ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

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Geographical Variability in *Thanatophilus sinuatus* (Coleoptera: Silphidae) Thermal Constants

Geografická variabilita termálních konstant u druhu *Thanatophilus sinuatus* (Coleoptera: Silphidae)

Diplomová práce

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

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Environmental Sciences
Applied Ecology

Thesis title

Geographical variability in Thanatophilus sinuatus (Coleoptera: Silphidae) thermal constants

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Objectives of thesis

Carrion beetles (Silphidae) are important group of necrophagous beetles with application in forensic entomology. This thesis will focus on the members of the genus Thanatophilus. The goal is to collect and process the data to find parameter estimates of the thermal summation models for the selected species. The next aim is to estimate and compare estimation the effects of geographical variation on development rate and thermal constants at various temperatures.

Methodology

Adults from different geographic regions (Czech Republic, Germany, Italy) will be collected. Specimens will be transported into laboratory facilities at CZU. Sampled individuals will be separated in breeding colonies (max. 10 adults per box) with equal number of males and females. The breeding colonies will be transferred inside climate chambers and bred at three different constant temperatures (14 ºC, 20 ºC and 24 ºC) with stable light regime (16/8 (L:D)). To follow the developmental times of the individuals bred at laboratory conditions, breeding colonies will be inspected daily for eggs. Eggs will be transferred into petri dishes (max. 10 eggs per petri dish).

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. All developmental milestones will be recorded. Third instar larvae will be separated individually to reduce cannibalism.

The proposed extent of the thesis

40 pp.

Keywords

Coleoptera, Silphidae, forensic entomology, thermal biology, thermal summation model

Recommended information sources

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DECLARATION

I hereby declare that this Diploma 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, 31 March 2022 _____

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ABSTRACT

Necrophagous beetles are an important but understudied group of insects, which are highly useful for forensic entomology. This thesis presents novel information regarding *Thanatophilus sinuatus* (Fabricius, 1775) developmental biology. *T. sinuatus* is frequently found in the carrion and is widely distributed throughout the Palearctic region. In this study, beetles from different geographic regions (Czech Republic, Germany and Italy) were collected and bred at three constant temperatures (14, 20, and 24°C). Development time for all developmental stages was gathered and analyzed. In total, 417 out of 733 specimens reached adulthood. Thermal summation models and standard errors were calculated for all developmental stages (data were excluded for egg) for the three different geographical localities. The obtained developmental lengths differed among temperatures and populations. When comparing the thermal constants, low values of LDT and high values of ADD were attained in colder locality in Czech Republic (LDT = 7.38; ADD = 346.37) dissimilar results were obtained in warmer localities as Italy (LDT = 9.49; ADD = 278.82), in accordance to the literature.

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

ABSTRAKT

Nekrofágní brouci jsou důležitou, ale málo prozkoumanou skupinou hmyzu, která je velmi užitečná pro forenzní entomologii. V této práci jsou uvedeny informace o vývojové biologii druhu *Thanatophilus sinuatus* (Fabricius, 1775). *T. sinuatus* se vyskytuje na mršinách a je velmi rozšířen v celé palearktické oblasti. Sebraní brouci z různých geografických oblastí (Česká republika, Německo a Itálie) byli chováni při konstantních teplotách (14, 20 a 24 °C). Doba vývoje pro všechna vývojová stadia byla sledována a následně analyzována. Celkem se povedlo odchovat 733 larev, z toho 417 pokusných jedinců se vyvinulo až do stádia dospělce. Termálně sumační modely, včetně standardních chyb, byly vypočteny pro všechna vývojová stádia (data pro vejce byla vyloučena) pro tři různé lokality. Získané délky vývoje se lišily mezi teplotami a populacemi. Při porovnávání termálních konstant bylo prokázáno, že nízkých hodnot LDT a vysokých hodnot ADD dosahuje populace z chladnější lokality (Česká republika), na rozdíl od populací teplejších oblastí (Itálie) (LDT = 7,38; ADD = 346,37).

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

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1. INTRODUCTION

The use of insect thermal biology is a standard procedure in forensic investigations and can be significantly biased due to environmental and biological factors affecting the accuracy of the estimates. The prior is especially important since some values of thermal biology such as the MP – Performance Measurements (e.g. development times) may vary across the species' geographical distribution. Carrion beetles of the genus *Thanatophilus* Leach, 1815 (Coleoptera: Silphidae) are an essential group of insects widely distributed and frequently found on carrion in temperate regions, incl. Europe. Nowadays, researchers have proven the importance of using the species of the genus (e.g., *Thanatophilus sinuatus* (Fabricius, 1775), *T. rugosus* (Linnaeus, 1758)) for solving forensic cases. This research aims to provide relevant information regarding *Thanatophilus sinuatus* thermal variation and its importance for forensic entomology.

Necrophagous beetles are an important but still under-examined group of insects, which is highly useful for forensic entomology. Information about larval development rate of selected species could be used for the postmortem interval (PMI) determination. The family Silphidae, which has a special focus in my thesis, is a relatively small family, with only 186 described species in two subfamilies (Nicrophorinae and Silphinae), and the greatest diversity of this group is concentrated in the Holarctic region (Newton, 2018; Sikes, 2008). Carrion beetles (Silphidae) are considered important to forensic entomologists, because this family can get an information on insect succession on corpse and time since death (Smith, 1986). 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.

The subject of my thesis is Geographical variability in *Thanatophilus sinuatus* (Coleoptera: Silphidae) thermal constants. The family Silphidae, which is described in more detail in the theoretical section of my thesis, was selected as our focal group for research. However, the principal subject of study is the species *Thanatophilus sinuatus*, to which the focus and laboratory research is devoted.

The study aims to identify a relationship between temperature and larval development rate. Calculations of the thermal summation model for each studied population will further determine the significance of geographical variability between geographically

distant populations of the same species. Also, the additional purpose would be to describe these relationships as much in 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 hypotheses are:

1. A relationship between temperature and larval development rate exists and larval development rate increases accordingly when the temperature increases, and larval stage duration is decreasing;

2. There is between-population variation in larval development rate. Populations from colder regions have significantly greater cold tolerance and have higher larval development rate at low temperatures, than populations of the same species from warmer regions and vice versa, populations from warmer regions have greater heat tolerance and have higher larval development rate at high temperatures, than populations from colder regions.

My master's thesis work is divided into two parts. The first part included a literature review about the family Silphidae in general and species *Thanatophilus sinuatus* in particular; the following necessary topics that I touched upon in my literature review are thermal summation models and forensic entomology.

The second part focused on laboratory research, analysis of the results and discussion. The analysis of the case studies was held during the writing of this thesis.

Regarding the relevance and uniqueness of my thesis, I would like to highlight that most of the research in forensic entomology is devoted to flies (Diptera), because 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. In that regard, necrophagous beetles are increasingly considered an interesting subject to study.

2. GOALS OF THESIS

1. Write a literature review of ecology of Silphidae with a focus on the members of the subfamily Silphinae. Provide a more detailed description of *Thanatophilus sinuatus*.
2. Supplement a theoretical part by information on insect thermal biology, thermal summation models and forensic entomology.

3. Conduct a laboratory investigation and analyze findings.
4. Create and describe in detail thermal summation models for different populations of *Thanatophilus sinuatus* within its European range.
5. Estimate the effects of geographical variation on development rate and thermal constants at various temperatures.
6. Compare the findings with existing studies and summarize the results.

3. SILPHIDAE

3.1. Systematics and phylogeny

Coleoptera is one of the largest orders containing approximately 400.000 described species (Lawrence & Ślipiński, 2013). Within Coleoptera, a recent phylogeny revealed that the superfamily Staphylinoidea is divided into six families standing out some important families such as Staphylinidae, Leiodidae and Silphidae (Zhang et al., 2018).

According to some authors, the family Silphidae should be re-arranged as a subfamily inside the rove beetles (Staphylinidae). Classification on whether Silphidae should be considered as monophyletic or as part of Staphylinidae is still under debate. The results are contradictory depending on which kind of information is being used to classify the group. Results collected by Grebennikov and Newton (2012), analyzing 260 morphological characters, concluded that Silphidae and Staphylinidae should be treated as two different groups separated no later than in mid-Jurassic. On the other hand, Song et al. (2021), after using next-generation sequencing mitogenomic data, place Silphidae and Scydmaenidae as subordinate groups of Staphylinidae (*also in* McKenna et al., 2015). Nevertheless, in this study, Silphidae will be treated as a family containing two subfamilies, Silphinae and Nicrophorinae.

Historically, the family Silphidae also included an independent lineague, Agyrtidae. Agyrtidae, or the primitive carrion beetles, were generally classified as a third subfamily of Silphidae even though they were not so closely related to Silphidae (Růžička & Jakubec, 2016). Agyrtidae was separated from the Silphidae because of the differences in the morphological characters of the adults and larvae, which was later supported by molecular investigation (McKenna et al., 2015). Nowadays, Agyrtidae is classified as a sister-taxon of Leiodidae (Lawrence & Newton, 1982; McKenna et al., 2015; Peck, 1990).

Nicrophorinae subfamily is represented by the following three genera: *Eonecrophorus* Kurosawa, 1985, *Nicrophorus* Fabricius, 1775 and *Ptomascopus* Kraatz, 1876 (Sikes et al., 2002). The genus *Nicrophorus* was taxonomically revised in the 1980s by Anderson & Peck (1985, 1986), describing one new species (*Nicrophorus hispaniola* Sikes & Peck, 2000) endemic of the Hispaniola Island (Sikes et al., 2002). By large, burying beetles (Silphidae: *Nicrophorus*) have been the focus of behavioral and ecological research since the 1980's, and detailed phylogenetic studies were presented by Sikes & Venables (2013) and Toussaint & Condamine (2016).

According to different sources, Silphinae, also called carrion beetles, consist of 12–18 genera and 112 species worldwide (Sikes, 2008). The subfamily Silphinae throughout Europe is represented by nine genera and 45 species (Růžička, 2015). Consequently, even though the Silphinae subfamily contained most of the generic and species diversity of the family Silphidae, research-related to its natural life history and its value for the forensic sciences is still understudied (Jakubec et al., 2021).

3.2. Distribution

Carrion and burying beetles (Silphidae) can be found in all zoogeographic regions, besides Antarctica, the only continent where they do not live (Sikes, 2005). They are most widespread in the Northern Hemisphere, in the temperate zone; whereas the species distribution is low in the Southern Hemisphere (Anderson & Peck, 1985; Ratcliffe, 1996). The family contains the highest species diversity in the eastern Palaearctic region, and the lowest is found in the Afrotropical region (Sikes, 2005).

