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Research

Developmental Models of the Forensically Important Carrion Beetle, *Thanatophilus sinuatus* (Coleoptera: Silphidae)

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Abstract

Carrion beetles of genus *Thanatophilus* (Leach, 1815) are an important group of necrophagous insects, with great potential for forensic entomology in temperate zones of Africa, America, Asia, and Europe. Developmental models for majority of *Thanatophilus* species remain unknown. In this study, we will provide new thermal summation models for all the developmental stages of *Thanatophilus sinuatus* (Fabricius, 1775), one of the most abundant and widespread species of the genus. The beetles were bred at seven different constant temperatures, and developmental time was measured for each developmental stage (egg, three larval instars, postfeeding stage, and pupa). Temperature–sex influence was tested, and thermal summation constants were calculated to be used for postmortem interval estimation during criminal investigations.

Key words: Silphinae, developmental biology, thermal summations model, forensic entomology

Forensic entomology studies arthropods related to criminal investigation (Catts and Goff 1992, Anderson 1995, Byrd and Castner 2010, Joseph et al. 2012). The insect community associated with corpses provides an important tool for estimating the length of time since death, expressed as the postmortem interval (PMI; Anderson 2010, Goff 2010, Amendt et al. 2011). A common method for PMI estimations gathered from insect evidence is based on the age calculation of the oldest immature stages collected from the cadaver (Higley and Haskell 2010, Amendt et al. 2011). Within insects, two major groups are generally used: beetles (Coleoptera) and flies (Diptera), together providing the majority of evidence for forensic investigations (Byrd and Castner 2010). Much of the research has focused on understanding the succession and colonization of cadavers by blowflies (Diptera: Calliphoridae), but there has been much less research of this process in Coleoptera, underutilizing the valuable information that this group can provide to criminal investigations (Matuszewski et al. 2008, Midgley and Villet 2009, Dekeirsschieter et al. 2011b, Ridgeway et al. 2014). Only recently, studies have shown that Silphid beetles could be as useful for criminal investigations (Midgley et al. 2010, Ridgeway et al. 2014). Two characteristics are crucial in that regards, namely swift colonization of cadavers soon after death (particularly in species of genera *Thanatophilus* Leach, 1815 and *Necrodes* Leach, 1815) and low development rate of the majority of known species (see Ratcliffe and Luedtke 1969, Midgley and Villet 2009, Ridgeway et al. 2014, Jakubec 2016, Frątczak-Łagiewska and Matuszewski 2018a, Jakubec et al. 2019).

PMI can be calculated based on the age of the oldest developmental stage of an insect collected from a corpse using mathematical models. These, among others, include the thermal summation model (TSM), which is the most widely used among forensic entomologists (Amendt et al. 2011, Villet 2011). TSM models are species and stage specific (Ridgeway et al. 2014); thus, accurate PMI estimation depends on the availability of these models for species found at the crime scene.

The family Silphidae comprises 187 species distributed almost worldwide (Sikes 2008, Newton 2020), with the highest diversity in the temperate zone of Eastern Palearctic Region (Růžička 2015). Adults and larvae are attracted by decaying organic matter, and majority of the species can be found on animal and human cadavers (Sikes 2008, Ridgeway et al. 2014). Developmental stages of silphids are nowadays recognized as an indicator of PMI in medico-legal studies (Midgley and Villet 2009, Joseph et al. 2012, Ridgeway et al. 2014).

Among Silphidae, genus *Thanatophilus* contains 24 valid extant species, distributed in Europe, Asia, North America, and Africa (Ratcliffe 1996, Newton 2020). Due to its wide distribution, fast

localization, and colonization of carrion within 24 h of death, some species of the genus have high applicability in forensic studies (Midgley and Villet 2009, Ridgeway et al. 2014). *Thanatophilus sinuatus* is a common carrion beetle frequently found on carcasses of large vertebrates in Europe (Matuszewski et al. 2008, 2010; Dekeirsschieter et al. 2011a,b; Jakubec and Růžička 2017; Jarmusz et al. 2020). Furthermore, the beetle was recorded on 13.27% of human remains, investigated by forensic entomologists in the Czech Republic (Jakubec et al. 2019). Based on its life cycle and natural history, the species meets most of the criteria to be used for PMI estimation as defined by Matuszewski et al. (2010). Recent studies conducted on *T. sinuatus* were related to its seasonal and daily rhythms (Kočárek 1997, 2002), preference of particular type of soil (Jakubec and Růžička 2015), behavioral responses (Dekeirsschieter et al. 2013), and larval morphology (Jakubec et al. 2019). Jakubec et al. (2019) additionally reared the species under artificial conditions and gathered preliminary data that have been supplemented and re-analyzed in the present work.

