal lability (Kipyatkov & Lopatina, 2010).

A major deficiency of a linear model is the inability to identify unambiguously the range of temperatures within which this model is applicable (Kipyatkov & Lopatina, 2010). If the analysis includes too low or too high temperatures, at which the dependence of species significantly deviates from the linear, it leads to an underestimation of the threshold values and the regression coefficient (Wermelinger & Seifert, 1998). Based on the analysis of the large body of literature on these subjects Honěk (1990) showed that the lower the temperatures used in the experiment, the lower the values of the threshold and coefficient of regression are received by calculations for the same species. Accordingly, the compliance of an amount of data with the requirements of linearity should be strictly monitored when experiments are conducting and results are processing (Sokal & Rohlf, 2013).

6. PRACTICAL PART

6.1. Diamesus

6.1.1. Taxonomic position

The following classification was taken from (Newton, 2019)

Order: Coleoptera

    Suborder: Polyphaga

        Infraorder: Staphyliniformia

            Superfamily: Staphylinoidea

                Family: Silphidae

                    Subfamily: Silphinae

                        Genus: *Diamesus* Hope 1840

                            Species: *Diamesus bimaculatus* Portevin 1914

                            Species: *Diamesus osculans* (Vigors 1825)

6.1.2. Taxonomy

The genus *Diamesus* includes two species: widely distributed *Diamesus osculans* and endemic *Diamesus bimaculatus* Portevin 1914, which is restricted only to Taiwan (Růžička et al., 2015; J. Růžička, unpubl.).

6.1.3. Distribution

Diamesus osculans, which was used in a laboratory study, is very widely distributed from India, Sri Lanka, Bhutan, Myanmar, Vietnam, Laos, China, Malaysia, Indonesia, New Guinea, New Zealand to eastern Australia (Peck, 2001; Růžička et al., 2002). It is supposed that *Diamesus osculans* entered northern Australia through New Guinea and the Indo-Malayan Island chains (Peck, 2001).

6.1.4. Appearance

Diamesus osculans is a fairly large beetle whose size varies from 30 to 45 mm (Tung, 1983; Lawrence & Slipinski, 2013). As a rule, they have a black or sometimes dark brown colouring with reddish marking (Tung, 1983; Lawrence & Slipinski, 2013). The elytra are short with two to four pink spots (which typically fade away in time), and a few abdominal segments are often left uncovered (Tung, 1983). The body is somewhat flattened and the hind legs are strong and muscular (Tung, 1983). There is also sexual dimorphism: the female is duller brown and generally smaller in size than male, and besides hind femora are more explained in males (especially in large specimens), there are simple in females (Tung, 1983; J. Růžička, unpubl.).

The larvae are broad and flat (Hatch, 1927). The obvious problem is the larvae of Silphinae are sometimes difficult to differentiate. Some larvae, however, have been described and compared among themselves. As examples, larva of *Diamesus osculans*, as opposed to the larva of *Necrodes littoralis* (Linnaeus 1758), has a more compact and more flattened body, relatively larger and more robust head, shorter and more robust legs, round lighter spots on laterotergites of abdominal segments and only short praescutum and scutellum on the dorsum of abdominal segments (J. Růžička, unpubl.).

6.1.5. Ecology

The genus *Diamesus* occurs in a wide spectrum of habitats: pastures, open and closed canopy forests, rainforests (Peck, 2001). An interesting feature is that *Diamesus osculans* is a pollinator of *Amorphophallus titanum* that grows at Indonesia (Davis et al., 2008). *Amorphophallus titanum* emits a rotting animal-like odour and for this reason, it attracts these beetles (Davis et al., 2008). And it is supposed that *Diamesus osculans* buries corpses of small animals by digging underneath them, burrows under or near the buried carcass and prepares a chamber where it lays its eggs (Hangay & Zborowski, 2010). This species prefers large carcasses, usually far too big to be buried (Hangay & Zborowski, 2010). Time span from egg to adult beetle is 38–40 days, longest life stage is the 3rd larval instar, that has lasted three weeks (J. Růžička, unpubl.).

6.2. Material and methods

Adults of *Diamesus osculans* were collected in 23–27 February of 2017 in the Philippines (Mindanao Island, Davao City, Marilog District, Malambo Range, Busay Resort, 07°29.0′N, 125°15.7′E, 1200 m) by P. Šípek and D. Vondráček. Beetles were collected using UV+white light traps, which were installed in the montane forest.