Despite the fact that most species of the Silphinae inhabit the Northern Hemisphere, they are more tolerant to warm habitats than Nicrophorinae (Sikes, 2008). Genera of Silphinae such as *Necrodes* Leach, 1815, *Oiceoptoma* Leach, 1815, *Necrophila* Kirby & Spence, 1828 and *Aclypea* Reitter, 1885 are mainly distributed throughout the Holarctic region (Sikes, 2005). On the other hand, genera such as *Diamesus* Hope, 1840, and *Ptomaphila* Portevin, 1926 are found inhabiting Australia (Sikes, 2005). *Diamesus* includes two species: *Diamesus osculans* (Vigors, 1825) distributed throughout India to Australia and *D. bimaculatus* Portevin 1914, an endemic species restricted to the island of Taiwan (Růžička, 2015). Nevertheless, the subfamily also contains a widely distributed genus *Thanatophilus* Leach, 1815, distributed throughout Europe, Asia, North America, and Africa (Sikes, 2005; Newton, 2018).

A large number of the *Nicrophorus* species inhabits the Northern temperate Hemisphere. They are absent in Antarctica, sub-Saharan Africa, and Australia (Sikes, 2005). They are rare in warmer climates, such as lowland rainforests, and practically never encountered in dry climates, such as deserts, because they are less tolerant of warm habitats, in contrast to Silphinae (Sikes, 2008). A small number of Nicrophorinae species are endemic to southeast Asia, New Guinea, and central and southern America (King et al., 2015). Moreover, there is only one genus of Nicrophorinae in the Western Palaearctic: *Nicrophorus* (Růžička, 2015). Some species of the subfamily Nicrophorinae have also been discovered in the Caribbean (Ratcliffe, 1996).

3.3. Silphidae species' protection

Multiple factors, such as anthropogenic land use, can cause a global decline in silphid beetles' diversity (Martín-Vega & Baz, 2012; von Hoermann et al., 2018). Studies have shown that silphid community and its diversity can be negatively affected by the increase of the mean ambient temperature (von Hoermann et al., 2018), hence, negatively affecting the role they play in the terrestrial environments.

Notwithstanding the wide distribution of the family Silphidae, there are endangered species that could be in danger of extinction as in the case of *Nicrophorus americanus* Olivier 1790, which inhabit the United States and Canada. Formerly, this species was distributed in the thirty-five eastern states of the U.S.A. from Canada to Texas. However, it was listed as a federally endangered species in 1989, remaining distributed in only six U.S. States (Jenkins et al., 2018).

There are some representants of the subfamily Silphinae listed in the Czech Red list of endangered species. Among them, there are three vulnerable species, *Ablattaria laevigata* (Fabricius 1775), *Aclypea undata* (O. F. Müller 1776), *Silpha tyrolensis* Laicharting 1781 and, one regionally extinct species *Thanatophilus dispar* (Herbst 1793) (Růžička & Jakubec, 2017). Moreover, the acquired data concerning the species *Aclypea souverbii* (Fairmaire, 1848) considered regionally extinct, is currently data deficient (DD) (Růžička & Jakubec, 2017).

3.4. Biology and ecology

Carrion beetles perform vital ecosystem functions contributing to the breakdown and recycling of the organic material in terrestrial ecosystems (Ratcliffe, 1996; Kočárek, 2003; Wolf & Gibbs, 2004). Their feeding activities on carrion are also known to help eliminate foci of infection of pathogenic bacteria (Sidor, 1979 *in* Peck, 1990) and

parasitic worms (Theodorides, 1950 *in* Peck, 1990), maintaining ecosystems health and productivity (Peck, 1990).

Silphids have developed strict niche differentiation, meaning that, depending on the dimensions of the season, habitat (biotope), and carcass size, silphids can reduce interspecific competition between silphid species and other carrion invertebrates (Anderson, 1982; Scott, 1998; Kočárek, 2001; Martín-Vega & Baz, 2012). Another strategy that Silphidae exhibits to avoid competition is different temporal activities. In a way, peak of activity for some species can be found during the spring as long as several other species has maximum activity during the summer and, just a few species are active during autumn (Růžička, 1994; Kočárek, 2001). They often also have various daily activities, Silphidae is primarily nocturnal insects, but some species can be diurnal (e.g., *Thanatophilus* spp.) or crepuscular (Scott, 1998; Kočárek, 2001).

Competition between carrion insects is rather undervalued, but it is an essential evolutionary factor for silphids (Scott, 1998; Matuszewski & Mądra-Bielewicz, 2021). Silphidae can display both competitions; within the species (intraspecific) and between different species (interspecific) (Anderson & Peck, 1985). Interspecific competitors of Silphidae can often be members of other insect orders (Diptera or Formicidae ants, for example) and even vertebrates. Furthermore, their strategy of finding, concealing and monopolizing small vertebrate carcasses underground in the case of *Nicrophorus* species is a strategy that occurred because of competition, especially caused by competing flies and other burying beetles (Ratcliffe, 1996; Scott, 1998; Sikes 2008). In addition to competitive relations, they also have specific relationships with nematodes (*Rhabditis stammeri* and *R. vespillones*) and mites of the genus *Poecilochirus.* These interactions could be mutualistic, commensalistic, or parasitic (Ratcliffe, 1996; Sikes, 2008).

3.4.1. Morphology of beetles

Adults

Adult carrion beetles are usually medium to large in size and their length ranges from 7 to 45 mm (usually from 12 to 20 mm) (Ratcliffe, 1996; Sikes, 2008). The species of genus *Diamesus* Hope, 1840 (Silphidae: Silphinae) are among the largest beetles of the family, their size varies from 30 to 45 mm (Tung, 1983; Lawrence & Slipinski, 2013). In contrast, the genus *Thanatophilus* Leach, 1815 has the smallest representatives of the family, and their size usually varies from 7 to 9 mm (Mroczkowski, 1966).

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Body shape can be oval (most Silphinae: *Silpha, Thanatophilus*) or rectangular (Nicrophorinae: *Necrodes, Nicrophorus, Diamesus*). The body can also be somewhat flattened or slightly curved (Sikes, 2008). Silphidae typically displays eleven antennomeres, although *Nicrophorus* species pedicel is greatly reduced and fused to the scape, making them appear to have ten antennomeres (Sikes, 2005). Silphid beetles do not have a frontoclypeal (epistomal) suture (Sikes, 2005). *Nicrophorus* species have a pair of stridulatory files on the fifth abdominal tergite (just under the apex of the elytra) (Sikes, 2005). Silphid beetles are generally dark in colour, but some (e.g., *Diamesus*, *Necrophila* Kirby & Spence 1828 *sensu lato*, *Nicrophorus* and *Oiceoptoma*) have bright orange colouring on the body which is a warning to predators (Ratcliffe, 1996). In contrast to Nicrophorinae, there are some flightless or some flight-dimorphic silphine species in *Silpha* and *Aclypea* (Ikeda et al., 2007).

Larvae

Silphidae larvae are flat, mobile and can be characterized as follows: Head: mandible without a molar lobe; maxilla with broad apically cleft mala bearing a dense setal brush on the outer lobe. Urogomphi present, articulated and usually with two segments (Newton, 1991). Length ranges from 12 to 40 mm (Sikes 2005).

Larval characters differ between the subfamilies. Larvae of Silphinae are usually oval or dorsoventrally flattened in shape. The body is highly pigmented and usually dark in colour (Sikes, 2005). Also, larvae are campodeiform and strongly sclerotized (Sikes, 2005; 2008). Bearing a cluster of 6 stemmata (ocelli) on each side of the head. On the other hand, larvae of Nicrophorinae are eruciform and weakly and sparsely sclerotized; the body is oval in shape (Sikes, 2005). Pigmentation is mostly absent, they are pale, white or yellowish in colour. Bearing only a single stemma on each side of the head (Sikes, 2005; 2008).

3.4.2. Reproduction and parental care

To understanding reproduction of carrion beetles, firstly, it is important to mention how they locate the nesting resource, in this case, vertebrate carcasses. Silphidae use specialized chemoreceptors on their antennae to find food; it helps them locate decomposing corpses and find carcasses at long distances (Scott, 1998). They also are one of the first invertebrates to colonize a fresh corpse. For example, if *Nicrophorus* is attracted to a dead animal, it can fly several kilometers (Petruška, 1975). Finding food can be accomplished either by active or passive methods. Adults

may passively wait on the ground and try to catch the smell of a decomposing corpse or actively fly over the ground looking for food (Pukowski, 1933).

Both subfamilies, Silphinae and Nicrophorinae, differ in strategies and behaviors linked with finding a carcass. Their reproductive strategies are divided into the ancestral condition of no parental care and the derived condition with biparental care provided throughout larval development (Sikes, 2005). Silphinae species commonly choose carcasses that weigh more than 300 grams (Sikes, 2005; 2008). They select large carcasses because they do not care for the offspring. Adults lay eggs near or under the carcass and later leave (Johnson, 1974). Eggs hatch in approx. 4–5 days and larvae feed on the carrion themselves until complete its development (Pukowski, 1933; Anderson, 1982; Newton, 1991). Larger carcasses should provide enough food for the larvae and allow them to finish development (Anderson, 1982). It should also be noted that Silphinae usually colonizes a carcass during the early or mid-stage of decomposition. Therefore, they must compete with flies (Diptera) for the food resource (Anderson, 1982).

Compared to Silphinae, Nicrophorinae species prefer small carcasses (< 100 grams); when found, they bury the carcass underground (Pukowski, 1933; Newton, 1991). Furthermore, in some *Nicrophorus* species, few individuals will work together in the burial, this is called "communal breeding" or "joint nesting" (Eggert & Müller, 2000), and later they fight to decide which one will lay eggs on the carcass. After burying the carcass, the female lays the eggs above and covers them with her hindgut secretions to inhibit the growth of bacteria and fungi (Pukowski, 1933). Once carcass is buried and eggs laid, they guard the offspring within a brood chamber with biparental care provided throughout larval development (Sikes, 2005). Burying beetles, particularly of the genus *Nicrophorus*, are known for their biparental care (Sikes et al., 2016). Both males and females provide parental care, and the major benefit of male assistance is to help defend the brood and carcass from competitors (Scott, 1996). To a certain extent, this could be considered as the highest level of sociability attained in the Coleoptera (Ratcliffe, 1996).

3.4.3. Feeding preferences

The members of the family Silphidae are primarily carrion feeder (necrophagous species) or prey on other carrion inhabitants including fly eggs or maggots and other carrion beetles (necrophilous species) (Ratcliffe, 1996; Sikes, 2005; Sikes, 2008).