In this study, we present the first TSM based on the developmental cycle of the widely distributed trans-Palearctic species, *T. sinuatus*. To achieve this, we reared *T. sinuatus* at seven different temperatures measuring the development rate for each of the temperatures and stages. Additionally, we tested whether sex influences the developmental length and whether the temperature affects the time proportions among the stages. Finally, we categorize the species as forensically relevant and applicable in accurate calculations of the PMI.

Materials and Methods

Laboratory Colony Establishment

Adult beetles of *T. sinuatus* were collected by pitfall traps baited with pork muscle tissues (*Sus scrofa* Linnaeus, 1758) and beef tissue (*Bos taurus* Linnaeus, 1758). The sampling was conducted in the Czech Republic during 2 yr. Between May and July of 2016, we sampled at three localities: Albeř (49°01′37″N, 15°08′43″E), Borová (49°44′30.05″N, 16°9′44.03″E), and Prague-Petřín (50°5′6.86″N, 14°23′11,60″E). During May and August of 2019, the collection took place around Kostelec nad Černými lesy (50°00′01″N, 14°51′33″E), Kolín-Štítary (50°0′49.78″N, 15°10′9.54″E), and Prague-Lysolaje (50°07′33.0″N, 14°21′44.0″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, identified, and sexed with the help of an identification key (Šustek 1981, Schawaller 1981, 1987). Individuals were separated into breeding colonies (maximum 10 per box), with an equal number of males and females. Each breeding colony came from the same locality avoiding cross-breeding and placed into a breeding box (Exo Terra: Art. #PT2250, dimensions 180 × 110 × 125 mm) filled with ca. 50 mm of sterilized moist gardening soil, used as the oviposition substrate. Beetles were provided with a piece of pork muscle tissue ad libitum. Additionally, a water source was provided in a form of 5-ml micro Eppendorf tube filled with water and stoppered by a piece of cotton.

Colonies were kept inside climatic chambers (custom made by CIRIS s.r.o.) at seven constant temperatures (14, 16, 18, 20, 21, 24, and 26°C) and 16:8 (L:D) h of dark photoperiod regime, maintained by fluorescent light (Osram L 8W/640) to simulate the light conditions during the species breeding season. Used temperatures and photoperiod were based on the previous rearing of this species by Jakubec et al. (2019). Breeding colonies were inspected once a day to remove newly laid eggs. Egg batches were placed into separate Petri dishes (100 × 15 mm) 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. Approximately 5 g of pork meat was also placed inside the dish and that was then secured with an elastic rubber band. The setup described here permits the larvae to start feeding immediately after hatching.

Once the first-instar larvae hatched, they were separated into smaller Petri dishes (60 × 15 mm). This layout followed the methodology suggested by Ridgeway et al. (2014). Each dish was filled up with moist gardening substrate to two thirds, placed on its side, and held closed by rubber band. Furthermore, the larvae were provided with a piece of pork meat, and the substrate was moistened regularly by submerging the bottom third of the dish into water for a few seconds. Unconsumed and moldy meat was regularly removed and replaced with fresh pieces. This layout enables observation of various life stages without unnecessary disturbances to the specimens. Newly emerged first-instar larvae hatched from the same clutch of eggs were placed in the Petri dish in groups of up to five specimens maximum. Developmental milestones (egg, first-instar larva [L1], second-instar larva [L2], third-instar larva [L3], postfeeding stage [PF], and pupae) were distinguished based on morphological features described by Jakubec et al. (2019). The whole breeding process was monitored once a day at 14, 16, 18, 20, 21°C and twice a day at 24 and 26°C.

Larvae were kept together in the same dish when their development rate in terms of instar level was the same or until reaching the final third larval instar (L3). This way, we minimized cannibalism and mortality that individual larvae often experience due to inefficient feeding. All newly emerged L3 were individually separated into Petri dishes (60 × 15 mm), so they would not be disturbed by other larvae in their postfeeding stage and especially once they created a pupation chamber. When a specimen reached pupal stage, the food was removed, and the Petri dish remained closed until its development to adult stage was completed.