Adult beetles were randomly assigned to breeding groups so that both female and male were present in the box. These groups were formed to produce offspring needed for further research. Adult and larvae specimens were kept in plastic boxes with a layer of soil and a small piece of fish meat (*Pleuronectes platessa* or *Cyprinus carpio*) as a food source. Food was regularly replaced to prevent the outbreak of the mould. The soil was tentatively disinfected and then was lightly sprayed with tap water every day for soil moisture conservation. Disinfection of the soil was carried out under the influence of high temperatures in the furnace.

Figure 4: Female and male of *Diamesus osculans* in the box

Boxes with adult and larvae specimens were evenly distributed between the climatic chambers at constant temperature (20, 22 and 24 °C) and 12 h of light and 12 h of dark photoperiod regime. Eggs were very small and were buried in the substrate, where adult specimens were based. It is precisely for this reason that it was difficult to locate and documented new eggs. As a result, the estimation of eggs development was not used in the models. Development of larvae specimens was documented every day and if it was possible at the same time.

Parameters of thermal summation model were estimated for L1 using the major axis regression method by Ikemoto & Takai (2000) mentioned in chapter 5.3. Data management and all analysis were completed using the R statistical program.

6.3. Results

In total, 16 adult specimens of *Diamesus osculans* were at the beginning of the experiment, and they produced 180 first instar larvae. In the breeding experiment, the duration of development of three larval instars (L1, L2 and L3) was registered. A number of larvae specimens in different stages of development and constant temperatures is given in Table 1. At the beginning of the experiment, we had 180 first instar larvae. Eighty of them completed the first larval stage and only eighteen completed the second larval stage. As can be seen in Table 1, the largest number of larvae were at 24 °C and the lowest number at 20 °C.

Table 1: Number of larvae specimens of *Diamesus osculans*

TEMPERATURE [°C]	L1	L2	L3
20	13	1	0
22	53	37	8
24	114	42	10
TOTAL NUMBER	**180**	**80**	**18**

Figure 5: L1 of *Diamesus osculans* in a Petri dish

Unfortunately, none of the observed larvae reached a pupal stage. It may be due to a nematode infection that arose in the laboratory during the L3 instar larvae. Moreover, the high mortality rate has been detected at the end of the experiment. In addition to the above nematode infection, the number of Acari increased significantly and food and the substrate were affected by mould. This could be caused by an unknown disease outbreak. However, the exact cause was uncertain. For this reason, data relating to the interval from the beginning of the experiment to the end of the second stage of larval development have been aggregated and used in the analysis, and all the rest of the data were not used.

The following table (Table 2) shows the sum of effective temperatures (k) and lower developmental threshold (t) values, which were calculated for L1 of *Diamesus osculans* with expected errors, it also includes coefficient of determination (R^2), degrees of freedom (Df) and p value.

Table 2: Summary of development constants for *Diamesus osculans* for L1

Stage	R^2	Df	p value	k	t
L1	0.9895	43	< 0.001	19.7±0.306	7.5±0.940

The graph (Fig. 6) shows the major axis regression for the first larval stage. The black line demonstrates the median and grey area around is a standard error. Black points illustrate outliers. DT is the time in days to reach the stage multiplied by the constant-rearing temperature.
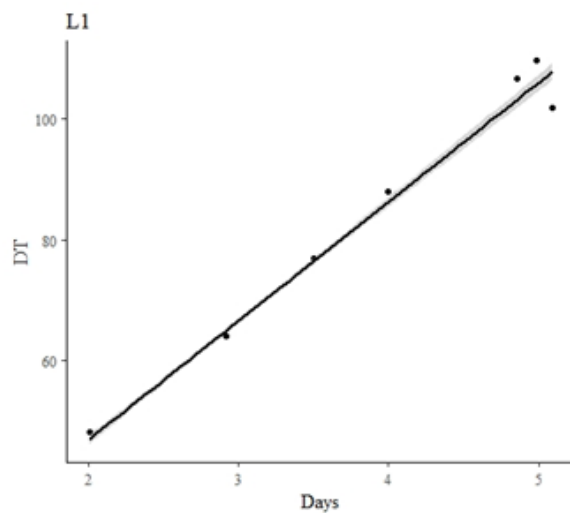
Figure 6: Major axis regression for L1 in *Diamesus osculans*

7. DISCUSSION

Mortality of the larvae was very high during the entire experiment, especially in the third instar. That could be attributed to a number of causes, the most obvious of which are a nematode infection, the substrate affected by mould and a significant increase in the number of Acari at the end of the experiment. The largest number of larvae were at 24 °C and the lowest number at 20 °C. This can be explained by the origin of this

genus and the preference for a warmer tropical climate similar to their natural habitat. However, the exact cause is uncertain.