There are several different feeding strategies within this family. Members of this family can be either carnivorous, phytophagous or necrophagous, as well as they can combine carnivory/necrophagy depending on food availability or developmental stage (Sikes, 2005). More often, however, they are necrophagous as adults and larvae (Sikes, 2005). Genus *Thanatophilus* and *Oiceoptoma* Leach, 1815, which are necrophagous as an adult and larvae, could be taken as an example (Portevin, 1926). Some Silphidae may be attracted by decaying fungi (e.g. *Phallus impudicus*), dung or rotten plant (Ratcliffe, 1996; Sikes, 2005). And some species of Silphinae prefer to consume different kinds of invertebrates (like predating on snails, caterpillars or slugs: *Silpha* spp., *Dendroxena* spp.) or are phytophagous (e.g. *Aclypea* spp.) (Sikes, 2005; Ikeda et al., 2007). An interesting feature of another burying beetle, *Nicrophorus pustulatus* Herschel, 1807, is that these beetle uses live snake eggs rather than carrion as resources for breeding (Blouin-Demers & Weatherhead, 2000; Smith et al., 2007).

There is an opinion that changes in feeding strategies of carrion beetles, especially the change from necrophagy to carnivory, is caused by the shift to predation and the inability of adults to fly (Ikeda et al., 2007). Ikeda (2007) conjectures that beetles, which were primarily predators and could not fly, were not able to successfully compete with other necrophagous beetles, and therefore they were forced to change their feeding strategy. In their study, Ikeda et al. (2007) also determined by comparing the carbon and nitrogen stable isotope ratios for them with those of necrophagous nicrophorine species and carnivorous carabid species, that the flight capabilities and feeding habits of adults can be correlate.

4. FORENSIC ENTOMOLOGY
4.1. General description

Forensic entomology is an integral part of forensic sciences (Smith, 1986; Byrd & Castner, 2009). It uses insects and carrion-associated arthropods information as a tool assisting investigations and judicial proceedings related to both humans and wildlife (Gennard, 2007). Insect specimens are physical evidence at a crime scene equal to fingerprints and DNA (Magni et al., 2013). Therefore, an increasing number of court systems need assistance from experts, represented by forensic entomologists (Magni et al., 2013). Modern forensic entomology includes urban entomology, stored product entomology, and medico-legal entomology (Catts & Goff, 1992). Forensic entomologists can also be involved in cases related to veterinary

entomology, livestock entomology, entomotoxicology and human or insect DNA (Byrd & Castner, 2009).

The basis of forensic entomology is the analysis of entomological evidence concerning insects and other invertebrates which sequentially colonize a corpse (Byrd & Castner, 2009; Gennard, 2007). Important information on the cadaver is collected as decomposition, developmental rate and invertebrates offspring stages progress (Wayne & Rodriguez, 1989). Insects can provide useful information about the manner of death, movement of a cadaver from one site to another, and length of the postmortem interval, also pre-appearance interval (PAI) and post-eclosion interval (PEI) (Wayne & Rodriguez, 1989; Catts & Goff, 1992; Tomberlin & Benbow, 2015). The post-mortem interval (PMI) represents the time between the death and finding of a corpse (Catts & Goff, 1992). And proper estimation of the postmortem interval is one of the most crucial aspects of legal medicine (Sharma et al., 2015).

4.2. History of forensic entomology

Insects have long been used in crime-solving (Benecke, 2001; 2008; Gennard, 2007). Some authors (Benecke 2001; 2008; Amendt et al., 2004; Gennard 2007; Magni et al., 2013) have compiled reviews of the history of forensic entomology (Greenberg & Kunich, 2002). It is known that the first cases of using insects for crime scene investigation are recorded as early as the mid-tenth century. The Chinese used the presence of flies and other insects as part of their investigative armory (Greenberg & Kunich, 2002). One of the first books devoted to this subject is a training manual on investigating the death, entitled *"Collected writings on the washing away of wrongs"*, published by Sung Tzu in 1235. This book describes 15 cases, the most popular one is the case where the suspect confessed of murdering a fellow farm worker with a sickle shortly after some blowflies landed on the murder weapon (Gennard, 2007).

Forensic entomology became a branch of scientific research between the thirteenth and the nineteenth centuries thanks to several centuries of discoveries in biology (Benecke, 2001). It can be evidenced in Linnaeus's work (1775), developing a system of classification and by Redi (1668) experiments using the flesh of different animals, confirming the development of larvae from the eggs laid by flies; the above mentioned, are amongst the most significant works during this period (Gennard, 2007). Linnaeus also found ways to identify insects, including the identification of forensically important flies as *Calliphora vomitoria* (Linnaeus) (Winsor, 1976).

The next important step in forensic entomology occurred in the nineteenth century. For the first time, Mégnin (1894) proposed a more or less predictable chronological sequence, known as insect succession. In his work La Faune des Cadavres: Application de l'Entomologie à la Médicine Légale (1894), he described the relationships between eight stages of human decomposition and the succession of insects colonizing the body after death. These important findings of insect succession on a corpse formed the basis for forensic entomologists' estimations of the time since the death (Gennard, 2007).

As it turns out in the twentieth century, insects can help solve crimes, even if the body parts were retrieved from the water. Third larval instar larvae of the blowfly *Calliphora vicina* Robineau-Desvoidy, 1830, were identified on parts of a corpse recovered from a river (Gennard, 2007). That directly confirmed that the eggs were laid before the body was dumped in the river. This information, coupled with other evidence, helped to solve the case.

Eventually, collaboration of academics and practitioners working together with the police and legal authorities provided the universal recognition of forensic entomology.

4.3. The stages of decomposition

Insects are well-known as consumers of carcasses (Wayne & Rodriguez, 1989). Different groups of insects can use corpses and decomposing organic materials associated with carrion in various ways: as a food source, as a place to rear the offspring, and among others (Wayne & Rodriguez, 1989). The succession of necrophagous insects colonizing carcasses can be used to indicate the carcass age (Smith, 1986). The speed in the stages of decomposition may usually vary depending on the environmental conditions, including the temperature.

Mégnin (1894) was the first to propose a predictable chronological sequence, known as insect succession. There are many different concepts for dividing the decomposition process into stages according to climate and other factors. Based on number of sources (Gennard, 2007; Joseph et al., 2011; Goff, L, M., 2010), decomposition process can be divided into five predictable stages related to the physical appearance of the carcass, the internal temperature, and the characteristic of insects populations:

1. **Fresh stage** (0–4 days);

   This stage initiates from the moment of death to the first signs of bloating of the body. The corpse appears fresh on the surface however is decomposing inside due to the activities of bacteria present before death. The blowflies (Calliphoridae), Sarcophagidae (flesh flies), Muscidae and adult ants are regarded as the first colonizers on this stage.

2. **Bloated** (putrefaction) stage (4–10 days);

   The dead body is swollen by gas produced internally through the metabolism of nutrients by anaerobic bacteria and accompanied by the odour of decaying flesh.

   During the second stage, there is an increasing number of flies, likely because of a specific smell. And the first beetles arrive during this stage, including members of the families Staphylinidae, Silphidae, Cleridae and Histeridae (Grassberger & Frank, 2004).

3. **Decay stage** (Active -Advance decay stages), also called black putrefaction stage (10–20 days);

   Typically, the flesh becomes into a creamy consistency. Exposed body parts turned black. Due to bacterial activity and maggots feeding the body breaks and gases are liberated. Fluids drain from the body and usually, an odour of decay is very strong. Silphid beetles, histerids and muscid flies may appear at this stage. Generally, beetles become dominant adult insects.

4. **Postdecay stage**, so-called advanced decay (20–50 days);

   In the later stages of decay, the cadaver is drying out, but there's still a bit of some flesh. A specific cheesy odour from butyric acid occurs. The major indicator of this

   stage is an increase in the presence of beetles and a reduction in the dominance of the flies (Diptera) on the corpse.

5. **Skeletal or dry remains stage** (50–365 days).

   The body is almost dry and may mummify. The rate of decay slows down. No typical groups of insects are associated with this stage.

The length of the stages is always approximate, and it is because there are a lot of factors that could affect decomposition and its speed. Despite that, an approximation of time since death can be estimated by observing the body's condition and noting the state of decay (Di Maio & Di Maio, 2001).

4.4. Characteristics of insects used in forensic entomology

The insects that can assist in forensic entomological investigations include (Smith, 1986; Goff, 2010):

1. **Necrophagous species** which feed on the dead tissue;

   This group includes many of the Diptera (Calliphoridae, called blowflies, and Sarcophagidae, called fleshflies) and Coleoptera (Silphidae and Dermestidae). These species may be the most significant for estimation of postmortem interval during the earlier stages of decomposition, defined as days 1-14.

2. **Parasites and predators of necrophagous species**, insects and other arthropods;

   An equally important group which include Coleoptera (Silphidae, Staphylinidae and Histeridae), Diptera (Calliphoridae and Stratiomyidae), and hymenopteran parasitoids of larvae and puparia of Diptera. Also, in some cases, dipteran larvae that are necrophages during their early development can turn into predators later.

3. **Omnivorous species** which are not obligate necrophages, but will use the corpse as a food source if available;

   This group includes ants, wasps, and beetles, which feed on the corpse and associated arthropods at the same time. They are capable of retarding the rate of carcass removal by depleting populations of necrophagous species if their population is too large.

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.

   It could be Collembola, spiders, and centipedes. Also, Acari in the families Acaridae, Lardoglyphidae and Winterschmidtiidae that feed on molds and

fungi growing on the corpse may be included in this category. And among the rare representatives are the various Gamasida and Actinedida, including the Macrochelidae, Parasitidae, Parholaspidae, Cheyletidae, and Raphignathidae, that feed on other acarine groups and nematodes

5. **Accidental species** which have no real relationship to the corpse but still can be found on the corpse.

These insects may have fallen onto the corpse from vegetation, and this possibly might give some information on the postmortem movement of a corpse.

In general, forensic entomologists mainly use the first two groups of insects for estimating the PMImin (Amendt et al., 2011).