Thermal Summation Model

To show the relationship between the beetles' developmental times and the accumulated degree days (DD) for all the developmental stages, we applied Ikemoto and Takai (2000) linear regression model ($DT = k + tD$) for calculating thermal summation parameters (k and t). In the equation, D is the duration of development, T is the environmental temperature, t is the lower developmental threshold, and k is the thermal summation constant. The main advantage of using this model is its robustness and simplicity. Both parameters of thermal summation are defined as a slope (t) and intercept (k) of the linear regression, respectively. Therefore, both t and k can be easily calculated along with their respective SE (Ikemoto and Takai 2000).

Effect of Sex on Development Length

A group of specimens from 14, 18, 20, 24, and 26°C that have completed development until adult stage was selected for the study of the effect of sex on development length, as only these specimens could be sexed. A binomial test was conducted to determine whether the observed sex ratio for the species is significantly different from the expected one (1:1).

To assess the potential differences in developmental time between males and females, two linear mixed effect regression models were fitted (null and 'sex' model). Response variables of both models were the developmental lengths of the six stages (egg, L1, L2, L3, PF, and

pupae). Null and alternative model also shared two fixed effect explanatory variables: temperature (14, 18, 20, 24, or 26°C) and developmental stage. Because the measurements of development times were done repeatedly on the same individual, the identity of that specimen had to be incorporated into the analysis; therefore, we used it as a random effect in both models. The only difference between the alternative and the null models was fitting the latter with sex as a fixed effect explanatory variable. The fit of these two models was compared via Akaike information criteria (AIC) to find out if the information about sex does significantly improve it.

Developmental Rate Isomorphy

Presence of developmental rate isomorphy (DRI) was tested using Dirichlet regression as suggested by Boukal et al. (2015). The main advantage of this method is that it does not require the temperatures to be within a range in which the development rate has a positive linear relationship with the temperature. The method consists of fitting three Dirichlet regression models with the same response variable (proportion of time spent in each developmental stage) and with several explanatory variables. The null model (mod0) was fitted without any explanatory variables to simulate the assumption that the proportions of time spent in individual instars are constant and independent of other factors (true presence of DRI in the species). The alternative models were fitted with temperature as explanatory variable (mod1) and the third model with expected quadratic effect of temperature (mod2). Both alternative models suggest that the DRI is not present in the species. Relative quality of each model was evaluated by AIC, to allow comparison. The one with the lowest value was considered as the most appropriate description of the underlying relationship.

Statistical Analysis

Data management and analyses were carried out using the R program (version 3.5.1; R Core Team 2016). Additionally, we used lme4 package for fitting mixed effect models, and visual outputs were processed via packages ggplot2 and sjPlot (Bates et al. 2015, Wickham 2016, Lüdecke 2018).

Results

Development of *T. sinuatus* at Seven Constant Temperatures

In this study, 918 individuals of *T. sinuatus* were used to gather information regarding to the developmental length at seven constant temperatures (14, 16, 18, 20, 21, 24, and 26°C). All tested temperatures, except for 21°C (see Discussion), allowed the completion of the life cycle from egg until adulthood. In total, 155 specimens reached adulthood. Observed developmental lengths differ between temperatures. The duration of the development at the lowest temperature (14°C) was 78.24 d (SD = 6.31; n = 23), while at the highest temperature of (26°C) was only 22.23 d (SD = 1.20; n = 16; Table 1). The greatest proportion of *T. sinuatus* development cycle was spent during the postfeeding (25%) and the pupal stage (33%), whereas the shortest time was spent in the L1 (6–7%) and L2 (8–10%) stages in all studied temperatures.

Mortality rate was calculated using a subset of specimens that were obtained during 2019 only, as the mortality was not tracked in the previous years. Obtained data spanned five temperatures (14, 16, 18, 24, and 26°C) and covered all of the developmental stages. The overall mortality rate was mostly associated with extreme temperatures. Higher mortality was found on both ends of the extreme temperatures tested: 14°C (73.19%) and 26°C (78.37%). Mortality rate across intermediate temperatures (16–24°C) oscillated between 35.0 and 50.0%. Mortality rate also varied between the developmental stages. The highest mortality was found during the egg development at all temperatures, in which 60.11% of the eggs did not hatch. In the follow-up stages, the mortality increased very little. Being 54.92% for L1; 53.27% for L2; 43.98% for L3; and 42.62% for pupae.