J. Růžička, H. Šípková and D. S. Sikes (unpublished) conducted a similar laboratory experiment, which was focused on *Diamesus osculans*, and noted that best results were obtained in the case larvae are reared together on a large piece of decaying fish meat, from first larval instar to mature larva. The same authors also noted that contamination with Nematoda was observed on carrion and larvae in most cases.

Such studies were already conducted on other species of the family Silphidae and other insect families. The results of this study are consistent with prior studies. The results of such studies also indicate influence of temperature on larval development rate. Velásquez &Viloria (2009) state that temperature increase leads to a decrease in total time needed to complete the development of *Oxelytrum discicolle* (Brullé 1836).

Other authors, like Kipyatkov & Lopatina (2002), explored ant populations. They indicated that temperature increase always shortened development time of all the populations studied. Anderson (2000) conducted research on determining the minimum and maximum developmental rates of some species of forensically significant blow flies of the family Calliphoridae. As a result, development has been faster at a higher temperature (Anderson, 2000).

8. CONCLUSIONS

The central topic addressed in my thesis is the thermal summation models in selected species of Silphinae (Coleoptera). It is usually beneficial to learn and then write a literature review about important theoretical topics related to the main subject of the thesis. When I was writing a theoretical part, one of the problems that I faced was the lack of information regarding the subfamily of interest to me, particularly as regards the genus *Diamesus*. As a rule, the relevant information about the subfamily Nicrophorinae was available in large number of publication than information about the subfamily Silphinae.

In the practical part, I have reviewed the species *Diamesus osculans* by providing all available information to date. In addition, there is a description of the laboratory

investigation, including the methodology of the work. Thermal summation model for the first instar of *Diamesus osculans* was created as a result of the experiment.

The necrophagous beetles in general, and the species *Diamesus osculans* examined by me in more detail, in particular, are extremely interesting for research. Very regrettably, the necrophagous beetles are underestimated by forensic entomologists, and in the past in studies and research on the issue, they have not received enough attention. First, there are difficulties in identifying these beetles and, secondly, there is an acute problem that is the lack of insufficient information about their ecology and development. But, fortunately, the situation is gradually changing and scientists are beginning to direct their attention to this topic. I am pleased to see that this gap can be eliminated. It will, however, take time, and a combined effort of many scientists.

9. REFERENCES

AMENDT, J., CAMPOBASSO, C. P., GAUDRY, E., REITER, C., LE BLANC, H. N., & HALL, M. J. R. 2007: Best practice in forensic entomology – standards and guidelines. *International Journal of Legal Medicine* **121 (2)**: 90–104.

AMENDT, J., CAMPOBASSO, C. P., GOFF, M. L., & GRASSBERGER, M. 2014: *Current Concepts in Forensic Entomology*. Springer, Dordrecht, 376 pp.

AMENDT, J., RICHARDS, C. S., CAMPOBASSO, C. P., ZEHNER, R., & HALL, M. J. R. 2011: Forensic entomology: applications and limitations. *Forensic Science, Medicine and Pathology* **7 (4)**: 379–392.

ANDERSON, G. S. 2000: Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *Journal of Forensic Sciences* **45 (4)**: 14778J.

ANDERSON, G. S., & VAN LAERHOVEN, S. L. 1996: Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences* **41 (4)**: 617–625.

ANDERSON, R. S., & PECK, S. B. 1985: *The Insects and Arachnids of Canada, Part 13. The carrion beetles of Canada and Alaska: Coleoptera: Silphidae and Agyrtidae.* Agriculture Canada, Ottawa, 126 pp.

ARCHER, M. S. 2004: The effect of time after body discovery on the accuracy of retrospective weather station ambient temperature corrections in forensic entomology. *Journal of Forensic Sciences* **49 (3)***:* 553–559.

BENECKE, M. 2001: A brief history of forensic entomology. *Forensic Science International* **120 (1-2)**: 2–14.

BENECKE, M., & LESSING, R. 2001: Child neglect and forensic entomology. *Forensic Science International* **120**: 155–159.

BLUNCK, H. 1923: Zur Kenntnis des "Breitrands" Dytiscus. *Zoologischen Anzeiger* **57**: 157–168.

BOUKAL, D. S., DITRICH, T., KUTCHEROV, D., SROKA, P., DUDOVÁ, P., & PAPÁČEK, M. 2015: Analyses of developmental rate isomorphy in ectotherms:

Introducing the dirichlet regression. *PLoS ONE* **10 (6)**: 1–16.

BOUREL, B., CALLET, B., HEDOUIN, V., & GOSSET, D. 2003: Flies eggs: a new method for the estimation of short-term post-mortem interval. *Forensic Science International* **135 (1)**: 27–34.