4.4.1. Diptera

When it comes to forensic entomology, Diptera (flies) is often the first thing that comes to mind. Diptera have the greatest role in the early decomposition process. The most common families of Diptera whose larvae inhabit of decomposing human carcasses and animal carrion are Calliphoridae, Sarcophagidae, Muscidae and Piophilidae (Smith, 1986). Members of families Calliphoridae, called blowflies, and Sarcophagidae are usually the first colonizers on dead bodies (Smith, 1986; Erhan & Beyarslan, 2013). Their larvae are most of the times, the first insects who will feed and colonize the decomposing remains of a carcass (Wayne & Rodriguez, 1989). Blowflies are attracted to the carrion in the first minutes to a few hours after death, as indicated by experimental studies (Wayne & Rodriguez, 1989; Watson & Carlton, 2003). They can begin oviposition immediately after arriving at a corpse or first feed on the protein-rich fluids emanating from the corpse and then start to lay eggs (Wayne & Rodriguez, 1989). After reaching the third instar, larvae of blowflies feed on the corpse before reaching post-feeding stage, and then leave the corpse, burrow down into the soil, and pupate (Wayne & Rodriguez, 1989). Nevertheless, they do not necessarily have to leave the corpse to pupate, they are also able to pupate directly on the body. So, examination of the soil in a place where corpse have decomposed can help track and identify valuable entomological evidence, not only in the initial stages of decomposition of the corpse. Moreover, Calliphoridae pupal cases, which are found at the crime scene, can provide valuable information relevant to

Postmortem Interval, even after the remains of the body have decomposed (Wayne & Rodriguez, 1989).

Diptera colonization speed is one of the reasons why they are the basic and most correct forensic indicators of time of death, and their development rates are needed to allow more correct PMI estimates (Grassberger & Reiter, 2001). [MMS1] As stated above, related to the assumption that Diptera colonizes corpses faster and may provide a more accurate estimate of minimum Postmortem Interval (PMImin), perhaps, a larger number of studies in forensic entomology is concentrated on flies. Further, flies are easier to handle in the laboratory conditions than other forensically relevant species, which could be why a significant part of developmental studies refer to Diptera (Amendt et al., 2014). Despite that, there are some certain limitations in determining PMImin if blowflies are used (e.g., difficulties in age determining of post-feeding larvae in connection with no satisfactory indicator of this instar was not found in previous morphological studies) (Greenberg, 1991).

4.4.2. Beetles

In the context of forensic entomology, beetles are undervalued, and still neglected or even ignored by many researchers. As the corpse decompose, other insect species, like Coleoptera (beetles), colonize the cadaver and continue the process that flies started. Silphidae and other beetle families (such as Dermestidae, Trogidae, Histeridae, Nitidulidae, Staphylinidae etc.) are regarded as important sources of information for the forensic entomology, as larvae or imago found in the corpse can help to estimate the postmortem interval (PMI) and the possible ways of death (Watson & Carlton, 2005).

Some species of the Silphidae family could help estimate PMImin during early decomposition, but also in later stages (Midgley & Villet, 2009). Is well known that flies are the first comers to colonize a corpse within few hours after death, but some beetles can also be among the first. For example, *Thanatophilus micans* (Fabricius, 1794), which is a useful model for estimating postmortem interval in southern Africa, can find corpses as quick as most fly species (Midgley & Villet, 2009). Recent observations (Midgley & Villet, 2009) indicate that *T. micans* can detect corpses and start breeding within 24 h since death. This observation concludes that *T. micans* has the same potential utility of estimates as flies. And in contrast to flies, beetles are

present in later stages of decomposition because of their long larval development (Midgley & Villet, 2009).

4.5. Estimating the minimum post-mortem interval (PMImin)

It is known that the post-mortem interval of human cadavers may be estimated based on insect evidence. Currently, forensic entomology is an integral part of a death investigation and is considered the most accurate method for estimating the time since death, especially if more than 72 h have passed (Kashyap & Pillay, 1989; Sharma et al., 2015). Methods for PMI estimation based on insect evidence are being developed, constantly improving, and applied by forensic entomologists (Matuszewski, 2021). Some species of insects, including flies, beetles, and moths, have been used as estimators of post-mortem interval since the late 19th century (Greenberg & Kunich, 2002). Recently, many studies have been devoted to forensic entomology and to better define the use of insect activity as a proxy for time since death. The rising number of scientific publications and countries where entomology-based estimation of PMI is regularly used is related to the active development of this field (Lei et al., 2019; Tomberlin & Benbow, 2015).
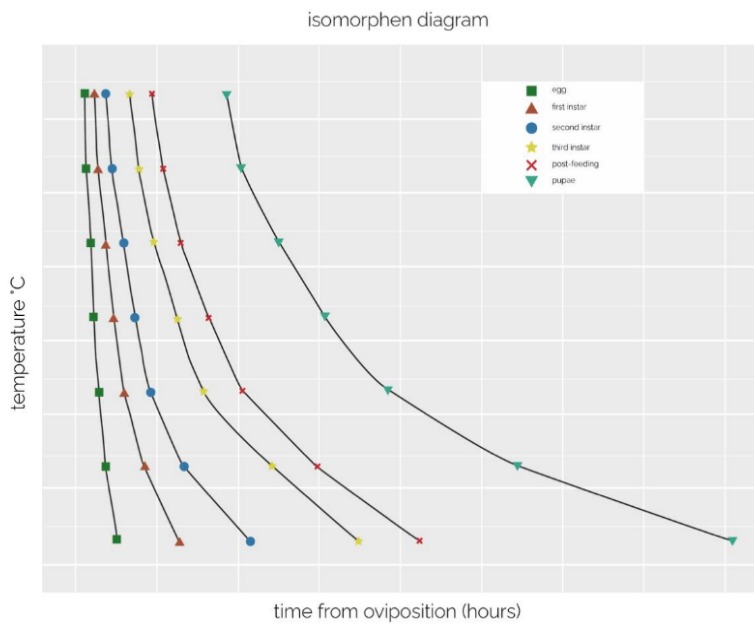
Insect development is directly related to temperature (Régnière et al., 2012; Pedigo et al., 2021). This relationship has been quantified for forensically important species, as the age of the specimen can be determined based on the stage of development and the thermal history at which the individual was reared (Introna et al., 1989; Harvey et al., 2016). In cases where no immature insects are available or they have completed development, as it often happens in later stages of decomposition, minPMI can be estimated based on the insects still present in remains at the crime scene, also, referred as the predictable process of insect succession (Goff, 1993).

There are different entomological approaches for age estimation. Current methods of age determination are predicated on the species-specific time needed for an immature insect to progress through developmental marks such as length, weight, and life cycle stage in relation to temperature. (Harvey et al., 2016). An essential part of estimating the PMImin is modelling insect development using all findings. Developmental data is information that indicates the development duration of immature stages observed at different temperatures (Amendt et al., 2011). Then, these data is summarized in one or more of the four developmental models: Isomorphen diagrams (Grassberger & Reiter, 2001), Isomegalen diagrams (Reiter,

1984), Thermal Summation Models (Ikemoto & Takai, 2000), and Curvilinear models (Amendt et al., 2011; Harvey et al., 2016).

The simplest method is an Isomorphen diagram **(Fig. 1)**. Isomorphen diagram is a scatter plot of the time from eggs hatching until hatchling plotted against constant temperature (Grassberger & Reiter, 2001; Amendt et al., 2011). Essentially, Isomegalen **(Fig. 2)** or Isomorphen dia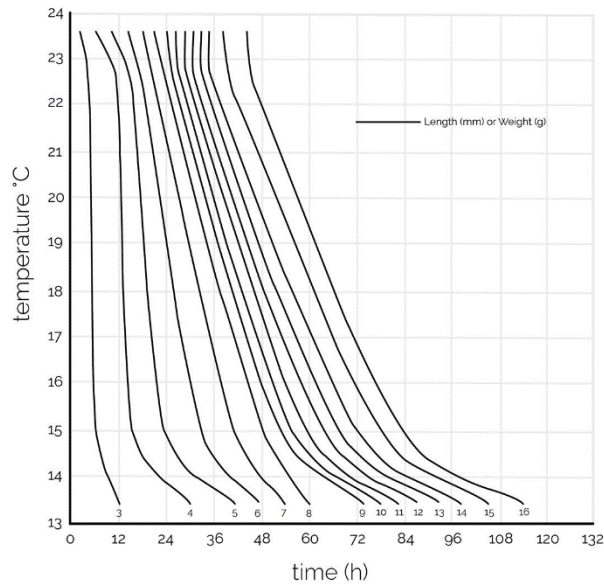grams constitute the lengths and the developmental stage of the larvae combined as a function of time and mean ambient temperature in a single diagram (Madea et al., 2014). Madea et al. (2014) state that both methods are suitable only when the body and the larvae were not

Figure 1. Isomorphen diagram

undergoing fluctuating temperatures, in other words, the temperature was nearly constant. However, Schmalensee et al. (2021) use this type of the linear degree-day model in their research, and their results showed that insect development rates are generally unaffected by thermal fluctuations, without taking into account direct effects of temperature.

Curvilinear or nonlinear models can describe the relationship between development and temperature in a more accurate way, by using the curvilinearity observed at the upper and lower temperature extremes, when the relationship between development rate and temperature is plotted (Logan et al., 1976; Briere et al., 1999). But so far, despite improved parameters for estimation, there is no curvilinear model, which consistently exceeds linear models across relevant species data (Zahiri et al., 2010). It should also be noted that the complexity of this kind of model limits its practical application to the forensic estimation of PMImin (Zahiri et al., 2010).

The thermal summation model is the most popular and commonly used method for modeling insect development rates. This model applies linear regression analysis to the positive relationship between temperature and development (Amendt et al., 2011). A

Figure 2: Isomegalen diagram

separate chapter will be devoted to this model's description and detailed analysis.

5. THERMAL SUMMATION MODELS

Temperature plays a key role in development time of insects (Taylor, 1981). Insects are poikilothermic animals which means that they are highly dependent on various environmental factors (Ju et al., 2011). But the temperature has probably the greatest effect on insect development (Taylor, 1981; Pedigo et al., 2021). Different studies show that temperature has an effect on various biological characteristics of insects such as sex-ratio (Zheng et al., 2008), adult life-span, survival, fecundity, and fertility (Yang et al., 1994; Dreyer & Baumagartner, 1996; Infante, 2000). And this close correlation leads to a temperature significantly impacting on colonization, distribution, abundance, behaviour, life history, and fitness of insects (Cossins & Bowler, 1987; James et al., 2002; Hoffmann et al., 2003).

5.1. Degree days/hours (ADD/ADH)

The degree day/hour theory is based on the fact that 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 curvilinear at high and low temperatures and linear only in between in the majority of cases (Sharma et al., 2015). The modern degree day model, which applied in forensic entomology, began to form around the 1950s (Arnold, 1960). So, an estimation of the accumulated degree days or hours (ADD or ADH) is one of the oldest

ways of calculating PMImin. ADD values represent a particular number of "energy hours" which are necessary for the insect development (Sharma et al., 2015). The equation for calculating ADD is as follows (Higley et al., 2006, fixed by Ipatova):

$$ADD = D \cdot (T - LDT)$$

where D is the development time (or the duration of development), T is the ambient temperature, LDT is the lower developmental threshold, a species value, the so-called development zero, which is the x-intercept, and is calculated by the linear approximation method.