Thermal Summation Model

Thermal summation models were established for all developmental stages (egg, L1, L2, L3, PF, and pupae; Fig. 1, Table 2). The (k) constant and (t) values were calculated with expected errors (Table 2). To complete the whole development from start to finish the individual needs to accumulate on average 360.46 DD with lower developmental threshold set at 9.85°C. Females needed only 330.24 DD, whereas males needed 375.36 DD and their respective t values were 10.21 and 9.35°C. Values of determination coefficient (R^2) for most of the models were above $R^2 > 0.91$, indicating good fit of the models on the data (Table 2). For egg and L2 stages, the determination coefficient was <0.91 ($R^2 = 0.857$ and $R^2 = 0.889$).

Effect of Sex on Development Length

Effect of sex on the developmental length was calculated using a group of beetles that were able to complete the development until the adult stage, from the temperatures of 14, 16, 18, 20, 24, and 26°C. Sex ratio (number of males related to number of females) was on average 1.15 in favor of males; however, the probability of males and females in the sample did not differ significantly from equality (exact binomial test, n = 168, 95% confidence interval [0.37, 0.53], P = 0.247). Sex ratio varied substantially between temperatures. The minimum observed at 16°C = 0.69 (n = 54) and 18°C = 0.67 (n = 5) and maximum at 20°C = 2.33 (n = 10) and 24°C = 2.20 (n = 32).

Development length was similar for both males and females, throughout all of the developmental stages and even as a total (see

Table 1. Mean developmental times (SD; *N*) of *Thanatophilus sinuatus* at seven constant temperatures for each developmental instar (d, °C)

Temperature	Egg	First instar	Second instar	Third instar	Post-feeding	Pupae	Total development cycle
14	7.51 (1.28; 47)	5.39 (0.73; 44)	5.77 (1.08; 37)	13.67 (3.38; 35)	20.60 (3.86; 25)	28.90 (3.73; 23)	78.23 (6.31; 23)
16	—	6.50 (1.85; 58)	6.16 (1.37; 58)	14.31 (2.50; 62)	19.64 (4.46; 56)	28.70 (4.38; 54)	NA
18	5.36 (0.9; 132)	3.89 (1.18; 128)	4.93 (1.80; 127)	9.29 (2.77; 121)	11.10 (4.10; 49)	14.84 (2.64; 38)	43.44 (7.40; 38)
20	3.47 (0.54; 82)	2.73 (0.73; 115)	4.21 (1.30; 92)	8.56 (1.55; 50)	11.17 (2.57; 29)	13.56 (2.44; 20)	42.04 (3.12; 21)
21	3.44 (0.66; 100)	2.61 (0.57; 91)	3.57 (0.85; 85)	6.18 (1.46; 65)	8.87 (1.82; 7)	NA	NA
24	2.96 (0.33; 37)	1.74 (0.44; 32)	1.86 (0.20; 24)	4.77 (0.90; 24)	5.94 (2.11; 32)	7.50 (2.89; 32)	24.77 (2.40; 32)
26	2.61 (0.43; 32)	1.50 (0.49; 22)	2.00 (0.73; 17)	4.52 (1.82; 17)	4.99 (0.87; 16)	7.03 (0.90; 16)	22.22 (1.20; 16)