BRIERE, J. F., PRACROS, P., LE ROUX, A. Y., & PIERRE, J. S. 1999: A Novel Rate Model of Temperature-Dependent Development for Arthropods. *Environmental Entomology* **28**: 22–29.

BUCHAN, M., & ANDERSON, G. S. 2001: Time Since Death : A Review of the Current Status of Methods used in the Later Postmortem Interval. *Canadian Society of Forensic Science Journal* **34 (1)**: 1–22.

BYRD, J. H., & CASTNER, J. L. 2009: *Forensic Entomology: The Utility of Arthropods in Legal Investigations, Second Edition*. CRC Press, New York, 705 pp.

CAI, C. Y., THAYER, M. K., ENGEL, M. S., NEWTON, A. F., ORTEGA-BLANCO, J., WANG, B., WANG, X.D. HUANG, D.Y. 2014: Early origin of parental care in Mesozoic carrion beetles. *Proceedings of the National Academy of Sciences* **111 (39)**: 14170–14174.

CAMPBELL, A., FRAZER, B. D., GILBERT, N., GUTIERREZ, A. P., & MACKAUER, M. 1974: Temperature Requirements of Some Aphids and Their Parasites. *Journal of Applied Ecology* **11 (2)**: 431–438.

CAMPBELL, D., FOGEL, S., & LUSNAK, K. 1975: Mitotic chromosome loss in a disomic haploid of Saccharomyces cerevisiae. *Genetics* **79 (3)**: 383–396.

CAMPOBASSO, C. P., DI VELLA, G., & INTRONA, F. 2001: Factors affecting decomposition and Diptera colonization. *Forensic Science International* **120**: 18–27.

CATTS, E. P. 1992: Problems in Estimating the Postmortem Interval in Death Investigations. *Journal of Agricultural Entomology* **9**: 245–255.

CERYNGIER, P., & HODEK, I. 1996: Enemies of Coccinellidae. Pp. 319–350. In: HODEK, I., & HONĚK, A.: *Ecology of Coccinellidae. Series Entomologica, Vol. 54*. Springer, Dordrecht, 458 pp.

CHAIKA, S. Y. 2003: *Forensic entomology. A manual for the students.* MAX Press, Moscow, 60 pp (in Russian).

DAVIS, C. C., ENDRESS, P. K., & BAUM, D. A. 2008: The evolution of floral gigantism. *Current Opinion in Plant Biology* **11 (1)**: 49–57.

DEKEIRSSCHIETER, J., VERHEGGEN, F., LOGNAY, G., & HAUBUGE, E. 2011: Large carrion beetles (Coleoptera, Silphidae) in Western Europe: a review. *Biotechnology, Agronomy, Society and Environment* **15 (3)**: 435–447.

DIXON, A. F. G., HONĚK, A., KELI, P., KOTELA, M. A. A., ŠIYLING, A. L., & JAROŠÍK, V. 2009: Relationship between the minimum and maximum temperature thresholds for development in insects. *Functional Ecology* **23 (2)**: 257–264.

DOBLER, S., & MÜLLER, J. K. 2000: Resolving Phylogeny at the Family Level by Mitochondrial Cytochrome Oxidase Sequences: Phylogeny of Carrion Beetles (Coleoptera, Silphidae). *Molecular Phylogenetics and Evolution* **15 (3)**: 390–402.

EGGERT, A. K., & MÜLLER, J. K. 2000: Timing of oviposition and reproductive skew in cobreeding female burying beetles (Nicrophorus vespilloides). *Behavioral Ecology* **11 (4)**: 357–366.

ERHAN, Ç., & BEYARSLAN, A. 2013: Identification of dipteran species of forensic entomology importance in summer season in Edirne. *Bitlis Eren University Journal of Science and Technology* **3**: 8–21.

ERNST, K.-D. 1969: Die Feinstruktur von Rieehsensillen auf der Antenne des Aaskäfers Necrophorus (Coleoptera). *Zeitschrift für Zellforschung* **94**: 72–102.

ERNST, K.-D. 1972: Sensillum coelosphaericum, die Feinstruktur eines neuen olfaktorischen Sensillentyps. *Zeitschrift für Zellforschung* **132***: 95–106.

EVANS, A. V., & HOGUE, J. N. 2004: *Introduction to California Beetles.* University of California Press, California, 299 pp.

FRAISSE, C. W., & PAULA-MORALES, S. V. 2007: Degree-Days: Growing, Heating, and Cooling. http://edis.ifas.ufl.edu (accessed 18 February 2019).