5.2. The effect of temperature on the insect development

As already mentioned above, since insects are exothermal animals, temperature has an important role in their growth and development. Consequently, the effect of temperature on the insect growth rate and duration of development can be considered one of the most important forms of dependence of ectothermal organisms on thermal habitat conditions.

Most of modern models are based on the concept of the "law of total effective temperatures", which abstracts the temperature responses of a particular species, in which a specific amount of thermal units should be accumulated above a temperature threshold, to complete a development (Damos & Savopoulou-Soultani, 2012). The sum of effective temperatures intended for assessing the effect of temperature on insect development (Dixon et al., 2009). The count is conducted from the pre-calculated values of the lower developmental threshold and the development is inhibited at lower temperatures (Kipyatkov & Lopatina, 2010). 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 (Dixon et al., 2009; Damos & Savopoulou-Soultani, 2012). At that, it is necessary to note, that the development of each insect species can occur only in a limited temperature range (Dixon et al., 2009).

It is widely known (Campbell et al., 1975; Wagner et al., 1984) that the duration of development of insects and other ectothermic organisms reveals an approximately hyperbolic dependence on temperature at the temperatures are favorable for living

activity (Kipyatkov & Lopatina, 2010). The main equation (Blunck, 1914; 1923, fixed by Ipatova) is as follows:
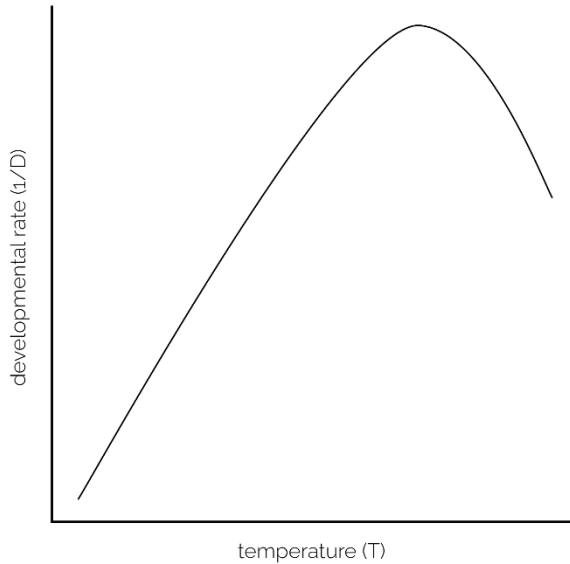
$$D = \frac{ADD}{T - LDT}$$

where D is the duration of development in days (hours, etc.), T is the temperature at which development occurs, LDT is the lower developmental threshold, and ADD 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. Thereunder, the duration of development hyperbolically increases with decreasing temperature and becomes infinitely large when the temperature reaches LDT. According to that, the development does not occur at the lower developmental threshold. Hyperbolic equations are difficult to work with, may not be easily visualized, and also are hard to compare (Kipyatkov & Lopatina, 2010).

5.3. Thermal summation models

Mathematical models constitute a language for formalizing the information obtained empirically after experimental observation and hypothesis testing (Damos & Savopoulou-Soultani, 2012). An empirical model, if it is properly described, can provide useful information about results and causes and can be further used to describe the behaviour of the system under different conditions (Damos & Savopoulou-Soultani, 2010). In applied entomology, empirical methods are commonly used in the creation of different developmental models (Damos & Savopoulou-Soultani, 2012). Broadly, this process includes the delimitation of all primary factors affecting development to the most limiting one, which is further chosen like the main one (i.e., temperature), in order to reveal empirical dependence of the developmental variable upon the limiting factor (Damos & Savopoulou-Soultani, 2012). A function that describes the data with high precision is plugged into this relation, and its prediction power is further evaluated by using another dataset as well (Damos & Savopoulou-Soultani, 2012).

There are a lot of various methods for estimation of minimum post-mortem interval (PMImin). One of these methods is the thermal summation model, which is the most frequently used method for calculating PMImin (Richards et al., 2008). The model is based on the principles 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 can process size and developmental data and also display temperature fluctuations (Amendt et al., 2011). Insect development may be estimated at close intervals over a temperature range, and where the development rate (calculated as reciprocals of

Figure 3. A generalized insect development rate curve

development time, 1/D) is plotted contrary to temperature, a sigmoid-shaped **(Fig. 3)** curve arises (Wagner et al., 1984). And normally, the correlation between temperature and developmental rate is linear between the lower developmental threshold and optimum temperature (Harvey et al., 2016). This leads to the conclusion that linear regression is capable of determining an x-intercept (lower developmental threshold, LDT) and an inverse of the slope of the linear regression (thermal summation constant, ADD) which permit prognosis of development time from the thermal history of a specimen (Amendt et al., 2011). In this way, if the development rate (R) is determined as the feedback values of duration of development (R = 1/D), then the above main equation (Blunck, 1914; 1923) can be converted to a linear form (Kipyatkov & Lopatina, 2010):
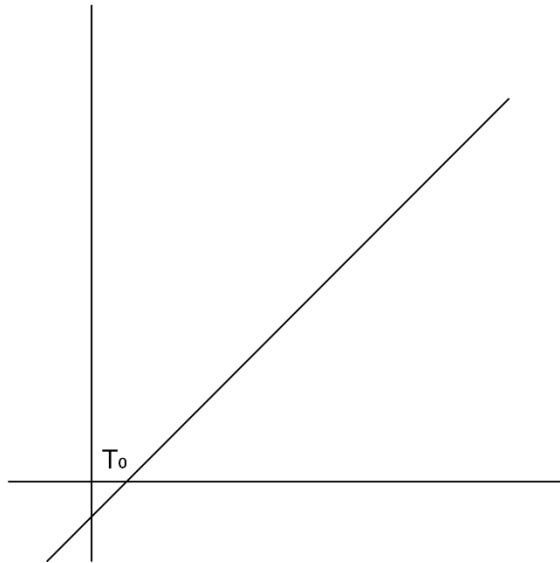
$$R = a + bT$$

where a is a constant and b is a linear regression coefficient of the development rate to temperature. The graph of this equation **(Fig. 4)** demonstrates the temperature
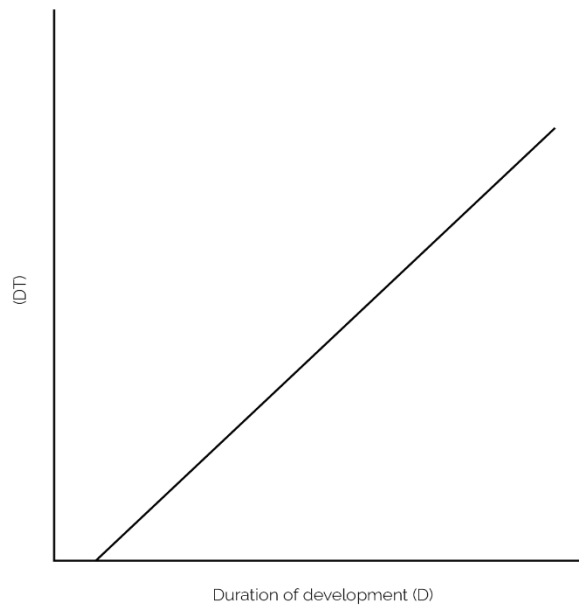
Figure 4. The graph of the linear equation:

$R = a + bT$

norm of the reaction of individuals or population, according to the development rate. It is a straight line that intersects the x-axis (the abscissa) at LDT.

This underlined the lower developmental threshold (LDT) is temperature at which development rate is zero. In this linear regression model, development is evaluated as physiological time with units of "degree days" or "degree hours" (Harvey et al., 2016), which were described in more detail in the previous chapter. One "degree day" or "degree hour" is typically equivalent to one degree above the lower developmental threshold over 24 hours or 1 hour, accordingly (Harvey et al., 2016). Every stage in the insect life cycle (egg, first, second and third instar, post-feeding and pupae) requires a certain amount of accumulated degree hours to move to advance to the next phase and complete development, that is ADD (Campbell et al., 1974). The central issue in linear models consists in that, they do not include the nonlinearity experienced in insect development at low and high temperatures, however, they are useful in their permitting estimation of lower developmental thresholds and thermal summation constants (Briere et al., 1999). It should be noted that a great difference between the upper and lower developmental temperature extremes would certainly hinder the accuracy of the PMImin estimation (Amendt et al., 2011). Ikemoto and Takai (2000) linear regression model is the most accurate and modern way for showing the relationship between the insect developmental times and the accumulated degree days (ADD). This revised regression model determines the point, which is closest to the extremes of the linear approximation that deviate significantly from it, and as result, the estimation of thermal summation parameters is becoming increasingly accurate (Ikemoto & Takai, 2000). A new linearized equation is as follows (Ikemoto & Takai, 2000): $(DT) = k + tD$

y-axis label: (DT)
x-axis label: Duration of development (D)

Figure 5. The graph of the new linearized formula: (DT) = k + tD (Ikemoto & Takai, 2000)

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. 5)** looks like a straight line with x = D (duration of development) and y = DT (Ikemoto & Takai, 2000). Thermal summation models are insect species and stage specific (Ridgeway et al. 2013), and it follows that accurate PMImin estimation rely on the fact if the thermal summation model is already created for the specific species.

The dependence of insect development rate on temperature for various species is practically the same as linear in a favorable for the development temperatures, and because of that linear model are used very broadly in studies on the ecology of insects (Campbell et al., 1975). The use of a linear model and thermal constants has the great potential in in comparative studies of the insect life cycle. The sum of degree-days and the regression coefficient of the development rate by temperature make it possible to compare same or different species according to their thermal requirements and the degree of thermal lability (Kipyatkov & Lopatina, 2010). But there is inability to identify unambiguously the range of temperatures within which a linear model can be applied (Kipyatkov & Lopatina, 2010), and this feature of linear models is perhaps the main drawback. Based on the analysis of the large number of relevant references, Honěk (1990) stated 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. This is why, 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, 2012).

6. PRACTICAL PART
6.1. Thanatophilus as a model taxon
6.1.1. Taxonomy and taxonomic position

Taxonomy and classifications of *Thanatophilus* species based on adult morphology were reviewed by several authors (Schawaller, 1981; Kozminykh, 1994; Růžička, 2002; Ji, 2012).