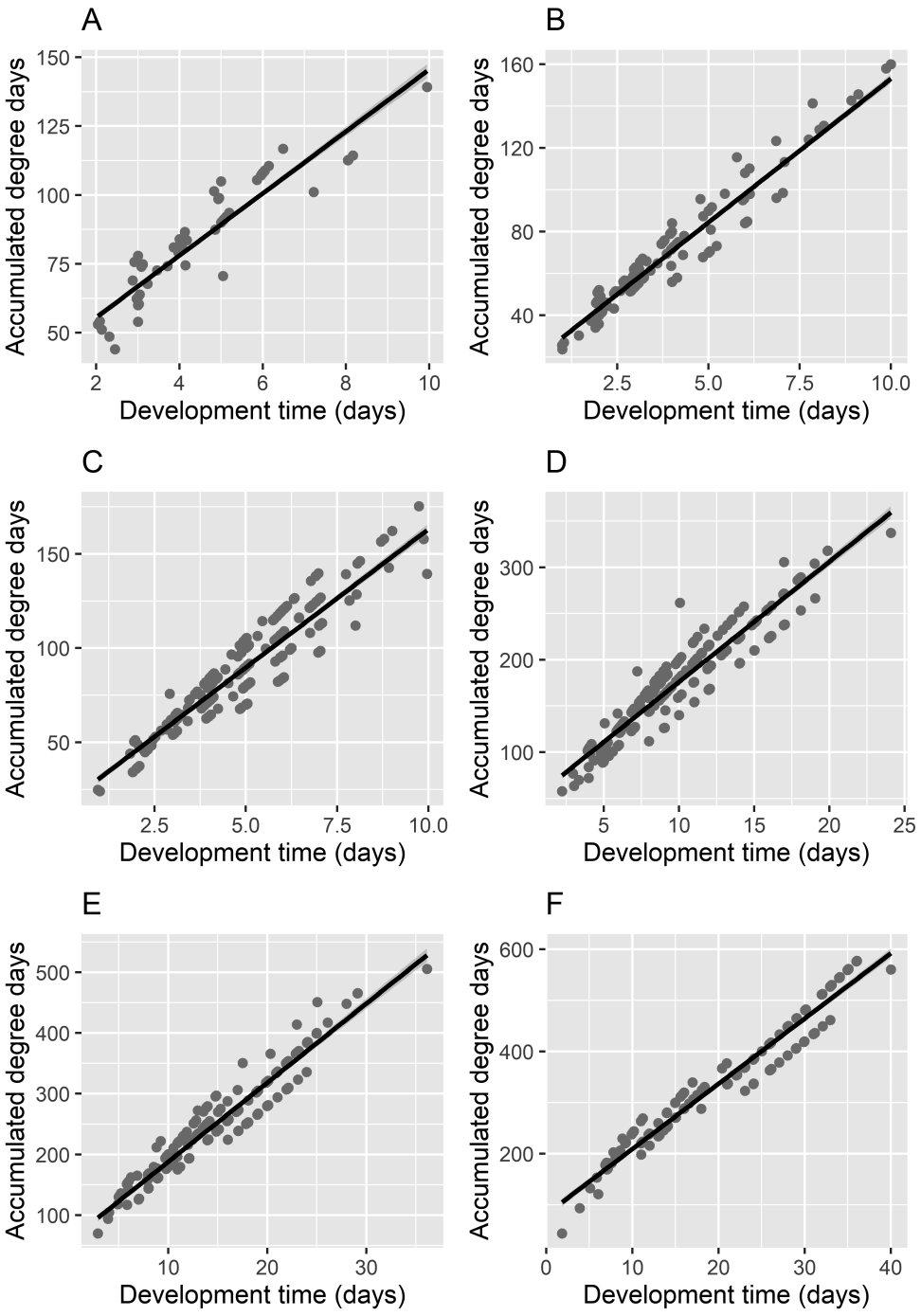

Fig. 1. Ikemoto and Takai's (2000) thermal summation model for all developmental stages of *Thanatophilus sinuatus*: (A) Egg, (B) first larval instar, (C) second larval instar, (D) third larval instar, (E) post-feeding, (F) pupae. The points indicate used data for the regression analysis.

Table 2. Overview of thermal summation models for six developmental stages of *Thanatophilus sinuatus*

Developmental stage	Temperature range of model (°C)	R^2	df	*P*-value	K (°C)	T_0 (°C)
Egg	14–26	0.857	428	>0.001	33.097 (1.03)	11.25 (0.22)
First-instar larva	14–26	0.933	488	>0.001	15.785 (0.66)	13.727 (0.16)
Second-instar larva	14–26	0.884	138	>0.001	16.171 (1.22)	14.719 (0.25)
Third-instar larva	14–26	0.903	372	>0.001	45.893 (2.24)	13.021 (0.22)
Post-feeding	14–26	0.936	212	>0.001	57.701 (3.44)	13.03 (0.23)
Pupa	14–26	0.957	181	>0.001	81.637 (4.18)	12.764 (0.19)
Complete development	14–26	0.933	128	>0.001	360.46 (10.75)	9.85 (0.23)

Number in parenthesis are the SE.

Fig. 2). Comparison of the null and sex model by AIC values showed that the null model had lower AIC values (null AIC = 2,785.5, sex AIC = 2,788.2); consequently, we can imply that the information about the sex of immature stages did not improve the fit of the model significantly.