FULLER, M. E. 1934: *The Insect Inhabitants of Carrion: A Study in Animal Ecology.*

H. J. Green, Melbourne, 62 pp.

GRASSBERGER, M., & FRANK, C. 2004: Initial study of arthropod succession on pig carrion in a central Europe urban habitat. *Journal of Medical Entomology* **41 (3)**: 511–523.

GRASSBERGER, M., & REITER, C. 2001: Effect of temperature on Lucilia sericata (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. *Forensic Science International* **120 (1–2)**: 32–36.

GRISENDI, A., DEFILIPPO, F., GATTI, F., DOTTORI, M., & BONILAURI, P. 2015: Estimation of Accumulated Degree Day Value of Six Landmarks within the Pupal Stage of Lucilia sericata. *Journal of Life Sciences* **9**: 311–317.

GROETERS, F. R. 1992: Geographic conservatism of development rate in the milkweed-oleander aphid, Aphis nerii. *Acta Oecologica* **13 (6)**: 649–661.

GUERROUDJ, F. Z., & BERCHI, S. 2016: Effect of temperature on the development of carrion beetle Silpha rugosa (Linnaeus, 1758) (Coleoptera: Silphidae) in Algeria. *Journal of Entomology and Zoology Studies* **4 (5)**: 920–922.

GUNN, A. 2009: *Essential Forensic Biology. Second Edition*. John Wiley & Sons, Hoboken, 424 pp.

HANGAY, G., & ZBOROWSKI, P. 2010: *A Guide to the Beetles of Australia*. Csiro Publishing, Collingwood, 238 pp.

HARVEY, M., GASZ, N., & VOSS, S. 2016: Entomology-based methods for estimation of postmortem interval. *Research and Reports in Forensic Medical Science* **6**: 1–9.

HASTIR, P., & GASPAR, C. 2001: Diagnose d'une famille de fossoyeurs: les Silphidae. *Notes Fauniques de Gembloux* **44**: 13–25 (in French).

HATCH, M. H. 1927: Studies on the Silphinae. *Journal of the New York Entomological Society* **35 (4)**: 331–371.

HAYMAN, J., & OXENHAM, M. 2016: *Human Body Decomposition. 1st Edition*. Academic Press, New York, 162 pp.

HIGLEY, L. G., NABITY, P. D., & HENG-MOSS, T. M. 2006: Effects of Temperature on Development of Phormia regina (Diptera: Calliphoridae) and Use of Developmental Data in Determining Time Intervals in Forensic Entomology. *Journal of Medical Entomology* **43 (6)**: 1276–1286.

HONĚK, A. 1990: Host plant energy allocation to and within ears, and abundance of cereal aphids. *Journal of Applied Entomology* **110 (1-5)**: 68–72.

IKEDA, H., KUBOTA, K., KAGAYA, T., & ABE, T. 2007: Flight capabilities and feeding habits of silphine beetles: Are flightless species really "carrion beetles"? *Ecological Research* **22**: 237–241.

IKEMOTO, T., & TAKAI, K. 2000: A New Linearized Formula for the Law of Total Effective Temperature and the Evaluation of Line-Fitting Methods with Both Variables Subject to Error. *Environmental Entomology* **29 (4)**: 671–682.

JAKUBEC, P. 2016: Thermal summation model and instar determination of all developmental stages of necrophagous beetle, Sciodrepoides watsoni (Spence) (Coleoptera: Leiodidae: Cholevinae). *PeerJ* **4**: e1944.

JENKINS, T., HOBACK, W. W., LEASURE, D., MUDLER, P., & DAVIS, C. 2018: Distribution of the Endangered American Burying Beetle at the Northwestern Limit of its Range. *Insect Systematics and Diversity* **2 (1)**: 1–8.

JOSEPH, I., MATHEW, D. G., SATHYAN, P., & VARGHEESE, G. 2011: The use of insects in forensic investigations: An overview on the scope of forensic entomology. *Journal of Forensic Dental Sciences* **3 (2)**: 89–91.

KING, J., RIEGLER, M., THOMAS, R., & SPOONER-HART, R. 2015: Phylogenetic placement of Australian carrion beetles (Coleoptera: Silphidae): Phylogeny of Australian Silphidae. *Austral Entomology* **54 (4)**: 1–10.

KIPYATKOV, V. E., & LOPATINA, E. B. 2002: Reaction norm in response to temperature may change to adapt rapid brood development to boreal and subarctic climates in Myrmica ants (Hymenoptera: Formicidae). *European Journal of Entomology* **99 (2)**: 197–208.