The following classification was taken from (Newton, 2018):

Order: Coleoptera

    Suborder: Polyphaga

        Infraorder: Staphyliniformia

            Superfamily: Staphylinoidea

                Family: Silphidae

                    Subfamily: Silphinae

                        Genus: *Thanatophilus* Leach, 1815

                            Species: *Thanatophilus sinuatus* (Fabricius, 1775)

The genus *Thanatophilus* includes 24 species, distributed in Europe, Asia, North America, and Africa (Anderson & Peck, 1985; Ratcliffe 1996; Newton, 2018).
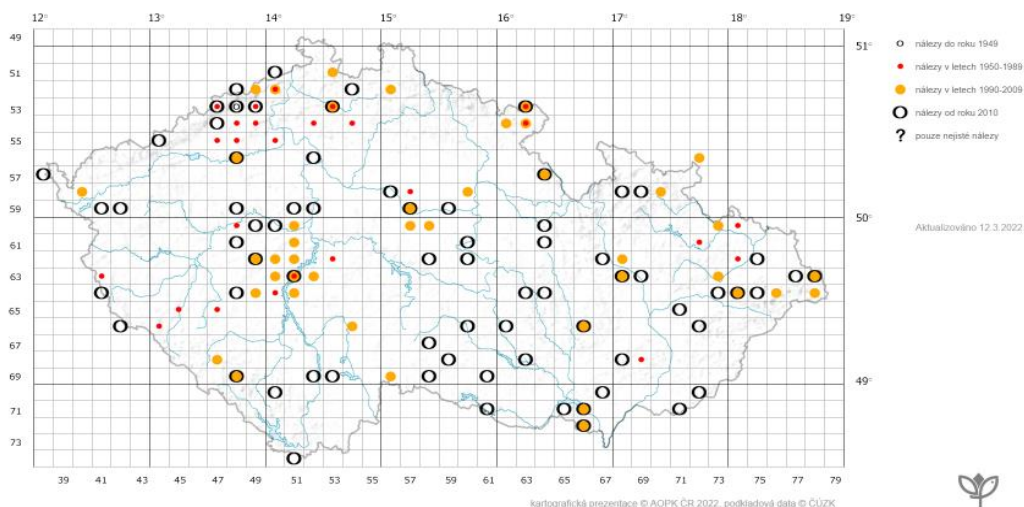
6.1.2. Distribution

The genus *Thanatophilus* inhabits Europe, Asia, North America and Sub-Saharan Africa (Schawaller, 1981; Newton 2018). There are two species of *Thanatophilus* in Africa: *Thanatophilus capensis* (Wiedemann, 1821) [with *T. mutilatus* (Laporte de Castelnau, 1840) being its junior synonym], which is endemic to the southern parts of South Africa, and *Thanatophilus micans* (Fabricius, 1794), which is widespread in Africa, reaching south of Arabian Peninsula (Schawaller, 1981; 1987; Newton, 2018). But the most of species inhabit the Northern Hemisphere.

Thanatophilus sinuatus, which was used in a laboratory study, is a common species and very widely distributed throughout the Palaearctic region (Schawaller, 1981; Růžička, 2002; 2015). And *Thanatophilus sinuatus* is one of the one of the most abundant and widespread species of necrophagous beetles in the Czech Republic **(Fig. 6)** (Růžička & Jakubec, 2017).

Figure 6: Occurrence of *Thanatophilus sinuatus* in the Czech Republic, based on observations and records © AOPK ČR 2022

6.1.3. Ecology

In general, members of the genus *Thanatophilus* are similar in appearance and, sometimes, ecology (Jakubec et al., 2019). So far, all known species of the genus are necrophagous in all stages of development (larvae and adults), and they are associated with the decomposition of large vertebrate carrion, including humans (Sikes, 2005; Jakubec et al., 2019). *Thanatophilus* species are considered important forensic bioindicators because they could provide valuable information about the time of death and possible body manipulation. Therefore, they have great potential for forensic entomology in temperate zones where they occur (Růžička & Jakubec, 2017; Montoya-Molina et al., 2021). *Thanatophilus* beetles usually prefer earlier stages of decomposition and can also swift colonization of carcasses soon after death (the first 24 h), making them even more valuable for forensic investigation (Midgley & Villet, 2009).

As for their habitat preference, the members of this genus may be considered as open land or non-forest inhabitants, associated to field grass lands or meadows (Růžička, 1994; Hastir & Gaspar, 2001; Kočárek, 2001; 2003).

Thanatophilus sinatus immature stages are frequently found feeding on cadavers decaying tissues during their development (Jakubec et al., 2019). Consequently, the species has been categorized as useful carrion taxa for forensic investigations (Matuszewski et al. 2010). *T. sinuatus* population dynamics are consist of a three-peaked growth curve (Novák, 1966). Adults begin to be active in the second half of April and have two generations more during the year (Novák, 1966; Růžička, 1994). Kočárek (2001), characterized the species as diurnal.

Additionally, the species can also be considered an important part of the North Hemisphere ecosystems because they can provide different valuable ecosystem services by promoting nutrient cycling and biological control of pests (Sikes, 2008; Růžička & Jakubec, 2017).

6.2. Material and methods

Adult beetles of *T. sinuatus* were collected in three different geographic regions (Czech Republic, Germany, Italy) in 2021. In the Czech Republic sampling was conducted near Včelná pod Boubínem, 49°01'19.3" N 13°51'33.6" E. In Germany near Frankfurt am Main, Griesheim, 50°05'41.5" N 8°37'06.4" E and Rüsselsheim am Main, 49°58'21.7" N 8°27'15.4" E. In Italy, Venezia Province, Caorle, 45°36.24'N 012°52.85'E. Due to the ecological preferences of the species, the traps were placed in open areas, in habitats such as harvested agricultural fields or grasslands.

All beetle specimens collected were transported into the laboratory at the Czech University of Life Sciences in Prague and identified. Individuals were separated into breeding colonies (maximum 10 per box), with an equal number of males and females from the same locality (Czech Republic, Germany, Italy). Breeding colonies consisted of plastic fauna boxes filled with a layer of gardening soil, used as the oviposition substrate. Inside the breeding colonies, beetles were provided with a piece of pork tissue as a food source. Water and humidity sources were provided in form of a small tube filled with water and stoppered by a piece of cotton. Food was regularly replaced to prevent the outbreak of the mold.

The breeding colonies were kept inside climatic chambers bred at three different constant temperatures (14, 20 and 24ºC) and 16:8 (L:D) h of stable light regime to simulate the light conditions during the species breeding season.

To be able to follow closely the developmental times of *T. sinuatus* bred at laboratory conditions, the breeding colonies were inspected daily to remove the eggs. The newly laid eggs were transferred into petri dishes (max. 10 eggs per Petri dish) and kept under the same conditions as their parents. Each Petri dish was filled with a thin layer of moist gardening soil, and the eggs were placed on the surface.

Emerging larvae of new generation were separated into Petri dishes. Each Petri dish was filled up with moist gardening substrate and held closed by rubber band. The larvae had received a small piece of pork meat, which was regularly replaced with fresh piece. Larvae were kept together in the same dish until reaching the third larval instar (L3). Third instar larvae were separated individually to reduce cannibalism.

A linear regression calculated the relationship between temperature and development length to obtain parameters and standard errors (lower developmental threshold and sum of effective temperatures) of thermal summation models for each developmental stage. Only the data of individuals completing the whole development was used in the analysis. Parameters of the thermal summation model were estimated for each developmental stage 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.
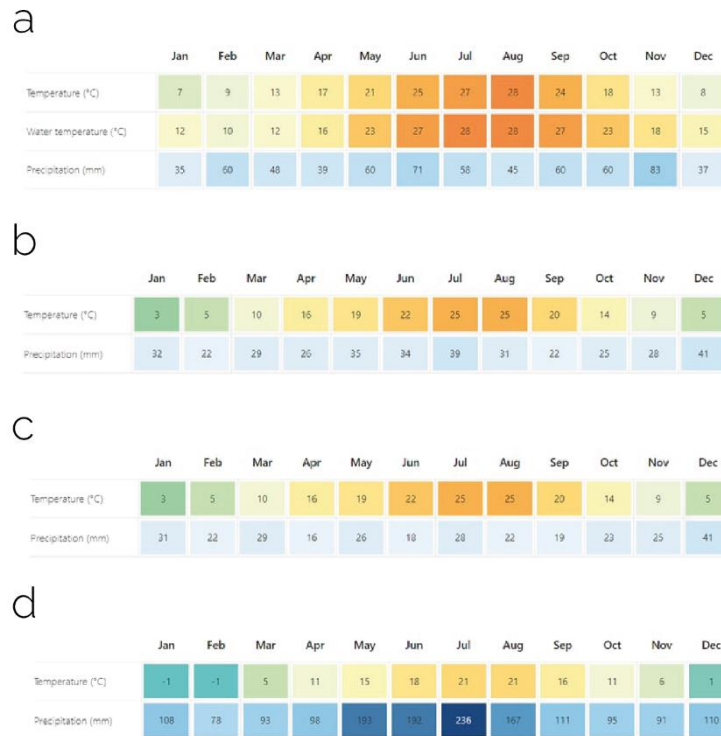
6.3. Climatic conditions of the selected locations

One of the goals of this work is to discover if there is between-population variation in larval development rate. Populations of the same species were collected in three countries with different climatic conditions. It should be understood for a further analysis and a better understanding of findings. To compare the average weather for each location by month, the closest localities from sampling locations were taken.

All locations have the oceanic climate prevailing. The highest average temperature is 28°C in August and the lowest is 7°C in January for Italy; the highest average temperature is 25°C in July and the lowest is 3°C in January for Germany; and the highest average temperature is 21°C in July and the lowest is -1°C in January for the Czech Republic.

According to the data available, it can be stated that the average temperature is 10.25 ºC and the average amount of precipitation is between 78 and 236 millimeters annually for Czech location; average temperature is 14.42 ºC and the average amount

of precipitation is between 16 and 41 millimeters annually for German location; and the average temperature is 19.92 ºC and the average amount of precipitation is between 35 and 83 millimeters annually for Italian location.

Figure 7: Average weather of locations by month: **(a)** Italian population – Caorle, **(b)** German population – Rüsselsheim, **(c)** German population – Frankfurt am Main, **(d)** Czech population – Včelná pod Boubínem (from https://www.besttimetovisit.co.in/, fixed by Ipatova, March 2022)

From this, beetles collected in the Czech Republic inhabited territories with colder and wetter climate compared to the German and Italian populations. The beetles collected in Germany inhabited an area with a warmer climate compared to the Czech Republic and the amount of precipitation in this area is the lowest of all the populations represented. In turn, habitat of the Italian population is the warmest and most temperate as far as precipitation is concerned.