Developmental Rate Isomorphy

Dirichlet regression was applied to individual development data for the egg, L1, L2, L3, PF, and pupae of *T. sinuatus* reared under the temperatures of 14, 18, 20, 24, and 26°C. When comparing all three models (mod0, mod1, and mod2), the model with quadratic effect of temperature had the lowest value of AIC (AIC = −2,334.8), followed by the model with temperature as an explanatory variable (AIC = −2,304.2). On the opposite side was the model implying DRI, which was accompanied by the highest value of AIC (AIC = −2,272.19), suggesting the worst fit to the data. These results imply that the proportion of the time spent in each developmental stage is not constant but has a quadratic relationship with the temperature.

Discussion

The effects of different temperatures on developmental times in Silphinae have been investigated only recently, and the number of works related to the topic is now increasing, thanks to the use of the taxa in the forensic investigations (Velásquez and Viloria 2009, Midgley et al. 2010, Matuszewski et al. 2013, Ridgeway et al. 2014, Novák et al. 2018, Jakubec et al. 2020).

In comparison with other species of genus *Thanatophilus*, our study shows that *T. sinuatus* spends similar or longer time on the corpses compared with Afrotropical species [*T. micans* (Fabricius, 1794) and *T. capensis* (Wiedemann, 1821)] (Ridgeway et al. 2014). More specifically, at 20°C, *T. sinuatus* takes 42.04 d to complete development from egg to adult. Under the same conditions, *T. capensis* takes 39.6 d and *T. micans* takes 22.64 d (Ridgeway et al. 2014). These species also differ in the lower developmental threshold values: *T. sinuatus* (t = 9.85°C), *T. capensis* (t = 9.04°C), and *T. micans* (t = 13.26°C; Ridgeway et al. 2014). In this case, it is clear that phylogenetical relatedness does not guarantee similarity in developmental characteristics.

Differences in developmental time between Afrotropical species of genus *Thanatophilus* and *T. sinuatus* could be attributed to differences in their areas of distribution and probably adaptation to local climatic conditions. However, comparison could be made between *T. sinuatus* and *T. rugosus* as these two species overlap in their occurrence patterns especially in Europe (Růžička 2015). These two species also share very similar habitat preferences (Kočárek 2001, Jakubec and Růžička 2015, Frątczak-Łagiewska and Matuszewski 2018b). Development length at 20°C seems to be slightly faster in *T. sinuatus*, as the development of *T. rugosus* takes on average 45.48 d (Novák et al. 2018). Deeper comparison between these two species is unfortunately not possible as TSMs for *T. rugosus* are not available.

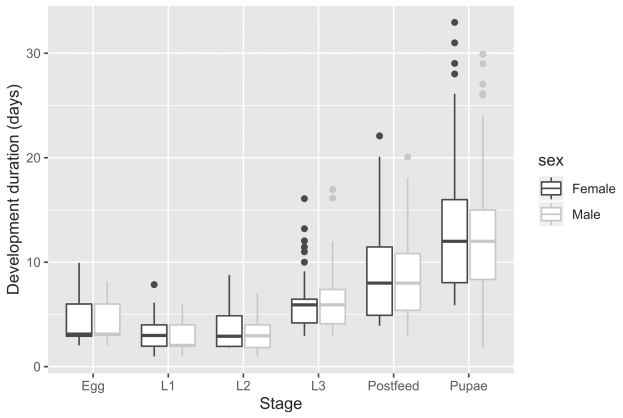

Fig. 2. Developmental length of males and females at each developmental stage (from egg until adulthood). Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively. Whiskers indicate the values within the 1.5 interquartile ranges. Small dots are outliers.

Our results agree with the general concept that development of beetles from genus *Thanatophilus* takes longer than development of forensically important blow flies (Diptera: Calliphoridae) (Ridgeway et al. 2014). Development of common European species of blow flies, such as *Calliphora vicina* Robineau-Desvoidy, 1830, *Phormia regina* Meigen, 1826, and *Lucilia sericata* (Meigen, 1826), can take approximately 21.45, 20.5, and 24.75 d, respectively, at 20°C (Anderson 2000, Nabity et al. 2006, Roe and Higley 2015), which is significantly faster than *T. sinuatus*. Therefore, in cases when the victim is found after more than 25 d, *T. sinuatus* can add additional information to the PMI estimates.