KIPYATKOV, V. E., & LOPATINA, E. B. 2010: Intraspecific variation of thermal reaction norms for development in insects: New approaches and prospects.

Entomological Review **90 (2)**: 163–184.

LAWRENCE, J. F., & NEWTON, A. F. 1995: Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). Pp. 779–1006. In: PAKALUK, J., & SLIPINSKI, S. A. (eds): *Biology, Phylogeny, and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson.* Muzeum i Instytut Zoologii PAN, Warszawa, 1092 pp.

LAWRENCE, J. F., & SLIPINSKI, A. 2013: *Australian beetles. Volume 1: morphology, classification and keys*. Csiro Publishing, Coolingwood, 576 pp.

LEE GOFF, M. 2000: *A fly for the prosecution: how insect evidence helps solve crimes.* Harvard University Press, Cambridge, 240 pp.

MADEA, B., GRELLNER, W., & KONDO, T. 2014: Vital Reactions and Wound Age Estimation. Pp. 237–252. In: MADEA, B.: *Handbook of forensic medicine.* Wiley-Blackwell, Hoboken, 1288 pp.

MATUSZEWSKI, S., FRATCZAK, K., KONWERSKI, S., BAJERLEIN, D., SZPILA, K., JARMUSZ, M., & MADRA, A. 2016: Effect of body mass and clothing on carrion entomofauna. *International Journal of Legal Medicine* **130 (1)**: 221–232.

MARCHENKO, M. I. 1987: Medico-legal relevance of cadaver entomaufauna for the determination of the time since death. *Acta Medicinae Legalis et Socialis* **38**: 257–302 (in Russian).

MCGAVIN, G. C., & LEWINGTON, R. 2001: *Essential Entomology. An Order-by-order Introduction*. Oxford University Press, Oxford, 328 pp.

MÉGNIN, J. P. 1894: *La faune des cadavres: application de l'entomologie à la médecine légale*. Gauthier-Villars et fils, Paris, 214 pp.

MIDGLEY, J. M., RICHARDS, C. S., & VILLET, M. H. 2010: The Utility of Coleoptera in Forensic Investigations. Pp. 57–68. In: AMENDT, J., LEE GOFF, M., CAMPOBASSO, C. P., & GRASSBERGER, M.: *Current Concepts in Forensic Entomology*. Springer, Dordrecht, 376 pp.

MIDGLEY, J. M., & VILLET, M. H. 2009: Development of Thanatophilus micans (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. *International*

Journal of Legal Medicine **123 (4)**: 285–292.

NEWTON A. F. J., 1991: Silphidae. Pp. 339–341. In: STEHR, F. W. (ed.): *Immature insects. Vol. 2*. Kendall Hunt, Dubuque, 992 pp.

PAYNE, J. A. 1965: A summer carrion study of the baby pig sus scrofa Linnaeus. *Ecology* **46 (5)**: 511–523.

PECK, S. B. 2001: Review of the carrion beetles of Australia and New Guinea (Coleoptera: Silphidae). *Australian Journal of Entomology* **40 (2)**: 93–101.

PEDIGO, L. P., HIGLEY, L. G., & OSTILE, K. R. 1986: Degday: A Program for Calculating Degree-days, and Assumptions Behind the Degree-day Approach. *Environmental Entomology* **15 (5)**: 999–1016.

PETRUŠKA, F. 1975: The effect of predominating winds on the flight of some species of beetles from the group of Silphidae into pitfall traps (Col. Silphidae). *Acta Universitatis Palackianae Olomucensis. Facultas Rerum Natur* **51**: 55–175.

PFENNIG, D. W., & LEVIS, N. 2017: Phenotypic Plasticity. *BioEssays* **19**: 519–525.

PUKOVSKI, E. 1933: Ökologische Untersuchungen an Necrophorus F. *Zeitschrift für Morphologie und Ökologie der Tiere* **27**: 518–586.

SOKAL, R., & ROHLF, F. 2012: *Biometry: the principles and practice of statistics in biological research, Fourth Edition.* W.H. Freeman, New York, 797 pp.

RATCLIFFE, B. C. 1996: *The carrion beetles (Coleoptera: Silphidae) of Nebraska*. The University of Nebraska State Museum, Lincoln, 100 pp.

RATTENBURY, A. E. 2018: Chapter 2 - Forensic Taphonomy. Pp. 37–59. In: RALEBITSO-SENIOR, T. K. (ed.): *Forensic Ecogenomics: The Application of Microbial Ecology Analyses in Forensic Contexts*. Academic Press, New York, 258 pp.