6.4. Results

In this study, 733 individuals of *T. sinuatus* from three different geographical regions (Czech Republic, Germany, Italy) were used to gather information regarding to the developmental length at three constant temperatures (14, 20, and 24°C). Life cycle from egg until adult was completed at all temperatures. In total, 417 out of 733 specimens reached adulthood. The observed developmental lengths differed among

temperatures and between population. Variation in the development rate was observed as well. Developmental rate increased with temperature.

Thermal summation models were established for five developmental stages **(Fig. 8)**. The accumulated degree days (ADD) and lower developmental threshold (LDT) values were calculated for all populations **(Table 1, 2, 3)**. To complete the whole development from the 1st larval instar to the emergence of the imago, the beetles from the Czech Republic (CZ) population needs to accumulate 346.38 °C ADD with a LDT set at 7,39 °C; German (DE) population needs to accumulate 307.94 °C ADD with a LDT set at 8.87 °C and Italian (IT) population needs to accumulate 278.83 °C ADD with a LDT set at 9.49 °C. Values coefficient of determination (R^2), for all inspected models were above 0.75, indicating good fit on the data.

Table 1: Summary of the developmental constants for *T. sinuatus* at five developmental stages.
Ikemoto and Takai
Czech (CZ) population

Developmental stage	Temperature range of model (°C)	R^2	df	p-value	ADD (°C)	LDT(°C)
1st instar larva	14-24	0,896	396	> 0,001	19,3115	11,065
2nd instar larva	14-24	0,782	396	> 0,001	9,8217	15,2093
3rd instar larva	14-24	0,762	396	> 0,001	55,1363	9,5555
Post-feeding	14-24	0,932	396	> 0,001	65,1701	9,2917
Pupae	14-24	0,919	396	> 0,001	109,4987	8,8708
Complete development	14-24	0,941	396	> 0,001	**346,3781**	**7,3874**

Table 2: Summary of the developmental constants for *T. sinuatus* at five developmental stages.
Ikemoto and Takai
German (DE) population

Developmental stage	Temperature range of model (°C)	R^2	df	p-value	ADD (°C)	LDT(°C)
1st instar larva	14-24	0,896	396	> 0,001	16,9926	11,8864
2nd instar larva	14-24	0,782	396	> 0,001	20,84	11,0431
3rd instar larva	14-24	0,762	396	> 0,001	64,55	7,9909
Post-feeding	14-24	0,932	396	> 0,001	70,6412	10,0729
Pupae	14-24	0,919	396	> 0,001	101,5656	9,6326
Complete development	14-24	0,941	396	> 0,001	**307, 942**	**8,867**

Table 3: Summary of the developmental constants for *T. sinuatus* at five developmental stages.

Developmental stage	Temperature range of model (°C)	R²	df	p-value	ADD (°C)	LDT(°C)
				Ikemoto and Takai Italian (IT) population		
1st instar larva	14-24	0,896	396	> 0,001	12,1813	13,7423
2nd instar larva	14-24	0,782	396	> 0,001	20,3022	10,7061
3rd instar larva	14-24	0,762	396	> 0,001	64,55	10,7611
Post-feeding	14-24	0,932	396	> 0,001	56,4515	11,1017
Pupae	14-24	0,919	396	> 0,001	102,7871	9,8867
Complete development	14-24	0,941	396	> 0,001	**278,827**	**9,494**

From this, Czech (CZ) population needs to accumulate the highest number of ADD (346.38 °C); Italian (IT) population – the lowest (278.83 °C). The opposite is, LDT is the highest for Italian (IT) population (9.49 °C) and the lowest for Czech (CZ) population (7,39 °C). Czech (CZ) beetles has the fastest development rate at a lower experimental temperature (14 °C), as opposed to German (DE) and Italian (IT) beetles, which developed more slowly at this temperature **(Fig. 9(f))**. In contrast, Italian (IT) beetles has the fastest development rate at a higher experimental temperature (24 °C) and Czech (CZ) beetles developed more slowly at this temperature.
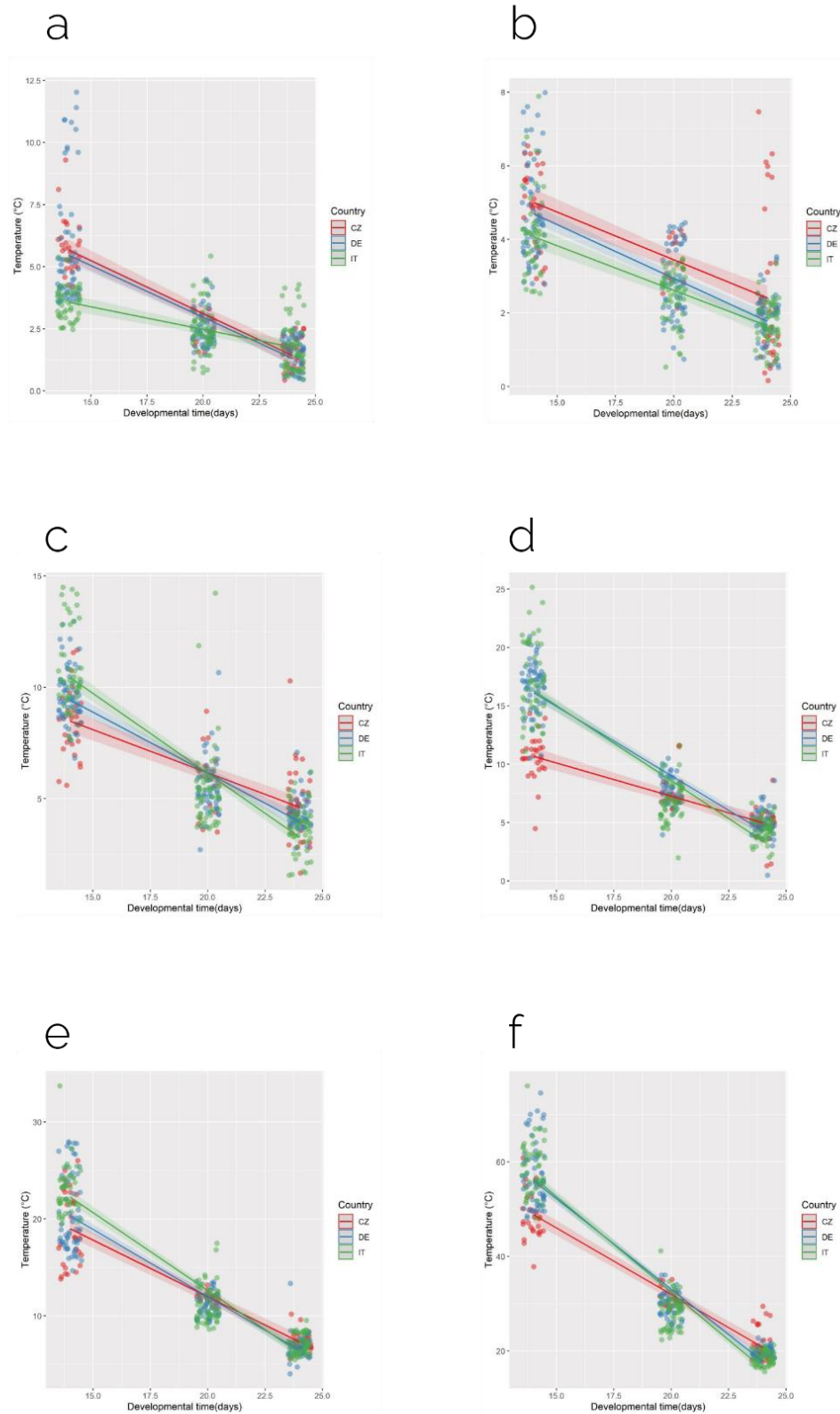
Figure 8. Ikemoto and Takai thermal summation model for five developmental stages of *T. sinuatus*:
(a) 1st larval instar, **(b)** 2nd larval instar, **(c)** 3rd larval instar, **(d)** post-feeding, **(e)** pupae, **(f)** summary.

Figure 9. Observed range of development times of *T. sinuatus* from three different geographic regions (Czech Republic, Germany, and Italy) over three experimental treatments (14, 20, 24 °C) for five developmental stages (data were excluded for egg): **(a)** 1st larval instar, **(b)** 2nd larval instar, **(c)** 3rd larval instar, **(d)** post-feeding, **(e)** pupae, **(f)** summary. The horizontal lines within the boxes indicate median values. The upper and lower boxes indicate the 75th and 25th percentiles, respectively. Whiskers indicate the values with the 1.5 interquartile ranges. Small dots are outliers.

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Figure 10. Effect temperature on development time for five developmental stages of *T. sinuatus* from three different geographic regions (Czech Republic, Germany, and Italy): **(a)** 1st larval instar, **(b)** 2nd larval instar, **(c)** 3rd larval instar, **(d)** post-feeding, **(e)** pupae, **(f)** summary

7. DISCUSSION

Many studies have highlighted the importance of *Thanatophilus sinuatus* for forensic investigations. Not long ago, Montoya-Molina et al. (2020) provided for the first time the thermal summation models for all the developmental stages for the species in the Czech Republic. Moreover, thermal summation models for species belonging to the *Thanatophilus* genus have been published by other authors (Midgley & Villet, 2009; Ridgeway et al., 2013; Montoya-Molina et al., 2021). For the first time in this work, the thermal summation model for all developmental stages of *T. sinuatus* is compared along geographical gradient (Czech Republic, Germany, and Italy).

Studies considering the effects of different temperatures on developmental rate were already conducted in other species of the family Silphidae and other insect families. The results of this work are consistent with those previous studies. The results of different studies also demonstrate the impact of temperature on development rate in insects. Velásquez & Viloria (2009), while breeding *Oxelytrum discicolle* (Brullé 1836), observed that while increasing the breeding temperatures, the total time needed for the beetles to complete the development decreases. Furthermore, when studying ant populations, Kipyatkov & Lopatina (2002) demonstrated that temperature increases always shortened the development time of all the populations studied. Anderson (2000) researched the minimum and maximum developmental rates of some species of forensically significant blowflies of the family Calliphoridae. His study showed that development has been faster at a higher temperature (Anderson, 2000).

Between-population variation effects were seen in this study. Developmental threshold (LDT) values for different populations ranged from 7.4°C for the Czech Republic to 9.5°C for Italy, and that is almost 2.1°C higher for *T. sinuatus* from Italian population. The observed differences are significant. Development of the Czech population of *T. sinuatus* from 1st instar larva to adult took 31.1 days at 20°C and 20.9 days at 24°C. This is longer than for another populations, including German population, which takes 27.7 days at 20°C and 20.3 days at 24°C, and Italian one, which takes 25.1 days at 20°C and 19.2 days at 24°C, which is the fastest result. However, the opposite trend is that development of the Italian population at low temperature took longer than the Czech population, which takes 52.4 days at 14°C, while Italian beetles takes 61.9 days at 14°C. It is likely that the observed differences between Czech, German and Italian populations of *T. sinuatus* are due to specific differences in their areas and probably adaptation to local climatic conditions.