To our knowledge, this study represents the second TSM of any member of the family Silphidae from Europe (see Gruszka and Matuszewski 2020), although TSMs of several nonsilphid necrobiont beetle species from the region were recently reported (e.g., *Sciodrepoides watsoni* (Spence, 1813) (Coleoptera: Leiodidae), *Creophilus maxillosus* (Linnaeus, 1758) (Coleoptera: Staphylinidae), and *Aleochara nigra Kraatz* 1859 (Coleoptear: Staphylinidae); Lin and Shiao 2013, Jakubec 2016, Frątczak-Łagiewska and Matuszewski 2018a, Wang et al. 2018).

Thanatophilus sinuatus oviposition was carried out mostly at night and into the substrate, thus preventing desiccation of the viscous layer that covers the eggs clusters. It differs from the closely related *T. capensis* that oviposits on the surface allowing the eggs to have contact with external air and light, influencing the activity hours of the species (Ridgeway et al. 2014). Nocturnal oviposition in *T. sinuatus* is not exceptional due to the fact that the adults of the species are also active during night, and explanation of the aforementioned authors has some logic, influencing the activity levels of the future larvae during the night.

The methodology suggested by Midgley and Villet (2009) and Ridgeway et al. (2014) has been successfully applied in this work with some variations. Main difference was that freshly hatched L1 was separated into groups of up to five individuals per one small Petri dishes. This approach was based on the premise that larvae up to L2 stage need to be among siblings for the successful development and survival as the aggregation may enable more efficient feeding (Scanvion et al. 2018). The communal feeding of *T. sinuatus* was observed under the field condition by Bonacci et al. (2011). Furthermore, Gruszka and Matuszewski (2020) hypothesized that this could be an ancestral trait of Silphidae. Our observations of high mortality of individually raised L1 larvae of *T. sinuatus* seemed to be independent of the environmental factors and further supports the need for aggregation in early developmental stages in this age group.

During the first year of the breeding (2016), we observed that no larvae were able to finish the development at 21°C. This could have been caused by severe infestation of the experimental animals at this temperature with mites of the genus *Poecilochirus* G. Canestrini et R. Canestrini, 1882 (Acari: Parasitidae), or by spread of a disease we were unable to identify. We did not observe this phenomenon in the

chambers with lower and higher temperatures, thus excluding temperature as the primary cause for the mortality.

The highest mortality was found in the egg stage. Often, the eggs did not hatch and died due to what was probably a bacterial or fungal infection. In addition, we suspect that some eggs may not have been fertilized. We also observed low mortality in early larval stages; nonetheless, L3 larvae often died during the process of building of pupation chamber while in postfeeding stage. In all of the cases where L3 interrupted the PF stage, due to a disturbance or without apparent reason, the L3 was unable to continue the normal developmental cycle and consequently died. Furthermore, we observed that specimens reared at extreme temperatures 14 and 26°C experienced complications during ecdysis from L3 to pupa (see Fig. 3B and C) or had malformations after emerging into adult stage (e.g., deformed wings and vestiges of pupae stage such as the bristles in the pronotum and visible thoracic sclerites (see Fig. 3A).

We did not find any statistically significant difference of developmental parameters between males and females of *T. sinuatus*, which is in line with our previous study of *Necrophila (Calosilpha) brunnicollis* (Kraatz, 1877) (Jakubec et al. 2020) and also conclusions of Gruszka and Matuszewski (2020) for *Necrodes littoralis* (Linnaeus, 1758; both Coleoptera: Silphidae). We were unable to confirm the presence of developmental rate isomorphy as well, following the methodology of Boukal et al. (2015). This also agrees with previously published conclusions for carrion beetles (Jakubec et al. 2020).

Fig. 3. Developmental malformation of *Thanatophilus sinuatus*: (A) Ventral and dorsal view of the deformed adult. (B) Lateral view of the larvae unable to leave the third-instar exuvia for pupation. (C) Pupa unable to complete the developmental cycle into adult; larval appendages and mouth parts are still attached to the pupa.

This study presents the first thermal summation models for all developmental stages of the species. The results here provided could enable forensic entomologists to calculate PMI in cases when the species is found on victim's cadaver. Using *T. sinuatus* for PMI calculations becomes especially useful in cases when the body is found in advanced stage of decay and other species with faster developmental rate already left the corpse. Our results are relevant mainly in the context of the Central Europe as our samples cover a limited geographic range, but they can be used in the future studies of geographic variability of developmental characteristics.

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