REITER, C. 1984: Zum Wachstumsverhalten der Maden der blauen Schmeißfliege Calliphora vicina. *Zeitschrift für Rechtsmedizin* **91 (4)**: 295–308.

RICHARDS, C. S., CROUS, K. L., & VILLET, M. H. 2009: Models of development for blowfly sister species Chrysomya chloropyga and Chrysomya putoria. *Medical and Veterinary Entomology* **23 (1)**: 56–61.

RICHARDS, C. S., PATERSON, I. D., & VILLET, M. H. 2008: Estimating the age of immature Chrysomya albiceps (Diptera: Calliphoridae), correcting for temperature and geographical latitude. *International Journal of Legal Medicine* **122 (4)**: 271–279.

RIDGEWAY, J. A., MIDGLEY, J. M., COLLTT, I. J., & VILLET, M. H. 2014: Advantages of using development models of the carrion beetles Thanatophilus micans (Fabricius) and T. mutilatus (Castelneau) (Coleoptera: Silphidae) for estimating minimum post mortem intervals, verified with case data. *International Journal of Legal Medicine* **128 (1)**: 207–220.

RŮŽIČKA, J., SCHNEIDER, J., SIKES, D., & HÁVA, J. 2002: Distributional records of carrion beetles (Coleoptera: Silphidae) from China, Part II. *Klapalekiana* **38**: 227–253.

RŮŽIČKA J., QUBAIOVÁ J., NISHIKAWA M. & SCHNEIDER J. 2015: Revision of Palearctic and Oriental Necrophila Kirby et Spence, part 3: subgenus Calosilpha Portevin (Coleoptera: Silphidae: Silphinae). *Zootaxa* **4013 (4)**: 451–502.

RŮŽIČKA J., & JAKUBEC P. 2017: Silphidae (mrchožrotovití). Pp. 417–418. In: HEJDA, R., FARKAČ, J., & CHOBOT, K. (eds): *Červený seznam ohrožených druhů České republiky. Bezobratlí.* Agentura ochrany přírody a krajiny, Praha, 611 pp.

SCOTT, M. P. 1989: Male parental care and reproductive success in the burying beetle, Nicrophorus orbicollis. *Journal of Insect Behavior* **2 (1)**: 133–137.

SCOTT, M. P. 1998: The ecology and behavior of burying beetles. *Annual Review of Entomology* **43**:595–618.

SHARMA, R., GARG, R. K., & GAUR, J. R. 2015: Various methods for the estimation of the post mortem interval from Calliphoridae: A review. *Egyptian Journal of Forensic Sciences* **5 (1)**: 1–12.

SIKES, D. S. 2005: Silphidae Latreille, 1807. Pp. 288–296. In: KRISTENSEN, N. P., & BEUTEL, R. G. (eds): *Handbook of Zoology. Vol.4.: Arthropoda: Insecta.* Waler de Gruyter, Berlin, 567 pp.

SIKES, D. S. 2008: Carrion Beetles (Coleoptera: Silphidae). Pp. 749–758. In: CAPINERA, J. L. (ed.): *Encyclopedia of Entomology*. Springer, Dordrecht, 4346 pp.

ŠÍPKOVÁ, H., & RŮŽIČKA, J. 2009: Preference různě staré mršiny u nekrofágních mrchožroutovitých brouků (Coleoptera: Silphidae) ve střední Evropě. *Klapalekiana* **45**: 213–219 (in Czech).

SMITH, A. N., BELK, M. C., SMITH, A. N., CREIGHTON, J. C., & BELK, M. C. 2017: Why does it take two to tango? Lifetime fitness consequences of parental care in a burying beetle. *PLoS ONE* **12 (10)**: e0186466.

SMITH, K. G. V. 1986: *A manual of forensic entomology*. British Museum (Natural History) and Cornell University Press, London, 205 pp.

SZPILA, K. 2010: Key for the Identification of Third Instars of European Blowflies (Diptera: Calliphoridae) of Forensic Importance. Pp. 43–56. In: AMENDT, J., LEE GOFF, M., CAMPOBASSO, C. P., & GRASSBERGER, M.: *Current Concepts in Forensic Entomology*. Springer, Dordrecht, 376 pp.

TUNG, V. W.-Y. 1983: *Common Malaysian beetles*. Longman Malaysia Snd. Berhad, Kuala Lumpur, 142 pp.

VELÁSQUEZ, Y., & VILORIA, A. L. 2009: Effects of temperature on the development of the Neotropical carrion beetle Oxelytrum discicolle (Brullé 1840) (Coleoptera: Silphidae). *Forensic Science International* **185 (1-3)**: 107–109.