It was evident the change in the duration of development. Hence, values of ADD and LDT differ between the studied populations. Is important to mention that, in accordance to the already published literature, populations presenting higher ADD and low LDT will develop faster at low temperatures, in this study the Czech population. On the other hand, populations featuring low ADD and high LDT will develop faster, as here was observed for the Italian population. This changes in the values of LDT and ADD are necessary for the development of the species and to successfully achieve adulthood. One explanation of this process on insects' populations is an adaptation to a better performance in environments where animals have to encounter long periods of low temperatures (Honěk & Kocourek, 1990).

Mortality of the larvae was very low during the entire experiment, however, there was the mortality in the egg stage, namely the eggs did not hatch. This was the reason for presented the thermal summation model just for five developmental stages of *T. sinuatus*, because the data were excluded for eggs. I encountered mortality problem when I did the experiment already for my bachelor's thesis. But it should be noted that I worked with another species and mortality of the larvae was high, especially in the third larval instar. I suppose it could be attributed to several causes, the most obvious of which are an infection and the substrate affected by mold. This time mortality has been low. Montoya-Molina et al. (2020; 2021) study, while working with the same species, and with *Thanatophilus rugosus* also faced similar mortality in the egg stage, and they noticed that it could be probably a bacterial or fungal infection.

Also, some deformations of adult beetles were detected at the highest experimental temperature (24°C).

8. CONCLUSIONS

The main subjects discussed in my master's thesis are the model species *Thanatophilus sinuatus*, the thermal summation models for this species and geographical variability of this species within three geographic regions with different climatic conditions (Czech Republic, Germany, and Italy). Primarily, it was necessary to examine in advance the relevant information and then write a literature review related to the key topics 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 Silphinae. I had the same problem when I was writing my bachelor's thesis, that is, the necessary information regarding the subfamily Silphinae, which were concern to me, but publications about the subfamily Nicrophorinae were available in large number.

In the practical part, I have reviewed the biology of *Thanatophilus sinuatus*. There is a description of the laboratory investigation, including the methodology of the work. Thermal summation models for the five stages of *T. sinuatus* for three different populations of the same species (Czech Republic, Germany, and Italy) was created as a result of the laboratory experiment. In addition, between-population variation in larval development rate were observed.

Both pre-set hypotheses were confirmed:

> Temperature has impact on larval development rate, and if the the temperature rises, this leads to the development rate increases and larval stage duration becomes shorter.
> Between-population variation in larval development rate exists.

Czech population from region with a colder and wetter climate compared to the German and Italian populations, has greater cold tolerance, and has higher larval development rate at lowest experimental temperature 14 ºC, than populations of the same species from warmer regions (Germany, Italy). The reverse is also true: Italian population from warmer region has greater heat tolerance and has higher larval development rate at the highest experimental temperature 24 ºC, than populations from colder regions (Czech Republic, Germany).

Silphidae is a fascinating family of insects, with interesting ecology and a high potential utility in forensic entomology. At the same time, very regrettably, most of the studies, especially in the past, has focused on flies (Diptera), so necrophagous beetles of the family Silphidae did not get as much scientific attention as they deserved. But fortunately, the situation is gradually changing, and I would like to point to this trend began to change, slowly but surely. Scientists are beginning to direct their attention to this topic. I am pleased to see that this gap can be eliminated. There is still a lot of work to be done, and it will take time, and a combined effort of many scholars.

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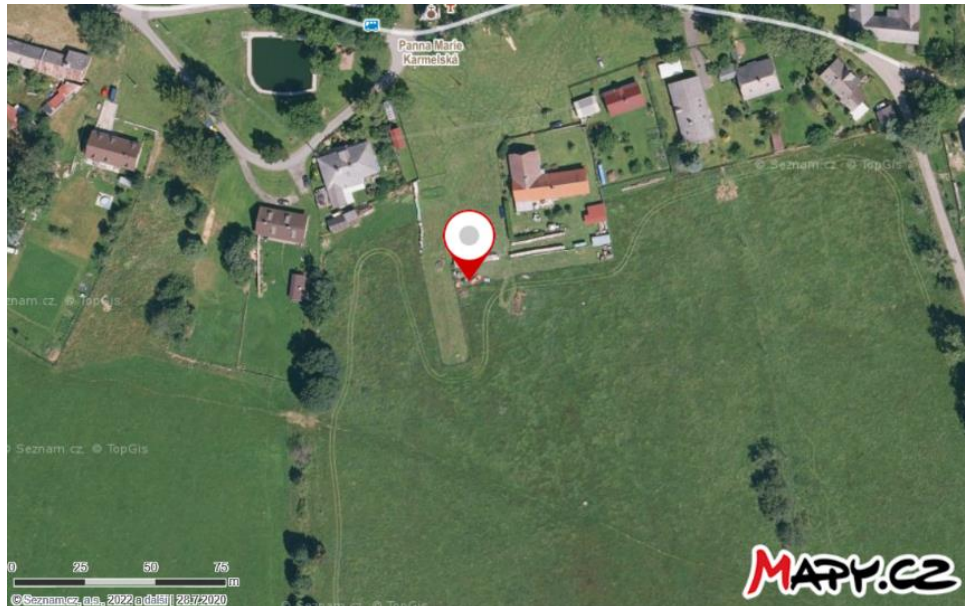
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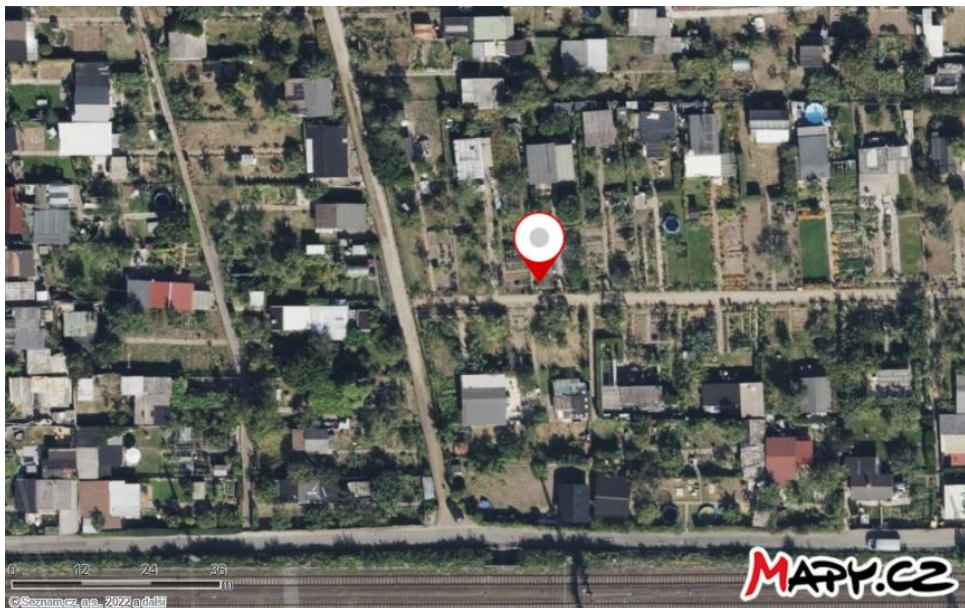
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10. ANNEXES

Annex 1: The sampling location of the Czech population. Near Včelná pod Boubínem, 49°01'19.3"N 13°51'33.6"E (from https://mapy.cz/, fixed by Ipatova March 2022)

Annex 2: The first sampling location of German population. Frankfurt Griesheim, 50°05'41.5"N 8°37'06.4"E (from https://mapy.cz/, fixed by Ipatova March 2022)

Annex 3: The second sampling location of German population. Rüsselsheim am Main, 49°58'21.7"N 8°27'15.4"E (from https://mapy.cz/, fixed by Ipatova, March 2022)

Annex 4: The sampling location of Italy population. Venezia Prov., Caorle, Via Strada Nuova street, 45°36.24'N 012°52.85'E (from https://mapy.cz/, fixed by Ipatova, March 2022)
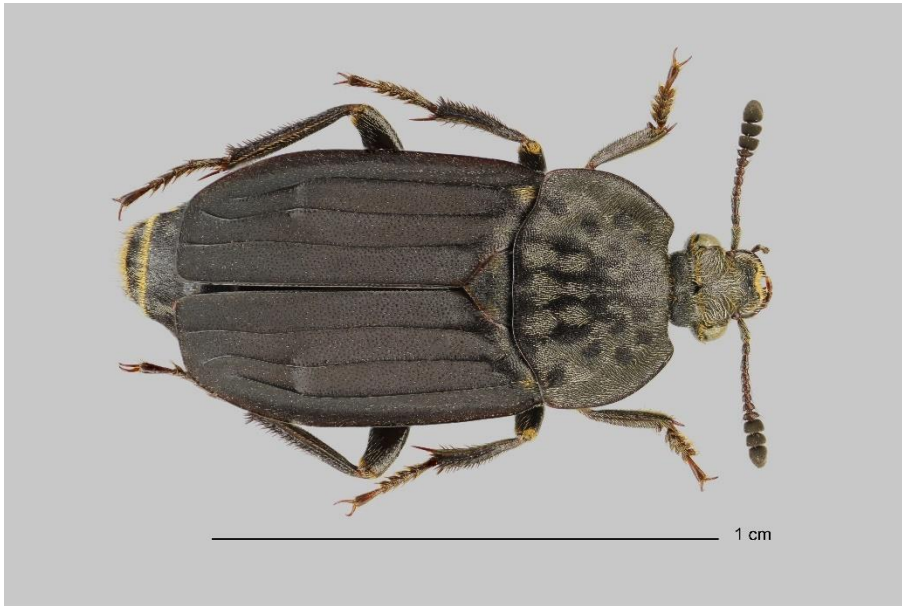
Annex 5: Adult carrion beetle. *Nicrophorus vespillo* (Linnaeus, 1758), female (photo by Pavel Jakubec).

Annex 6: Silphidae larvae. *Nicrophorus (Nicrophorus) nepalensis* Hope, 1831, 3rd instar larva (photo by Hana Šípková).

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Annex 7: Male of *Thanatophilus sinuatus* (photo by Pavel Jakubec).

1 cm

Annex 8: Female of *Thanatophilus sinuatus* (photo by Pavel Jakubec).

1 cm

Annex 9: Larvae of *Thanatophilus sinuatus* (photo by Pavel Jakubec).

Annex 10: Larvae of *Thanatophilus sinuatus* (photo by Pavel Jakubec).