WAGNER, T. L., WU, H. I., SHARPE, P. J. H., SCHOOLFIELD, R. M., & COULSON, R. N. 1984: Modeling insect development rates: a literature review and application of a biophysical model. *Annals of the Entomological Society of America* **77 (2)**: 208–220.

WATSON, E. J., & CARLTON, C. E. 2003: Spring succession of necrophilous insects on wildlife carcasses in Louisiana. *Journal of Medical Entomology* **40 (3)**: 338–347.

WATSON, E. J., & CARLTON, C. E. 2005: Succession of Forensically Significant Carrion Beetle Larvae on Large Carcasses (Coleoptera: Silphidae). *Southeastern Naturalist* **4 (2)**: 335–346.

WELLS, J. D., & GREENBERG, B. 1992: Interaction between Chrysomya rufifacies and Cochliomyia macellaria (Diptera: Calliphoridae): the possible consequences of an invasion. *Bulletin of Entomological Research* **82**: 133–137.

WERMELINGER, B., & SEIFERT, M. 1998: Analysis of the temperature dependent development of the spruce bark beetle Ips typographus (L) (Col., Scolytidae). *Journal of Applied Entomology* **122 (1-5)**: 185–191.

ZAKHVATKIN, Y. A. 2001: *General Entomology Course*. Kolos, Moscow, 376 pp. (in Russian).

ZUMPT, F. 1965: *Myiasis in Man and Animals in the Old World: A Textbook for Physicians, Veterinarians, and Zoologists*. Butterworths, London, 267 pp.

10. ANNEXES

Annex 1: Male (on the left) and female (on the right) of *Diamesus osculans*.
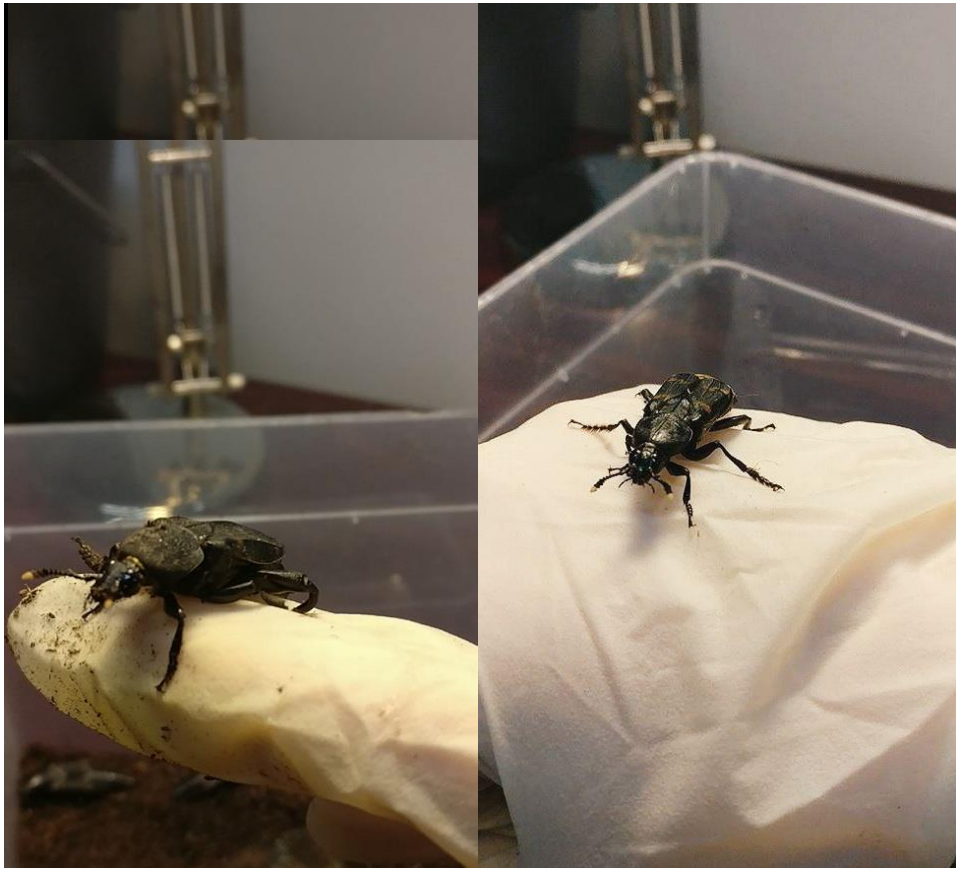
Annex 2: The first instar of *Diamesus osculans* in a Petri dish.

Annex 3: The laboratory and the climatic chambers.